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KHAT (*CATHA EDULIS* FORSK) AND ITS EFFECT ON ANTI-MALARIAL CHEMOTHERAPY

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

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DEDICATION

To my father, Brigadier of War and Art, and my mother who have always been there for me with their mind and soul

ABSTRACT

Millions of Yemenis and East Africans habitually chew daily the fresh leaves and twigs of *Catha edulis* commonly known as khat; it produces a stimulating amphetamine-like effect. Most people believe that khat leaves relieve fatigue and reduce body temperature; therefore it is a common practice to chew khat during sickness and during treatment. The effect of khat chewing on the bioavailability of certain antibiotics has been studied; the outcome of these studies showed that khat chewing significantly reduced the bioavailability of the antibiotics, however, khat and its effect on anti-malarial drugs has not been studied. The aim of this study was to evaluate the effect of khat on anti-malarial chemotherapy and to explore possible pharmacokinetic interactions between chloroquine (CQ) and khat during co-administration in healthy adult Yemeni volunteers. Furthermore, we wanted to determine the effect of khat on CQ concentration, parasitaemia and parasite clearance in *Plasmodium falciparum* infected patients. In addition, anti-malarial activity was tested *in vitro* against CQ sensitive-resistant strains.

In a two-phase cross-over study, 15 healthy adult male volunteers were given a single dose of 600 mg of CQ with and without khat. Plasma concentrations of CQ were determined during a 24 h period following drug administration on both occasions. CQ plasma concentrations were determined by a validated HPLC-UV method. Pharmacokinetic parameters of CQ were calculated using compartmental analysis. In the two periods of treatment, the mean (SD) peak plasma concentrations (C_{max}) were 415 (103) ng/ml (CQ with khat) and 508 (106) ng/ml (CQ alone). The total areas under the curve (ACU_{0-24}) were 2108 (682) ng/h/ml (CQ with khat) and 2797 (845) ng/h /ml (CQ alone). The time taken to reach C_{max} (T_{max}) was 3.8 (0.41) h and 3.6 (0.51) h. and elimination half-life ($t_{½}$) was 7.7 (2.10) h and 7.5 (2.77) h respectively. Statistically significant differences were observed for both (C_{max}) ($p < .001$) and (ACU_{0-24}) ($p < .003$) of CQ when comparing values with or without khat. These results demonstrate a pharmacokinetic interaction between CQ and khat and suggest that the observed interaction may be clinically significant, although the CQ levels were above the therapeutic level for *P. falciparum*.

CQ concentration in 103 *P. falciparum* malaria patients who either chewed or did not chew khat during treatment was assessed. The CQ plasma concentrations were determined over the three days of treatment using HPLC method. A significant reduction in CQ concentration in malaria patients was found ($p = <0.001$), the mean plasma CQ concentrations were 319 (101) ng/ml and 204 (110) ng/ml in khat and non khat chewers respectively.

Parasitaemia and parasite clearance were investigated in khat and non khat chewers. The results showed that there was no significant association between khat use and parasitaemia or parasite clearance ($P > 0.05$). Overall resistance was 24.6 % and 28.3 % in khat and non khat chewers respectively.

Soxhlet -khat extracts were tested for their *in vitro* anti-malarial properties in chloroquine sensitive (CQS, 3D7) and resistant strains (CQR, Dd2 and V1/S) of *P. falciparum*. Chloroform and water extracts showed mild activity against CQS (3D7) with an IC_{50} of 21 and 39μg/ml respectively. No other khat extracts showed activity against CQS or CQR strains. Further investigation is needed in order to separate and identify the active constituents in the chloroform and water extracts.

The findings in this thesis are very important in terms of the public health concern. The work in this thesis provides evidence that taking khat can result in CQ reduction that represent a potential risk for patients taking conventional medicine. Policy makers should increase public awareness of the potential risk of khat interaction with chemotherapeutic drugs. The study also provided an evidence of continuing emergence of CQ resistance in *P. falciparum*.

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ABBREVIATION

AUC	Area under curve.
C_{max}	Maximum concentration.
CQ	Chloroquine
CQS	Chloroquine sensitive
CQR	Chloroquine resistant
DCQ	Deseythlchloroquine.
EMRO	Eastern Mediterranean Region.
GDP	Gross Domestic Product.
HPLC	High-Performance Liquid Chromatographic.
HQC	High Quality Control.
IC_{50}	50 % inhibitory concentration.
IS	Internal standard.
LLQ	Lower Limit of Quantification.
LOD	Limit Of Detection.
LQC	Low Quality Control.
mM	Millimolar.
MoPH	Ministry of Public Health.
MQC	Middle Quality Control.
ng	Nanogram.
NMCP	National Malaria Control Program.
QND	Quinidine.
QCs	Quality Control.
RBC	Red Blood Cells.
RBM	Roll Back Malaria.
Rt	Retention time.
T_{max}	Time to reach maximum concentration.
$t_{½}$	Elimination half-life.
ULQ	Upper Limit of Quantification.
UV	Ultraviolet.
WBC	White Blood Cells.
WHO	World Health Organization.

CHAPTER 1

LITERATURE REVIEW

1.1 BACKGROUND OF YEMEN

Yemen, officially Republic of Yemen, is a country on the Arabian Peninsula in South Asia and is a part of the Middle East, bordering the Arabian Sea and Gulf of Aden on the south and the Red sea on the west. It borders Oman to the northeast and Saudi Arabia to the north (**Figure 1.1**). The present nation of Yemen was formed in 1990, when the northern and southern of Yemen were unified. Sana'a is the nation's capital and Aden is the country's commercial capital. Yemen is an Arabic country in both languages and culture and is nearly 100 % Muslim. It has an area of 555000 km^2. Yemen is one of the most highly populated countries in the Middle East. Based on the latest national census in 2005, the population of Yemen is 20,727,063. Administratively Yemen is divided into 21 governorates in addition to the capital Sana'a City. 73 % of the total population are in the rural areas where poverty is widespread and 27 % of total population are in urban areas. Family average size is 7.4. Male life expectancy and female expectancy are 57 and 61 respectively in 2004. The population growth rate is 3 % and the total fertility rate is 6.2 in 2004, these are considered the highest among developing countries (WHO, 2006).

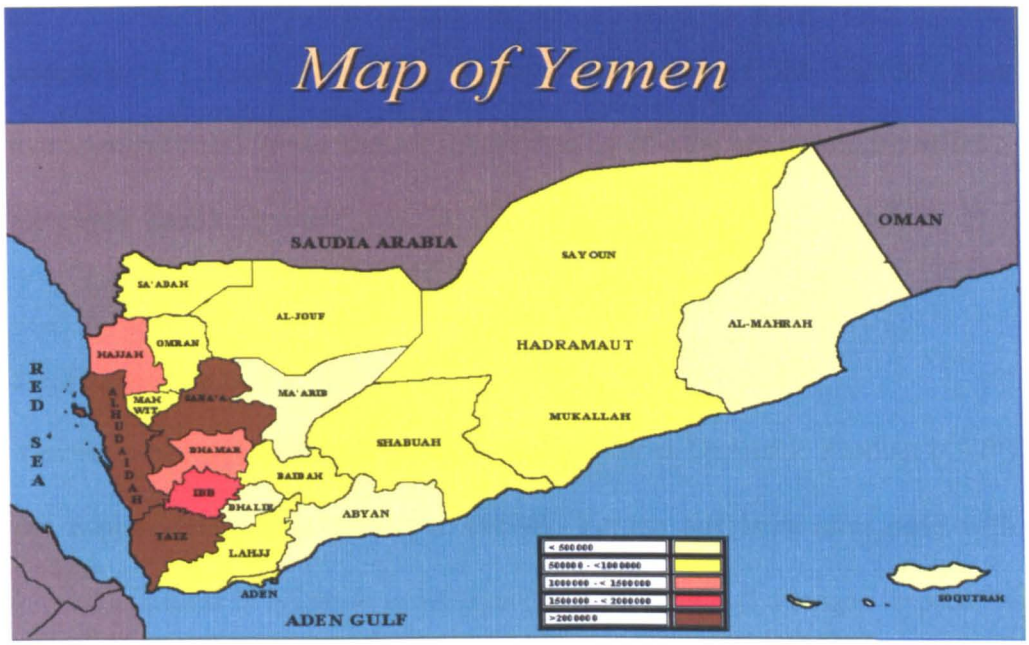


Figure 1.1 Map of Yemen showing population densities (source: National Malaria control Program, Yemen, 2005).

1.1.1 Health service in Yemen

The quality of health services are poor in public health facilities, despite all the efforts made before and after the unity of south and north of Yemen to improve the situation. The financial allocation for health was 6 % of the total government expenditure in 2003 and the Ministry of Public Health budget as % of government budget was 5.2 (WHO 2006) but even this small amount was not spent fully. The rugged topography and the thinly distributed population in the rural areas, make it difficult for the government to establish static health facilities. Overall in 2003, it was estimated that fifty percent of the population have access to local health service with 80 % of urban population and 25 % of rural population having access (WHO, 2006). The

private service especially in-patients facilities have greatly expanded during 1990's encouraged by government and facilitated through subsidy and tax concessions (Unicef & 1998). However, the cost of the service, poor managements and most importantly the quality of care are not different from the public health services.

1.1.2 Economy in Yemen

Yemen is one of the poorest Arab countries. Gross Domestic Product (GDP) per capita is less than $650 per annum. Yemen has been struggling with several conflicts in addition to its significant economic challenges especially after the Gulf war in 1990 when about 1 million of Yemeni workers in Saudi Arabia and Gulf States had to return. In addition, Gulf States and their allies significantly reduced or completely stopped the financial support which the country used to receive. Furthermore, the 1994 civil war added more loads on to the Yemen economy. These factors have affected the GDP and the national currency with high inflation rates. In 1995, the government began a five-year-reform program restructuring the economic and administrative system. The reforms were favourably received by the World Bank and International Monetary Fund (IMF), which agreed to provide aid. However, limited progress led IMF to suspended funding between 1999 and 2001 (World Bank, 2006).

In December 2005 the World Bank announced that because of the government's continued inability to effect significant economic reforms and

stem corruption, funding would be reduced by more than one-third. In the November 2006 meeting in London, a group of bilateral and multilateral donors agree to support the Yemen economy and pledged US$4.7 billion over four years (2007–10). The goal of the meeting, which was jointly chaired by the World Bank and the government of Yemen, was to provide sufficient economic aid to Yemen to enable it to qualify for future Gulf Cooperation Council (GCC) membership (World Bank, 2006).

Agriculture is the mainstay of Yemen's economy, generating more than 20 percent of GDP in 2005 according to the Central Bank of Yemen. However, the scarcity of water and frequent periods of drought and the extension land areas for khat (a stimulant-containing shrub) cultivation in place of exportable grain, fruits, vegetables, cotton, and coffee have also damaged Yemen's economy (World Bank, 2006).

1.2 KHAT PLANT (*CATHA EDULIS* FORSK)

Catha edulis (family Celastraceae) is a plant discovered by a Swedish botanist Peter Forsskal has a number of local names, in Ethiopia, it is commonly known as "chat" or "tschat", or "Mirra", (Kalix, 1984, Kalix, 1984c), and in Yemen it is known as khat or Qat. In this study, the local name (khat) will be used instead of *Catha edulis*. Khat is a psychostimulating herbal drug and is used as a recreational drug in East Africa and Yemen. Chewing of khat leaves appears to be a deep-rooted social and cultural traditional habit in Yemen (Drake, 1988). There are no recent data about the prevalence of the khat habit

in Yemen. Kennedy (Kennedy, 1987) estimated that 80-85 % of the men and 50-60% of women in northern Yemen chewed khat more than once a week. Another survey from Sana'a and nearby villages estimated that 90% of adult men and 20 % of adult women where regular khat chewers (National Conference on Qat, 2002). Fresh young short leaves of khat are customarily chewed to attain a state of stimulation and pleasurable effect (Kalix, 1984). The most important active ingredient of khat is cathinone which has a close structural similarity to amphetamine (**Figure 1.2)** and is considered to be mainly responsible for the psychoactive stimulation, the pleasure and euphoric effect (Hollister, 1995). The khat plant grows in many countries in Asia (Yemen, Turkistan, and Afghanistan) and in Africa (Ethiopia, Kenya, Madagascar, Somalia, Djibouti, Tanzania, Uganda, Rwanda, and Zaire) (Geisshusler, 1987). These countries are also an endemic of malaria, therefore, concurrent administration of anti-malarial drug with khat is a real possibility, and however, no previous reports have been found in this field.

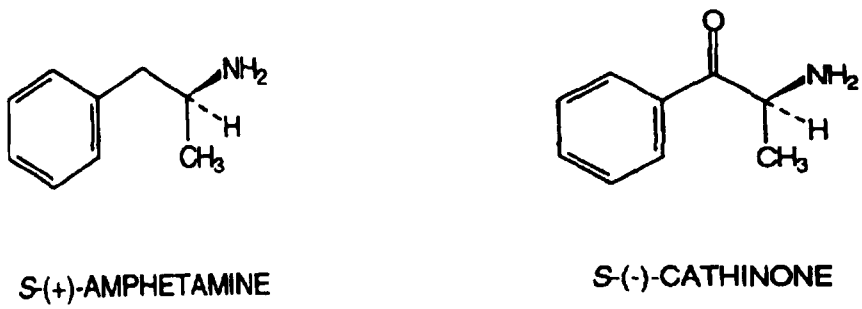


Figure 1.2 Structures of amphetamine and cathinone.

Khat is an evergreen flowering tree or shrub plant which usually reaches 6-7 metre in height under favourable conditions; on moist slopes at 3500 and 8000 feet above sea level (Kennedy, 1980). In Yemen, there are 44 types of khat from different geographic areas. Although it considered to be a thirsty plant, it grows when others crops fail during droughts (Al-Motarreb, 2002a).

After planting, khat is ready for harvesting after 3-4 years. Khat is not cultivated in a hot climate; therefore, coastal hot cities like Aden, Al-Hudaydah and Al-Mokalla receive their khat from the surrounding villages and provinces. The leaves are brownish and somewhat leathery, the upper surface is glossy (**Figure 1.3**). The odour is faintly aromatic and the taste either sour or sweet. During the khat sessions, the young and smooth leaves and the bark of the plant are chewed slowly over several hours and the juice of the masticated leaves is swallowed and at the end of khat session, the residues are discarded (Kalix, 1985).

Figure 1.3 *Catha edulis* plant (khat).

1.2.1 The origin of khat

The first scientific report that described and named the khat plant was in the eighteenth century by Peter Forskal, a Swedish botanist who travelled to Yemen with his friend the geographer Karsten Niebuhr. Forskal identified the plant and name it *Catha edulis* in the family Celastraceae. Niebuhr who was the only survivor of the European scientific expedition team to Arabia, edited and published the work of Foskal in a botanical paper in 1775 (Raman, 1983). Although khat has grown for many centuries in Yemen, there is no certainty of its origin and the date for its introduction (Al-Ra'dee, 1992). There is a historical debate about the origin of khat; whether it originated in Yemen or Ethiopia. Some researchers agree that khat originates from Ethiopia and from there the practice was brought during the Ethiopian occupation of Yemen in 525 AD. However, this date is believed by others to be too early. The earliest

reference to this plant appears to be dated around 1053 AD by Al-Biurni, who compiled a list of all contemporary drugs, including one called Qat that was imported from Turkistan. It was used to relieve biliousness and to cool down the stomach and liver (el Tahir *et al.*, 1990). Khat is also mentioned in an Arabic medical book *the complex Drugs* that was written in 1237 AD in which the khat was recommended as a treatment for depression, because it led to happiness and excitement (Al-Attas, 1981). In view of the above, it can be conclude that the historical evidence for the beginning of khat practice originated in Ethiopia and chewing of khat was probably introduced into Yemen as social habit in the 13th century (Al-Motarreb, 2002a). The opposite view, that khat is of Yemen origin. This is supported by some Ethiopia legends that are recounted by Getahun (Getahun, 1973).

1.2.2 Constituents of khat plant

Since the 19th century, many studies have been conducted to establish the chemical constituents of khat. Fluckiger and Gerock were among the first to isolate a new alkaloid of unknown structure from khat, which they called katine (Dhaifalah, 2004, Fluckliger, 1887). Later on many substances were isolated and cathine was identified as (+) norpsedoephedrine.

In 1975, United Nation Narcotic Laboratory (UNDL, 1975) carried out analytical study using fresh leaves. This study led to the identification of new alkaloid, S-α-aminopropiophenone and the name (-) cathinone was suggested for this substance which is found to be accumulating in young leaves. This

might explain the preference use of the young leaves among khat chewers. From these finding and other studies, khat, contains three main alkaloids, cathinone (S-ά-aminopropriophenone), cathine (norpseudoephedrine), and norephedrine, which structurally related to amphetamine and noradrenaline (**Figure 1.4)** (Schorno, 1979, Szendrei, 1980). In addition, other studies resulted in the characterization of further types of alkaloids called cathedulins. Recently, 62 cathedulin alkaloids have been identified in crude extracts using liquid chromatography/mass spectrometry (Kite et al., 2003).

Many other chemical constituents were found in khat. Tannins were found in considerable quantities (7 – 14%) and vary among different types of khat (Halbach, 1972) and flavonoids. These compounds in some plant extracts have been found to have anti-malarial activity. Other constituents were found in small amounts: essential oils, thiamine, niacin, riboflavin, iron and amino acids, ascorbic acid and triterpenes (Getahun, 1973, Luqman, 1976, Raman, 1983, Szendrei, 1980). An analysis of 22 khat samples of various origin demonstrated that the total amount of cathine and cathinone in different types of Yemeni khat, ranges from a maximum of 343 mg/100g fresh edible khat in Nahamy type to a minimum of 78mg/100g in fresh Sery type (Ramadan *et al.*, 1981). The phenylalkylamine composition varies between the plant region and country of origin. A comparison of *Catha edulis* from Kenya, Madagascar and Ethiopia (Ahmed, 1984) showed that the highest level of cathinone was found in the Kenyan *Catha edulis* leaves (Brenneisen, 1985).

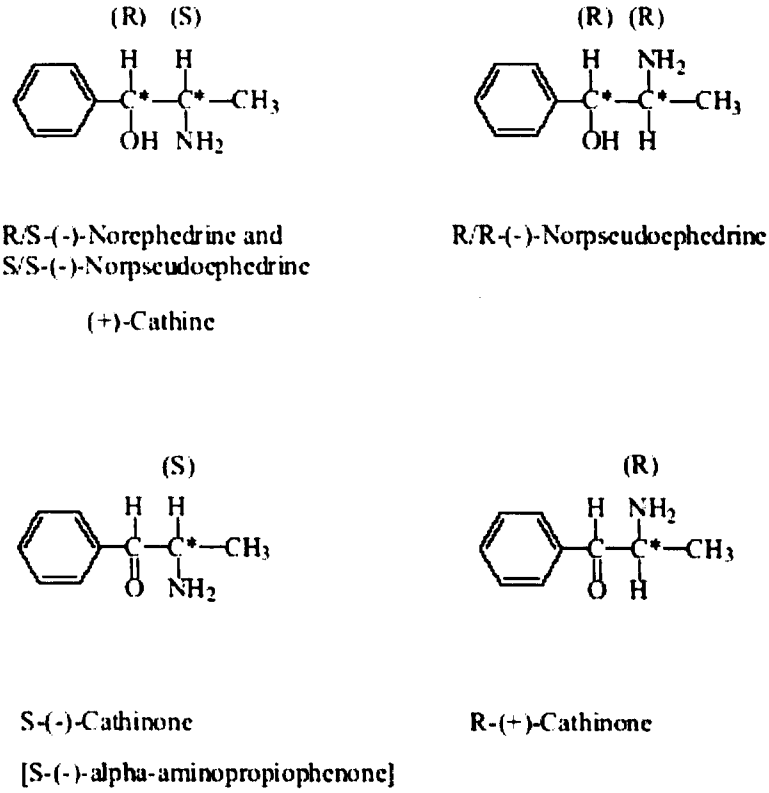


Figure 1.4 chemical structures of cathinone and cathine.

Most compounds in nature are chiral and nature usually produces these single enantiomers. Enantiomers are stereoisomers that are mirror images; a pair of enantiomers is mirror-image forms of the same compound and have opposite absolute stereochemistry. The starred carbon in **Figure 1.4** represents a chiral centre. This means that the carbon has four different substituents that can exist in a mirror image form known as enantiomers. The (-) means that the compound rotates plane of polarized light to the left (negative rotation,

levorotatory) and the (+) means that the compound rotates plane of polarized light to the right (positive rotation, *dextrorotatory*). For chemists, the R/S system is the most important nomenclature system for denoting enantiomers. Each chiral centre is named R or S according to a system by which its substituents are each assigned a priority based on atomic number, if the centre is oriented so that the lowest-priority of the four is pointed away. If the priority of the remaining three substituents decreases in clockwise direction, it is labelled R (Rectus), if is decreases in counterclockwise direction, it is labelled S (Sinister).

Many chiral drugs must be made with high enantiomeric purity due to the potential side effects of other enantiomer. A laboratory synthesis will always give a mixture of enantiomers is called a racemic mixture which means both enantiomers are present in solution in equal proportion. For example Thalidomide is racemic drug used to treat morning sickness in the late 1950s and early 1960s. It was found while the R-enantiomer is effective against morning sickness, the S- enantiomer found to have teratogenic effect leading to birth defect. Even if just the R-form was given, it was found that *in vivo*, the enantiomers could inter convert leading to presence of both enantiomers in the serum.

1.2.3 Pharmacokinetics of cathinone

Khat is always used on the same day of harvesting the branches. To keep the leaves fresh, farmers and consumers usually wrapped them in plastic foil, wet

clothes or shawls, or banana leaves. Commercially, the value of the leaves drops dramatically after the first day of harvesting (Al-Motarreb, 2002a). Cathinone, the mean active component, is relatively unstable and decomposes into (+) norpseudoephedrine (cathine) and norephedrine within a few days of picking or if the leaf is dried (**Figure 1.5**). Thus, only freshly picked leaves have the full efficacy and only fresh of leaves are preferred by khat chewers (Brenneisen, 1986).

In humans, after oral administration of cathinone, it is rapidly absorbed and metabolized into norpsedoephdrine (25-52%), and norphedrine (20-35%), only 2% of absorbed cathinone is excreted in urine unchanged (Brenneisen, 1986). In contrast, norpseudoephedrine and norephedrine are absorbed slowly, with a half-life of 3 hours and then excreted mainly in the unchanged form within about 24 h (Frosch, 1977). Cathinone is transformed mainly to norpseudoephedrine in khat leaves.

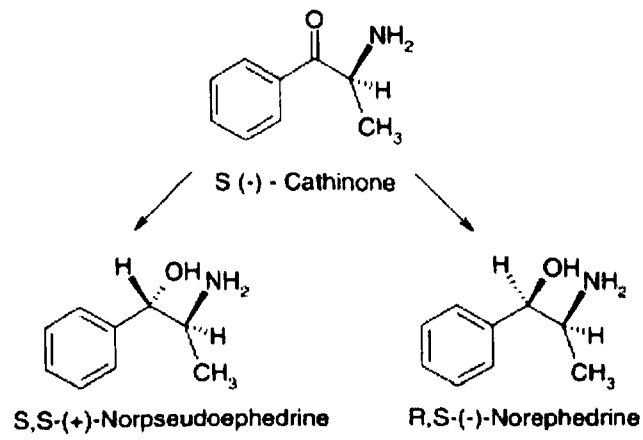


Figure1.5 Metabolism of S-(-)-cathinone.

Cathinone has been determined in spiked human plasma (Morad, 1989), in the plasma of volunteer after administration of pure cathinone (Kalix *et al.*, 1991b) or after chewing of khat leaves (Halket, 1995, Widler *et al.*, 1994). Cathinone was reported to have a mean half-life 1.5 ± 0.8 h and a mean residence time of 4.5 h, and was therefore, not detected in blood plasma samples ≥ 10 h after ingestion (Toennes, 2002, Toennes *et al.*, 2003, Widler et al., 1994). The maximum cathinone concentration was achieved at 1.5-3.5 h (Halket, 1995).

1.2.4 Mechanism of action of khat

The pharmacological profile of S-(-)-cathinone has been shown both *in vitro* and *in vivo* to closely resemble that of amphetamine (Brenneisen *et al.*, 1990), the mechanism of action has been found to be linked to the release of monoamines (Kalix, 1985). The cellular and subcellular effects of cathinone were studied by Rosecrans *et al.* who showed that cathinone had little effect on brain noradrenaline turnover in mice, but increased dopamine turn over significantly, although to a lesser extent than (+) amphetamine (Rosecrans *et al.*, 1979). Most of the pharmacological effects of alkaloids are suggested as being mediated by release of biogenic amines through preferential binding to the noradrenaline receptor/transporter, but also partly through binding to dopamine and 5-hydroxytryptamine receptors (Rothman *et al.*, 2003). Evidences of β-adrenergic receptors involvement were observed in cathinone and amphetamine induced thermogenesis in brown adipose tissue (Rothman *et al.*, 2003, Tariq *et al.*, 1987). In addition, amphetamine derivatives and

cathinone were shown to potentiate the actions of noradrenaline on rat right ventricle contraction; it was suggested that this occurred as a result of cathinone preventing the uptake of noradrenaline from the nerve terminal by an action that involved competitive blockade of the noradrenaline transporter (Cleary, 2002, Cleary, 2003).

1.3 KHAT EFFECT ON THE BODY SYSTEM

Khat chewing causes considerable adverse health effects in various body systems. Previous studies showed that khat chewing leads to several peripheral effects that include the increase in blood pressure and heart rate (Duke, 1985, Widler *et al.*, 1994). Chronic use of chewing khat has also been associated with the high incidence of acute coronary vasospasm and myocardial infarction (Al-Motarreb *et al.*, 2002b).

Khat chewers complain of a number of gastrointestinal tract problems including gastritis, constipation, oesophagitis, duodenal ulcer, and delay in gastric emptying (Halbach, 1972, Heymann *et al.*, 1995, Luqman, 1976, Raja'a *et al.*, 2000). In addition, khat chewing affects the urinary system and urine flow rate is reduced (Nasher *et al.*, 1995). A strong correlation between khat chewing and oral cancer has been reported as well as the development of oral keratotic white lesions at the site of chewing, which could also be related to the insecticides used for the plant (Ali *et al.*, 2004, Halbach, 1972).

Liver cirrhosis was found to be frequent in chronic khat chewers but the role of khat remains to be determined (Halbach, 1972). After oral administration to white rabbits, khat induces cytotoxic effects in cells (Al-Ahdal, 1988, Al-Habori *et al.*, 2002, Al-Mamary *et al.*, 2002, al-Meshal *et al.*, 1991, Dimba *et al.*, 2003), and in lymphoid tissue and in the liver and kidney (Al-Habori et al., 2002, Al-Mamary et al., 2002, al-Meshal et al., 1991), but the mode of cell death has, so far, not been addressed.

Euphoria, excitation, alertness , anorexia and cheerful sensation are some of the effects on central nervous system (CNS) (Nencini, 1986). The CNS-stimulation effect may progress to agitation accompanied by aggressive and manic behaviour (Kalix *et al.*, 1990c). Development of tolerance is not usual in khat users because of the impossibility of increasing the amount of khat beyond certain limit due to the capacity of the buccal cavity (Halbach, 1972). There is no clear abstinence syndrome after prolonged khat use, although a mild depressive reaction during withdrawal from khat is sometimes seen (Halbach, 1972 and Luqman *et al.*, 1976). Psychic symptoms are contradictory,one study showed a significant association of psychic symptoms with khat use (Odenwald *et al.*, 2005) another study disprove such an association (Litman *et al.*, 1986, Numan, 2004). Psychic effect was not common on normal individuals.

Few studies have been conducted to show the possible effect on productive health and there are conflicting opinions regarding khat effect on the

reproductive system in animals. One has shown a negative impact of khat on sexual organs and behaviour such as degenerative changes in testicular morphology and reduction in plasma levels of testosterone in male rats, and reduce the fertility of female mice (Islam *et al.*, 1990, Tariq *et al.*, 1986) and rats (Islam *et al.*, 1990, Jansson, 1988b, Quereshi, 1998, Tariq *et al.*, 1986, Tariq *et al.*, 1987, Tariq *et al.*, 1990). Other studies have showed positive effect by increase level of testosterone and decreasing level of prolactin and cortisol (Al-Mamary *et al.*, 2002, Mwenda *et al.*, 2003). In humans, long–term chronic use may cause spermatorrhoe and lead to permanent impotence. It was found also that mothers who chewed khat during pregnancy tend to have lower mean birth weight children (Abdul Ghani *et al.*, 1987, Jansson, 1988b). A recent study showed that cathine and norephedrine, accelerated capacitation and inhibited spontaneous acrosome loss, suggesting that they may enhance natural fertility (Adeoya-Osiguwa, 2005). In fact, according to the WHO statistical information system, Yemen has the highest fertility and annual population growth rates in EMR.

1.4 ANTI-MICROBIAL EFFECT OF KHAT

Numerous of plants have anti-malarial properties, but few of them have been scientifically evaluated. Some of these plants have alkaloids as the active constituents found to be active against *P. falciparum* (Steele *et al.*, 1999). Many alkaloids have been identified in khat extract; therefore, it is likely that some of these alkaloids may have anti-malarial activities. There is no information on its use as anti-malarial agent and no studies have been carried

out to investigate the anti-malarial activity although its use widespread in endemic malaria countries. However, recently, a few studies have shown that khat has anti-microbial properties against a number of bacterial species (Al-Hebshi, 2005b, Elhag H, 1999). In addition, aqueous khat extracts have been found to exhibit selective antibacterial activities against oral bacteria, and give a preliminary evidence for presence of one or more water-soluble constituents with antibiotic resistance-modifying properties (Al-Hebshi, 2005b).

Furthermore, from reviewing the literature on plant and its medical use in the past, we found that khat was used as in folk medicine. Also in Ethiopia, leaves and roots are used to treat influenza, cough, gonorrhoea, asthma and other chest problems (Lemessa, 2001). Additionally, it is used to improve performance and increase work capacity. These studies showed the possible positive aspect of khat use which is usually ignored.

1.5 SOCIAL AND ECONOMIC ASPECTS OF KHAT CHEWING

Khat chewing is a deep-rooted cultural tradition habit in Yemen and it has been used as recreational drug. Many houses have a room in which people gather each afternoon to consume the khat in a special setting (**Figure 1.6**). These private gatherings are of great social significance. Khat chewers consumed about 100-300 grams of fresh leaves during continuous sessions every afternoon which last for 4-6 hours but it may extend up to 8 hours (Date, 2004). During khat session the fresh leaves and the bark of the plant are chewed slowly and masticated over several hours till the juice is extracted

and swallowed. The residue of mastication is then discarded at the end of the khat session (Kalix, 1996) (**Figure1.7**).

This habit was almost unknown outside the regions where plant grows since only fresh leaves are active, however during the last two decades, due to the development of modern transportation and immigration, the habit has spread considerably including United State, United Kingdom, and several European countries (Kalix et al., 1991b). Yemenis consider that khat sessions represent an important social occasion, and provides the environment for people to gather and socialize. Different social classes are usually gathered together especially in sessions attracting large numbers such as wedding parties, funeral gathering and election campaigns. At these gatherings, social class distinctions are forgotten. Khat sessions are used also in formal meeting where government officials and businessmen are engaged in discussion and work. Khat is also used to improve performance, stay alert and increase work capacity (Kalix, 1984c). College and university students consume khat to increase mental alertness and to work hard in their academic endeavours.

In addition to the reported health problems of khat chewing habit; it is also associated with a variety of social and economic problems. Khat chewers devote many hours in buying the leaves and in the khat sessions. It affects work time and quality family time. The relationship of the family members is weakened. The father returns home at lunchtime in a hurry, has his lunch,

Figure 1.6 A typical room (Majleas) for khat session.

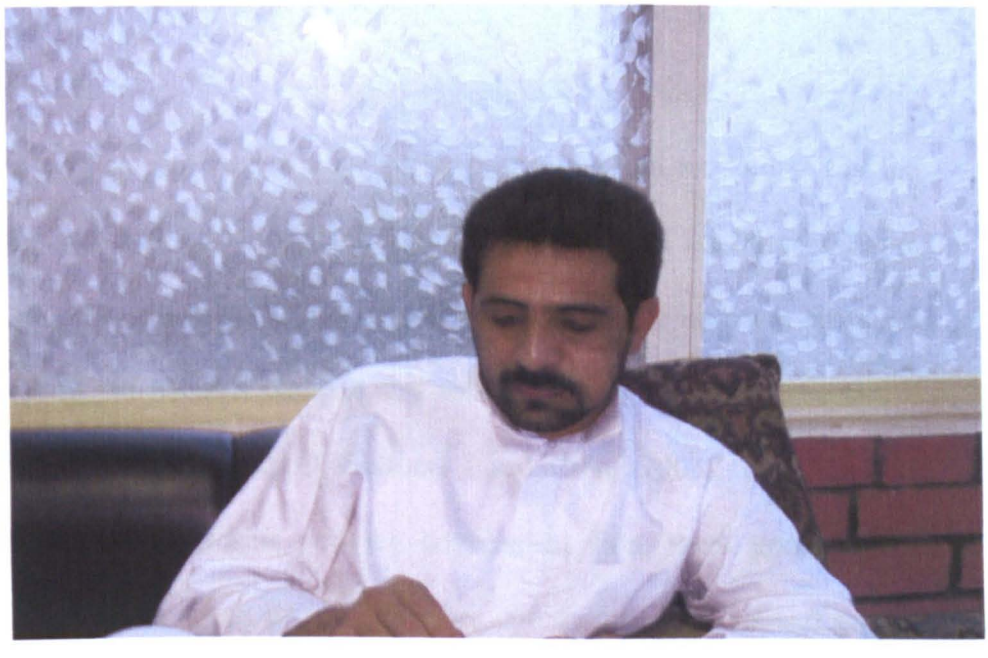


Figure 1.7 A khat chewer with large ball of leaves in the cheek.

which the main meal in Yemen and then leaves the house without having enough time to see either his children or wife.

Therefore children do not see their father in most cases and fathers do not have effective influence on their children. Recently, khat chewing among women has increased sharply which affects the family in particular children, who are left alone and most of the times play unsupervised in the street. Moreover, khat influences the family budget in a great deal. The price of khat varies according to the type of khat; fine quality is expensive. It is estimated that daily cost of khat exceeds one-third of the family budget. However, the demand for khat has been of economic benefit to the farmers and their families in the village.

Economically, khat is the most important cash-crop in Yemen. It is profitable to many people who are involved in its production and marketing and the reason for cultivating khat is the high income it provides for farmers. Some studies in 2001 estimated that the income from cultivating khat was about 2.5 million Rials per hectare, while it was only 0.57 million Rials per hectare if fruits were cultivated (Alafif Cultural Foundation, 2003). It is also an important source of revenue to the government from taxes upon it. The growing of khat is not as difficult as other crops such as coffee and grapes. However, khat is consumed locally and its production is not exported and thus it does not contribute to the country's foreign exchange earnings. The chewing of khat habit has been accelerated by a cycle expanding of khat

production, increased demand from chewers, increased area for khat cultivation and increase economic benefits to local financial system. The area in which khat cultivated increased in Yemen from 76,059 hectares in 1989 to 97,772 hectares in 1998 (Date, 2004). Cultivation of khat consumes much of the country's agricultural resources. It is estimated that 40% of the country's water supply goes towards irrigating it, with production increasing by about 10% to 15% every year (Date, 2004) **(Figure 1.8).**

Figure 1.8 khat cultivation.

About $700 million is spent on khat annually (Kassie *et al.*, 2001). In addition, it has been estimated that Yemenis spend about 14.6 million person-hours per day chewing khat and the estimated expenditure has been increased from 14.6 billion Yemeni Rials in 1990 to 41.2 billion in 1995 (Alafif Cultural Foundation, 2003).

1.6 KHAT AND POLICY ISSUES

Although khat chewing habit is a common daily activity and deeply rooted in Yemen, there is an increasing awareness about its social, medical and economic problems. Many people, among them government officials realized that the problem is so deeply rooted that a solution will be extremely difficult, probably requiring a vigorous effort spanning several generations to get rid of it. In 1972, a time before the unification of the country, Prime Minister Mohsen Al-Aini's Government in North Yemen passed a resolution to up-root khat trees on land owned by the state and at the same time promulgated the harm of the khat habit. The resolution was backed by large scale mass media campaigns. Unfortunately, such action had very limited response from the public and was a total failure. In the Southern part of Yemen (former People's Democratic Republic of Yemen) prior to the unification with the North in 1990, there were also serious steps taken to fight khat. In December 1976, the government passed a legal ban on khat prohibiting its sale and purchase except at the weekend, but this regulation is no longer in force after unification. On 16 January 1992, non government organization, an anti-khat association, was formed. The main objectives of this association being to draw society's attention to the social, economic and medical effects of the khat chewing by using the information media such as television, regular journals, booklets and posters, to create a social climate to discourage khat chewing especially by young people, and to use all possible means towards achieving a khat-free society. Yemeni society does not consider that khat habit is a real problem and this revealed that the problem was much too deep

to be resolved by a simple resolution or legal action either by the government or by the non government organizations.

From the above review khat not only has direct effect on the health, social and economic but also has indirect effect helping in transmission of some diseases such as malaria due to the bad practice of cultivation. Due to local farming practices in cultivation of the khat tree may cause population growth in vectors of malaria, *Anopheles arabiensis*. Therefore, the government should increase the awareness of the effect of khat use on the public health.

1.7 EPIDEMIOLOGY OF MALARIA IN MEDITERRANEAN REGION

Malaria remains to be one of the most sever infectious disease threat to the world and still one of the devastating global health care problems, with an estimated 300-500 million cases and 1 million deaths occurring each year most of them children under age of 5 (WHO, 2005). As of 2004, malaria was endemic in 107 countries and 3.2 billion people still at risk. Malaria slows a country economic growth and it depletes human resources. It is estimated that malaria responsible for a loss of 1.3 % of economic growth annually in malaria endemic countries (WHO, 2005).

In the Eastern Mediterranean Region (EMR) malaria is also a major health problem. It is estimated that about 10 million of malaria cases and 49,000 malaria related deaths occurs annually in this region, out of which 95% occur

in four countries: Afghanistan, Somalia, Sudan and Yemen (Beljaev, 2000). About 45% of the population in this region lives under the risk of both *Plasmodium falciparum* and *Plasmodium vivax* malaria, and an additional 15% at risk of *P. vivax* alone. After Afghanistan, where *P.vivax* is the predominant species (Sadrizadeh, 2005), Yemen has the highest incidence of malaria in the EMR and *P. falciparum* is responsible for a high proportion of reported cases (WHO, 2005).

There are three eco-epidemiological zones of malaria in EMR: the first one is Afrotropical, in which *P. falciparum* is the predominant species, which is highly endemic (Sadrizadeh, 2005). It is impractical to eliminate malaria from the Afrotropical areas, except in areas in Southern Africa and ocean islands, in the near future because this leads to a decrease in population immunity. A decrease in the force of infection of *P. falciparum* malaria may increase incidence of cerebral malaria in children (Gupta et al., 1999).

The second zone is the Oriental type of malaria, which is present in Pakistan, Afghanistan south from the Hindukush, south-eastern Iran, Oman and adjoining areas of the United Arab Emirates. This type of malaria characterized by the presence of both *P. falciparum* and *P. vivax* in roughly equal proportions and it is more difficult to control compared to Palaearctic malaria.

Many areas in North Africa, which belongs to Palaearctic region, were highly endemic for *P. falciparum* in the pre-eradication era, but by the end of the 1980s, malaria transmission was severely reduced under the impact of control/eradication activities and the total number of cases in some areas was less than 100 cases annually in 1996-98 (Sadrizadeh, 2005).

In the countries with Afrotropical malaria, the situation is quite serious especially if health systems are disrupted. Wars and political instability lead to a breakdown of malaria control and the re-emergence of the diseases, this clearly occurred in Afghanistan and Iraq. On the other hand, past experience shows that in the rest of the region, a rapid decline of malaria is achievable provided that a normal political situation prevails.

Many countries in the EMR have expressed their commitment to malaria eradication. Morocco, Egypt, Oman and United Arab Emirates now have only residual malaria and a complete interruption of transmission is deemed feasible and sustainable. In other countries, achievements of malaria-free status is the main objective; this requires the development of a common approach through updating the malaria eradication strategy (Sadrizadeh, 2005) among these countries is Yemen, which endorsed a strategy of malaria control, which was integrated into the general public health program and became a National Malaria Control Program (NMCP). The program started in 2001 with ultimate goal to reduce incidence of malaria in the country by 50 % by 2010 (**Table 1.1**).

Table 1.1 Classification of the countries in the EMR by status of malaria control program.

Countries where malaria does not occur or sporadically occurs after importation	Bahrain, Cyprus, Jordan, Kuwait, Lebanon, Libya, Palestine, Qatar, Tunisia
Countries with strong health system and effective malaria control program, where malaria is quite well contained	
a) Eradication of malaria is feasible and sustainable if achieved	Egypt, Morocco, Oman, UAE
b) Malaria morbidity may be brought down	Iran, Pakistan, Saudi Arabia, Syria
Countries with a very serious problem	
a) countries with Afrotropical malaria	Djibouti, Somalia, Sudan, Yemen
b) countries outside of the area of Afrotropical malaria but with compromised health system	Afghanistan, Iraq

Source: EMRO

Malaria control strategies in the EMR, including Yemen, concentrate on diminishing the severity of the disease and the elimination of its inherent mortality, which is a technically feasible goal, even in the presence of drug resistance.

1.7.1 Epidemiology of malaria in Yemen

Approximately 60 % of the population of Yemen is at risk of malaria. It is estimated that between 800,000-900,000 malaria cases occur each year (Khalifa, 2006). Malaria is one of the leading causes of death in Yemen, about 0.9 % of the malaria cases die each year (NMCP, 2006) although the

true number of malaria cases and death from malaria is not known. Malaria is responsible for high significant child morbidity and mortality, 10 % deaths of Yemeni children are because of malaria (Unicef & 1998). Furthermore, malaria was estimated to cause 12.5% of school absenteeism (Bassiouny, 2000). According to the current epidemiological situation in the EMR, malaria is one of the most serious problems affecting the public health. Yemen is one of the five EMR countries classified as having severe malaria problems and a weak health system **(Figure 1.9)**. Within group 4, Sudan had the highest estimated number of malaria cases in 2005 (**Figure 1.10**).

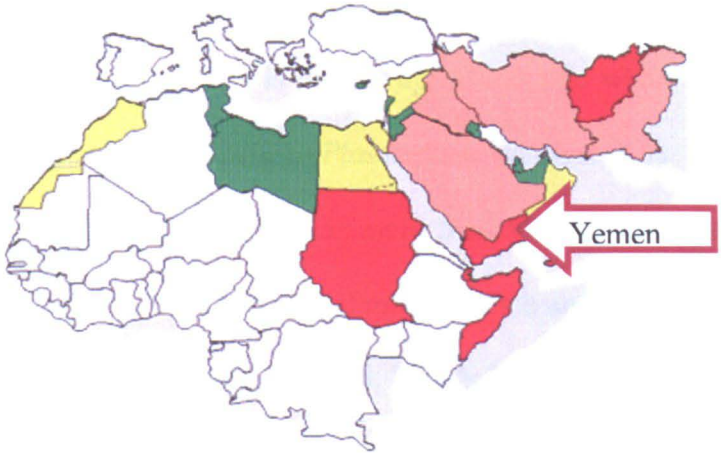
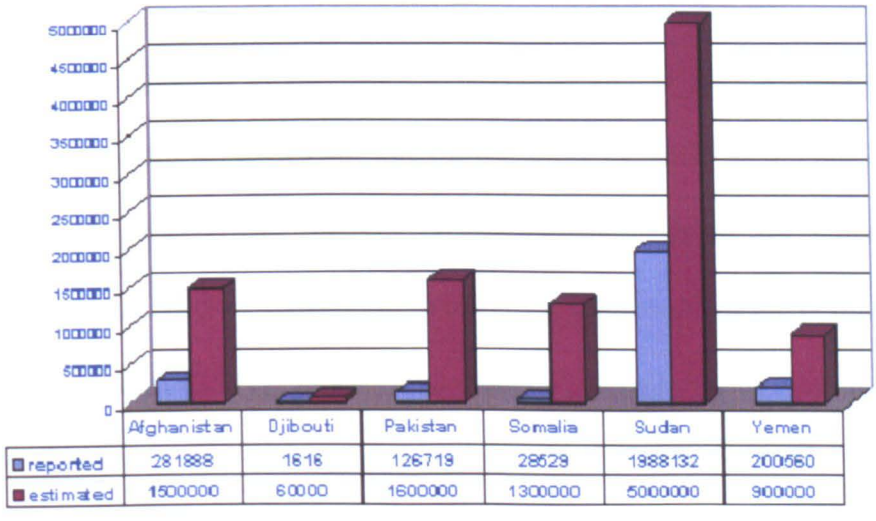


Figure 1.9 Epidemiology situations of countries in Eastern Mediterranean Region (EMR). Countries are classified into 4 groups according to the epidemiology situation and malaria control. Group1: (green): Countries which have eliminated malaria, Group 2: (yellow): Countries with very limited malaria transmission in residual foci, Group 3 (pink): Countries with a moderate endemicity with low malaria burden limited to certain areas and with effective malaria programmes, and Group 4 (red): Countries with moderate/ high malaria burden, weak health system and/or complex emergencies among them Yemen.
N.B.: malaria situation in the countries with white color is not discussed here.



	Afghanistan	Djibouti	Pakistan	Somalia	Sudan	Yemen
reported	281888	1816	126719	28529	1988132	200560
estimated	1500000	60000	1600000	1300000	5000000	900000

Figure 1.10 Reported and estimated malaria cases in countries with serious endemicity. Source: http://www.emro.who.int/rbm/Epidemiology-current.htm

According to the WHO, malaria in Yemen belongs to the Afro-tropical epidemiological zone with an *Anopheles arabiensis* as the main vector except in Sokotra Island; malaria is mostly hyper-endemic where it belongs to the oriental type (WHO, 2002). *Plasmodium falcipurm* is the predominant species, which is responsible for more than 90% of the malaria cases and responsible for the vast majority of malaria death and the rest are caused by *P. vivax* or *P. malariae* (NMCP, 2000, WHO, 2002). Arid and semi-arid hyop- and mesoendemic areas are prone to outbreaks of malaria following heavy or prolonged rainfall. Local mosquitoes (especially *Anopheles arabiensis*) are efficient vectors of malaria, and local farming practices, such as cultivation of the khat tree, may favour transmission (NMCP, 1998).

The endemicity of malaria has been divided into five areas: 1) The coastal region (Tihama) where the transmission level is meso to hyperendmic and has a peak of transmission in the winter (October-April) as the hot weather in summer is not appropriate for malaria transmission; about 25 % of population live in this region; 2) The western mountains region which has a peak of transmission in summer (May-September), and where 30 % of population live; 3) The highlands region with transmission all year-round, it is meso or hyperendemic and 5% of the population live in this region; 4) The islands region (including Socotra Island) which has a peak of transmission in summer (May-September). Before 2000, transmission was high, however, the Island is now considered being free of malaria except for some imported cases; 5) The desert region in the east and the mountains more than 2000 metre above the

sea level (including Sana'a city) are believed to be malaria free or hypoendemic (NMCP, 2006).

Routinely collected data are not complete and the information of malaria situation in Yemen is scarce. However, cases reported to the Ministry of Public Health (MoPH) suggest malaria as a major public health problem. The numbers of clinical malaria cases that were reported are 111,651, 214,212, 127,750 and 200,560 in the years 2002, 2003, 2004 and 2005 respectively (NMCP, 2006). According to the number of laboratory confirmed cases that were reported to MoPH in 2004, malaria was considered the second common infectious disease after gastroenteritis (NMCP, 2004).

1.7.2 The National Malaria Control Programme in Yemen

The first National Malaria Control was established in Aden in 1960. In the northern governorates the program was initiated in Al-Hudaydah in 1978. In the 1990's, malaria control suffered serious setbacks due to discontinuation of organized vector control activities, climatic changes, heavy rain falls and social instability. This probable contributed to malaria epidemics in 1996 and 1998 with 1552 deaths reported from several malaria endemic governorates in Yemen. In addition, the spread of *P. falciparum* resistance to CQ and the weakening of the organizational structure of malaria control aggravate the situation. In January 2001, the National Malaria Control Program (NMCP) was established with a 5-year plan of action for malaria control with the broader Roll Back Malaria (RBM) partnership with WHO and other

international organizations. The RBM control programme initially focused on high-risk areas including the Tihama coastal belt, selected districts in foothill and mountainous areas and Socotra Island. The main goal of NMCP was to reduce the incidence of malaria by 50 % by the year 2010. The NMCP's strategy were: measures for capacity, strengthening early and diagnosis, prompt and effective treatment, integrated vector management, surveillance and information systems, community participation and prevention of malaria during pregnancy. In high-risk areas, vector control and strengthened surveillance with active community participation have succeeded in reducing the number of malaria cases 10-fold since 2001 (WHO, 2005). According to NMCP, the epidemic infection in the Tihama region, some 226 km west of Sana'a, had dropped from 48.3% in 1998 to 11% in 2004, 9.6 % in 2006 and 8.3 % in 2007 and in Socotra; an island in the Indian Ocean, the prevalence rate had fallen from 36% to less than 1%. However, challenges remain; according to a WHO-EMRO report, the main factors leading to the deteriorating malaria situation in the country are: the discontinuing of organised vector controlled activities, the increase in breeding places due to water resource development projects, mistaken diagnosis due to poor laboratory quality control, and the availability of sub-standard anti-malarial drugs in the market (Abdo-Rabbo, 2005).

Yemen is poised for reform not only in its pattern of government, in socio-economic development, and in Civil Service sector but also in the health sector. The Ministry of Public Health is putting forward a Health Sector

Reform strategy designed to address the challenge of the current health system. Widespread rural poverty, low coverage of public health service and the country's few resources relative to its neighbour are among other factors that impede health system reforms.

1.8 CHLOROQUINE (CQ)

CQ (7-chloro-4(4-diethylamino-1methylbutylamino) quinoline) is a basic amine and a synthetic anti-malarial agent (**Figure 1.11**). It is available for oral use as tablets either as 250 mg or 500 mg of the diphosphate equivalent to 150 mg and 300 mg of base respectively (Keystone, 1990).

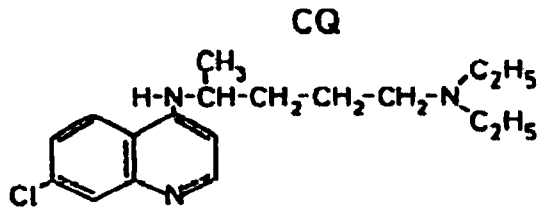


Figure1.11 Chemical structure of chloroquine (CQ).

In spite of the increasing prevalence of CQ-resistant against *plasmodium falciparum*, CQ has been the drug of choice for treatment and prevention of malaria in many developing countries including Yemen, in which CQ is the recommended drug by the NMCP as first-line drug in the country and it is

given both as a therapeutic and as a prophylactic agent in public health centres and it is free of charge. The low cost, easy availability and safety makes CQ the drug of choice for most patients in Yemen, on the other hand, unwise use and massive use of the drug has lead to the development and spread of CQ resistance in the world as well as in Yemen (Ducharme, 1996). CQ resistance to *P. falciparum* has been reported in some part of the Yemen (Mamser, 1989, Mubjer, 2006, NMCP 2003, NMCP, 2005, WHO, 1999).

The standard treatment regimen of uncomplicated malaria in CQ sensitive areas is a total of 25 mg base/kg. Two standard schedules are used, either given as a total dose of 10 mg/kg, followed 1 day later by 10 mg/kg and then 5 mg/kg on the third day or as an initial dose of 10 mg/kg followed by 5 mg/kg at 8, 24, 48 h later. These regimens provide adequate plasma and red cell concentration-time profile and avoid toxicity (Krishna, 1996).

1.8.1 General Pharmacokinetics

1.8.1.1. Absorption and bioavailability

The bioavailability of CQ is excellent and it is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration in either healthy subjects or in malaria patients (Gustafsson *et al.*, 1983b). The bioavailability is about 80 % for solution and 90 % for tablets ustaafter a single dose in heathy volunteers (Gustafsson et al., 1983b). Peak plasma levels of CQ are reached within 1-6 h with wide individual variation in blood concentration. Maximum concentration was ranges from 56 to120 ng/ml after

a single oral dose of 300 mg CQ base (Rombo *et al.*, 1985, Walker *et al.*, 1983). Following oral administration, whole blood concentration is 5-10 times higher than observed in plasma (Frisk-Holmberg *et al.*, 1984), with a high proportion of the drug and its main metabolite bound to platelets and granulocytes (Bergqvist, 1983, McChesney *et al.*, 1966, Rombo *et al.*, 1985a). In assessing inter- individual variation in CQ kinetics, Hellgren *et al.* studied 90 subjects treated for arthritis and found that CQ concentration varied 2.3-5.6- fold in plasma, and up to 11 fold in whole blood. These variations were related to age and weight factors (Hellgren, 1995).

1.8.1.2 Distribution

CQ has an extremely large apparent volume of distribution (100-1000 L/kg) because of its extensive binding to a variety of tissues (Frisk-Holmberg *et al.*, 1984, Gustafsson et al., 1983b), especially those containing melanin, such as skin and eye and it also occurs in the liver, spleen and kidney (Peters 1984), however, the apparent volume of distribution of the central compartment is relatively small (0.2 L/kg). *In vitro*, in plasma from healthy volunteer, ultrafiltration or equilibrium dialysis techniques reveal that 58-61 % of CQ is bound to plasma proteins (Augustijns, 1992, Augustijns, 1993, Ofori-Adjei *et al.*, 1986, Walker *et al.*, 1983).

It takes 3-4 weeks for equilibrium to be reached between the plasma and tissue levels, this coupled with CQ's slow rate of excretion, means that during

treatment of malaria blood levels are determined by distribution rather than by elimination or metabolism (White, 1988a).

1.8.1.3 Elimination and Excretion

Urinary excretion is the main mechanism of elimination of CQ and its principle metabolite. CQ is slowly eliminated from the body; with a multiexponential decline in plasma concentration and elimination pattern as equilibrium is reached between the small central and the large peripheral compartment. About 50 % of CQ is excreted unchanged in the urine, and approximately 10 % is excreted in faeces (Peters 1984).

In healthy volunteers, after a single dose of 300 mg, CQ concentration could be detected in blood and urine up 52 and 119 days post dose, respectively (Gustafsson et al., 1983b). Initial half life was 5 days, but the terminal phase was up to 2 months (Gustafsson *et al.*, 1987). Similarly, after a 10-week malaria prophylaxis regimen of 300 mg/week, CQ was still present in serum after 70 days and in urine up to 1 year after the last dose (Gustafsson *et al.*, 1987, Ofori-Adjei, 1985).

1.8.2 Pharmacokinetics in malaria

1.8.2.1 Absorption and Distribution

Pharmacokinetics is the study of the changes in drug concentration in the body and it involve absorption, distribution, metabolism and elimination and

the availability is the amount of drug administrated drug reach the systemic circulation intact.

The pharmacokinetics properties of CQ, which are similar in adults and in children, are not significantly altered by disease severity (Krishna, 1996). Because of the extensive tissue binding and slow elimination, patients are never in a stable condition for long enough to gather the required information. However, some observation can be mad from the concentration profile.

In Nigerian children with acute uncompleted malaria; (Adelusi, 1982, Walker *et al.*, 1983a) CQ was well absorbed and peak concentration was achieved within 30 min, both in plasma and red cells, which does not differ from previous studies on healthy adults, (Gustafsson et al., 1983b). Therefore, the authors concluded there was no difference between healthy individuals and patients with malaria and the pharmacokinetics properties of CQ were similar in children and adults (White, 1988a, White *et al.*, 1987a). Also, apart from the higher C_{max} in patients with malaria, there was no pharmacokinetic differences between Thai healthy volunteers and malaria patients following an intravenous infusion of CQ or oral administration (Edwards *et al.*, 1988). However, in another study, a comparison between healthy volunteers and patients with *P.vivax* malaria, infection significantly increased C_{max} and AUC and also prolonged t½ (8-28 days) of both CQ and DCQ (Na-Bangchang *et al.*, 1994).

1.8.2.2 Elimination

After a single dose of CQ, the differences in the elimination half- life and clearance between healthy individuals and patients were significant (Edwards *et al.*, 1988, White *et al.*, 1987a). Although it has not been investigated with CQ, plasma concentrations of quinine and quinidine were increased and their elimination half-lives prolonged in patients with malaria indicating that malaria can induce changes in drug elimination which could become significant following multiple drug therapy (Trenholme *et al.*, 1976).

It has been reported that liver dysfunction has been observed in malaria patients (Trenholme *et al.*, 1976, White *et al.*, 1982). Also in experimental models, liver blood flow was decreased (Skirrow, 1964a) and histopathologcical evidence of liver damage has been seen (Aikawa, 1980). A variation in hepatic metabolism is a probable explanation of the variability in CQ pharmacokinetics.

1.8.3 Drug Metabolism

Clinically there is marked variation in pharmacokinetics of CQ following single dose or multiple doses (Ette *et al.*, 1989, Gustafsson et al., 1987, Gustafsson et al., 1983b, Wetsteyn *et al.*, 1995). These differences may reflect diversity in metabolism. Following administration, CQ is rapidly dealkylated into the pharmacological active desethylchloroquine, bidesethylchloroquine and 7-chlorodesethylchloroquine (Ette et al., 1989, Frisk-Holmberg et al., 1984, Gustafsson et al., 1983b, White, 1993) (**Figure 1.12**). DCQ is rapidly

detected in blood and plasma reaching concentration of about 20-30 % of the parent drug (Frisk-Holmberg et al., 1984, Gustafsson et al., 1983b). CQ main metabolite can contributes to the drug's efficacy when it is used as a prophylactic, but is probably unimportant in determining the response to therapy in acute malaria (White, 1987, White, 1988a).

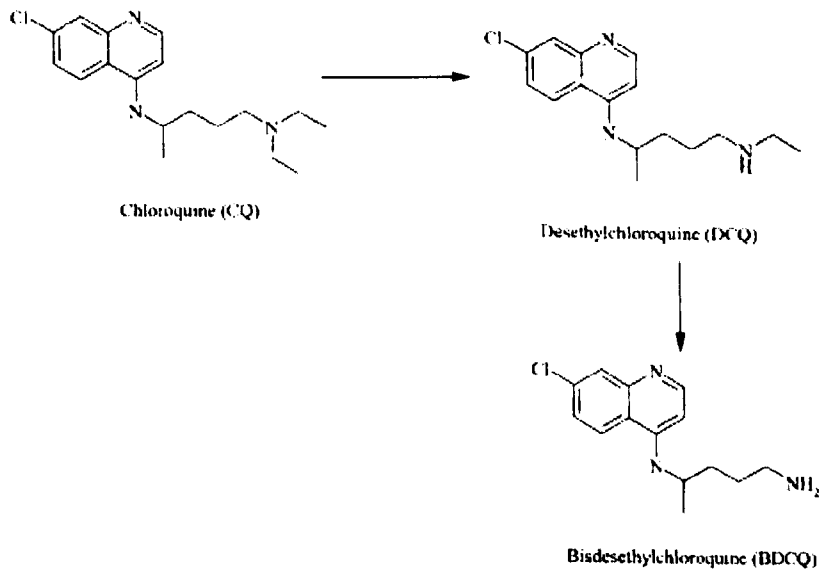


Figure 1.12 Metabolism of chloroquine.

1.9 PHARMACOKINETIC DRUG INTERACTION

Drug-interaction may alter drug concentration and pharmacokinetic paramaters. This pharmacokinetic interactions can occur when one drug alters the rate or extent of absorption, distribution, metabolism, or excretion of another drug (Leucuta 2006).

1.9.1 Interference with drug absorption

Drug interaction may result in the formation of insoluble complexes which result in a reduction of absorption, some drugs e.g. tetracycline, form insoluble complexes with antacids containing divalent and trivalent cations. In addition, some drugs delay gastric emptying e.g. anticholinergics, tricyclic antidepressants, antihistamine, and phenothiazines reduce bioavailability of co-administrated drugs (karbwang, 1993). In addition, alteration in gastric pH may increase and decrease drug absorption. Food can enhance or impair drug absorption.

1.9.2 Interference with drug metabolism

Most of the drugs which are metabolized in the liver by cytochrome P-450 may be affected by either enzyme induction or enzyme inhibition. Chronic administration of drugs or co-administration with other drugs leads to enzyme induction which results in increased activity of cytochrome P-450 which accelerates the metabolism of many drugs and consequently decreases drug levels. Enhancement of drug metabolism can happen not only with drugs but also with diet constituents and tobacco smoking. The consequences of enzyme induction normally affect drug efficacy, however it may also alter the toxicity of certain substances (karbwang, 1993).

Enzyme inhibition will lead to inhibition of drug metabolism and lead to a prolonged half-life and accumulation of the drug leading to significant adverse effect in the case of some drugs. Inhibitors can act at many different

sites on the drug metabolizing enzymes system. However, with regard to drug interaction in humans, the most likely mechanism is competition for the same substrate-binding site. In general, induction leads to a decrease in pharmacological response whereas inhibition leads to an enhancement with prolonged pharmacological responses but this is dependent on the relative potencies of parent drug and of the metabolites derived from the routes of metabolism that are altered (karbwang, 1993).

1.9.3 Interference with drug excretion

The rate of elimination of drugs by any excretory pathways, for example, faeces, bile, sweat, tear and lung may be impaired by drug-drug interaction. Renal excretion is the type of excretion that has received greatest attention in relation to drug interactions. Drug–drug interactions may change the rate of glomerular filtration; tubular secretion and tubular reabsorption and rate of blood flow. This mechanism may increase or decrease drug clearance which then results in a higher or lower concentration (karbwang, 1993).

1.9.4 Interference with drug distribution

After absorption, the drug is distributed throughout the body, including plasma proteins. When drug interactions occur, one drug replaces another drug from it its sites on plasma proteins. This displacement interaction results in a transient rise in the displaced drug concentration which results in a compensatory rise in metabolism or excretion. This interaction rarely results

in clinically significant changes in drug response, but it can be clinically important if the displaced drug is bound in tissue or when the drug has a small volume of distribution (karbwang, 1993).

1.10 CQ PHARMACOKINETIC INTERACTIONS

The pharmacokinetics of CQ is susceptible to different types of drug interactions. Food, drug and plant interactions can effect and exaggerate pharmacokinetics variability of CQ at any level.

Food was found to enhance CQ bioavailability. When a typical breakfast together with CQ tablets (600 mg CQ base) was given to healthy Indian volunteers, the mean plasma concentration and AUC_{0-12} were increased by 52 and 42% respectively (Tulpule, 1982). Furthermore, AUC and C_{max} were significantly elevated when CQ was administered with rice-based meals (Tulpule, 1983b). Grapefruit consumption in mice increased the plasma concentration of CQ and altered some kinetics parameters of CQ (Ali, 2002). However, CQ kinetics has been reported to be significantly reduced by the consumption of certain dietary substances. Some beverages indigenous to Sudan including lemon squash were co-administered with 600 mg base to healthy individuals; the area under curve (AUC) and the maximum drug concentration in plasma (C_{max}) were considerably reduced. Mean CQ peak plasma concentration and AUC_{0-24} were deceased by 62% to 73% respectively (Mahmoud *et al.*, 1994)

There have been few drug interaction reported for CQ, as might be expected from its metabolic profile. AUC and peak concentration of CQ were reduced by 99 % when activated charcoal was given 5 minutes after ingestion of CQ (Neuvonen *et al.*, 1992). In animal studies, aspirin reduced the absorption of CQ, but clinical relevance of these results has not been established (Na-Bangchang, 1993). Rengelshausen (2004) found that co-administration of methylene blue with CQ as a treatment for malaria resulted in a small reduction of CQ exposure which is not expected to be clinically relevant and thus represents no concern for further development as an anti-malarial combination (Rengelshausen *et al.*, 2004). Antacids and antidiarrhoeals were found to reduce bioavailability of CQ. Other substance like calcium carbonate, kaolin, magnesium trisilicate were found to decrease the absorption of CQ significantly. The hepatic biotransformation of CQ may affected by some anti-malarial such as amodiaquine and primaquine by inhibiting hepatic drug metabolizing enzyme system (Na-Bangchang, 1993).

Khat- anti-malarial interaction has not been study, however, khat and its effects on bioavailability of some antibiotics has been studied and it was found that the bioavailability of these antibiotics were reduced significantly and that was attributed to forming insoluble complex with khat which reduced their absorption (Attef, 1997). Furthermore, khat was found to delay gastric emptying (Widler *et al.*, 1994) and that may result in reducing drug absorption.

1.11 ANALYTICAL METHODS OF CQ

The older fluorescence and ultraviolet methods for CQ measurements lacked the necessary sensitivity to detect low plasma concentrations accurately; therefore, they have been replaced by highly sensitive High-Performance Liquid Chromatography (HPLC) techniques. It is the most sensitive and reproducible technique and can accurately measure concentration in the range 3μg/L using UV detection and as low as 0.15 ng/ml using fluorescence detection (Bergqvist, 1980).

CQ plasma concentrations in this study were measured by an HPLC method which was developed by D.J. Bell (2006) who reported that this method has several advantages over the previous reported methods (Gitau *et al.*, 2004, Winstanley *et al.*, 1987, Winstanley *et al.*, 1992, Mihaly *et al.*, 1985). Firstly, the same instrumentation and columns can be used for different anti-malarial drugs. Second, this method is applicable for the analysis of small volume of samples (50-200 μl of blood) stored at -80 °C after collection.

1.11.1 Validation of the method

It is important to validate the method before it is used in the analysis. The validation ensures the performance of the method and should include all the validation parameters. Accuracy, precision, specificity, detection limit, qualification limit, and linearity and range are considered essential factors in the validation process (USFD, 2001). The validation of the method can be full

or partial validation. The method which has been used in this study was partially validated.

1.11.2 Calibration curve

The calibration curve is a linear relationship between the instrument response and known concentration of the sample and it should be prepared in the same biological matrix as in the intended study by adding known concentrations to the sample. In addition, the calibration curve should consist of six to eight samples, a blank (no internal standard is added) and zero sample (internal standard is added). The concentration standards should be chosen on the basis of the concentration range expected in study samples (USFD, 2001).

1.11.3 Accuracy and precision

Accuracy, together with precision, determines the error of the analysis and is therefore an important criterion in the evaluation of an analytical method. Accuracy is defined as the degree of closeness between the test value and a reference value or true value and is determined by replicate analysis of samples of a known concentration. The accuracy of the method can be determined by analyzing spiked control samples with analyte concentrations around the lower limit of quantification (LLOQ), 2-5 times the LLOQ, 0.5 times the upper limit of quantification (ULOQ), and the ULOQ. (Wieling *et al.*, 1996, Dadgar *et al.*, 1995). Each concentration should be evaluated with at least 5 replicates in a minimum of 3 analytical runs together with a

calibration curve, independently prepared from the control samples (Braggio *et al.*, 1996, Karnes & March, 1993). Accuracy is often expressed in the term of a mean value, standard deviation and difference between the mean and the true value. The mean value should not be exceeding ± 15 % of the nominal value except at LLOQ, where it should not exceed ± 20 % (USFD, 2001).

The precision is defined as the distribution or agreement (degree of scatter) between a series of measurements of multiple sampling of the same homogenous sample under the prescribed conditions. The precision can be further classified into, intra-assay (within- day, repeatability) and inter-assay (between-day, reproducibility) (Rosing *et al.*, 2000). Repeatability is the closeness between the measurements of the same sample in the analytical method in which the variability in measurement should be kept in a narrow range over the same analytical run, whereas the reproducibility is the agreement between the results obtained with the same method over a long time interval (Karnes, 1991).

1.11.4 Specificity and selectivity

Specificity and selectivity evaluation are used to verify that a method is specific when it only measures the analyte without any kind of positive or negative interferences (Smolec *et al.*, 2005). Because it is almost impossible to develop a chromatographic assay for a drug in a biological matrix that will respond to only the compound of interest, it is advisable to concentrate on the selectivity parameter. The selectivity is the ability of a method to produce a

response for the target analyte in the presence of other compounds (e.g. endogenous compounds) (Buick *et al.*, 1990, Dadgar *et al.*, 1995, Karnes, 1991b). Selectivity of the method with respect to interferences from biological fluids can be obtained by processing a blank sample from six independent sources of the same matrix (USFD, 2001).

1.11.5 Limit of detection

The limit of detection (LOD) is defined as the lowest amount of analyte that can be detected but not quantified under the prescribed conditions (Wahlich & Carr, 1990, Buick *et al.*, 1990). There is an overall agreement that the LOD should represent the lowest concentration that can be detected in the sample of interest. LOD is dependent on the background signal, whether it is due to endogenous substance or electronic noise. It is preferred that LOD is determined by measuring the lowest amount in a blank biological sample rather than the reference solution (Buick *et al.*, 1990). LOD influenced by minor changes in the conditions of the analytical method, like temperature, purity of reagents and instrumental system changes. For these reasons is not of great important for the analysis of drugs in biological samples and should not be included in the calibration curve (Peng *et al.*, 1990).

1.11.6 Lower limit of Quantification

The lower limit of Quantification (LLOQ) is the lowest amount on the standard calibration curve which can be measured with acceptable accuracy and precision by the method (Braggio *et al.*, 1996, Carr, 1990, Peng, 1990).

The LLOQ-value is determined by the presence of background signal (accuracy) and the reproducibility of the analytical method (precision). The LLOQ can be determined by analyzing at least five replicates of the control samples prepared independently from the calibration samples in separate runs. The LLOQ samples should be prepared in a biological matrix at known concentration, around the expected LLOQ. In terms of accuracy and precision of the method, the deviation from the known concentration should not exceed 20% within each run (Dadgar *et al.*, 1995, Shah *et al.*, 1992). LLOQ values which do not meet the precision and accuracy criteria may lead to unreliable results. To avoid this, LLOQ value should be increased to that concentration where the criteria are met.

1.11.7 Range and linearity

The range of bioanalytical assay is the concentration interval between the upper and the lower concentration of analyate that can be measured with acceptable accuracy and precision. Linearity of a method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. To prove the linearity, at least six non-zero standard concentrations run in duplicate should be used, a zero sample (sample with internal standard) and a blank sample (sample processed without internal standard (USFD, 2001).

The standard curve samples can either be prepared independently or by serial dilution. An independent preparation standard makes it easier to discover any

error in the preparation, however, in a serial dilution; an error in the highest standard will be present in the entire calibration standard. Although serial dilution gives a better correlation coefficient, it could give a false regression equation. Many calibration curves are calculated by least-squares linear regression, to obtain the y-intercept, slope and correlation coefficient (r) which should be as high as possible. When the standard deviation is not constant, the calibration data ought to be weighted to increase the accuracy of the lower concentrations (Bressolle, 1996, Karnes, 1991b).

A high correlation coefficient (>0.99) of the calibration curve is often used to state linearity (Braggio *et al.*, 1996, Causey, 1990, Karnes, 1991b, USFD, 1994). Although good linearity always provides a correlation coefficient close to 1, a high correlation coefficient does not necessarily imply linearity. The correlation coefficient merely gives an indication of the absence or presence of a response-concentration relationship (Aarons, 1987, Carr, 1990). Therefore, by assessing an acceptable high correlation coefficient alone the linearity is not guaranteed and further tests on linearity will still be necessary.

1.12 AIMS AND OBJECTIVES

The aim of this study is to determine whether khat (*Catha edulis* Forsk) has an effect on anti-malaria chemotherapy (chloroquine).

Specific objectives of the study were to determine:

1. The effect of khat on pharmacokinetics of the anti-malaria drug, chloroquine by measuring the concentration of chloroquine in the components of blood of volunteers chewing khat.
2. The effect of khat chewing during treatment on the concentration of chloroquine in malaria patients.
3. The effect of khat chewing during the treatment with chloroquine on parasitaemia and parasite clearance.
4. The effect of khat extracts *in vitro* on drug sensitive-and resistant strains of *Plasmodium falciparum*.

CHAPTER 2

EFFECT OF KHAT ON THE PHARMACOKINETICS OF CQ IN HEALTHY VOLUNTEERS

2.1 INTRODUCTION

Drug-drug, drug-food and drug-herbal interactions are factors that can effect and exaggerate pharmacokinetic variability of the drug (Leucuta, 2006). Drug-drug interactions may result in alterations of pharmacokinetics of the drug at different levels. This alteration includes Absorption, Distribution, Metabolism, and Excretion (ADME) of a drug. The consumption of certain dietary substances can affects the absorption of many drugs (Welling, 1989) including the anti- malarial, chloroquine (CQ) (Tulpule, 1982), atovaquone (Rolan *et al.*, 1994) and mefloquine (Crevoisier *et al.*, 1997). In Sudan, concomitant ingestion of CQ with lemon juice or two other local beverages caused a significant reduction in the area under the curve (AUC) and the maximum drug concentration in plasma (C_{max}) (Mahmoud *et al.*, 1994). However, another study showed that grapefruit juice co-ingestion in mice increased the plasma concentration of CQ and altered some of its kinetic parameters (Ali, 2002). Herb-drug interaction can also affect the pharmacokinetics and pharmacodynamics of the drug. Interaction of plants with chemotherapeutic drugs could increase or decrease the action of the drug, either of which could result in adverse effects (Izzo, 2004).

The leaves and stem tips of khat plant are chewed every day by millions of people in East Africa and Yemen to obtain the stimulating effects (out lined in chapter 1). Khat leaves contain more than 40 alkaloids among them cathinone and cathine which are the main stimulate active constituents (Dhaifalah, 2004). The plant also contains tannins, flavonoids, and essential oils.

Recently, analysis using liquid chromatography/mass spectrometry revealed the presence of 62 cathedulin alkaloids in crude methanolic extracts of fresh khat (Kite *et al.*, 2003). All of which may potentially participate in drug-drug interactions. Most of the studies that have been conducted on khat have concentrated on its pharmacological effect, its effect on the various body systems and have overlooked khat-drug interaction and its effect on the bioavailability of drugs. However, using urine excretions method, khat chewing was found to interfere with the absorption of some orally administered antibiotics and the bioavailability was significantly reduced (Attef, 1997). The mechanism of interference was attributed to forming insoluble complex with khat constituents.

Because it is common to take chemotherapeutic drugs together with khat in Yemen, therefore, the concomitant use of khat and CQ is a real possibility. Nonetheless, the possibility of an interaction between khat and CQ has, as far as we are aware, never been tested in humans or animals. Therefore, the present study was conducted to investigate the effects of khat chewing on the pharmacokinetic parameters of CQ in healthy adult Yemeni volunteers.

2.2 MATERIALS AND METHODS

2.2.1 Chemicals and standards

Extraction solutions, contains sodium hydroxide (NaOH), 0.2M hydrochloric acid, hexane-tert-butyl methyl ether (1: 1) were obtained from Sigma® and mobile phase from BDH (Poole, England). The mobile phase was prepared by mixing distilled water, acetonitrile (85: 15 %) and 1 % triethylamine (BDH) and pH adjusted to 2.8 with concentrated orthophosphoric acid (pH/Ion meter, PHM 95 Radiometer Copenhagen). CQ phosphate and internal standard (IS), quinidine (QND), were obtained from Sigma–Aldrich (St. Louis, USA) and were prepared in distilled water and methanol respectively.

2.2.2 Instrumentation and chromatographic conditions

Samples were analysed by high-performance liquid chromatography (HPLC) with chromatography LC 2010 C system used at room temperature in the range of 20-23 °C (Shimadzu LC 2010 C, Kyoto, Japan). Autosampler temperature was 10°C and column temperature was 25°C. The chromatographic separation was carried out using a reversed phase Hypersil BDS C18 150 mm X 4.6mm column, 5µm particle size (Thermo Hypersil-keystone Runcorn, UK). The mobile phase was pumped at a constant flow rate of 1 ml/min and the run time was 8 minutes and the column effluent has monitored at 340nm. Peak areas and retention times were calculated using Chromelion software version 6.70, (chromatography management system Dionex Ltd. 2005).

2.2.3 Study area

The study was conducted in Sana'a city, the capital city of Yemen Republic. The mountains of Yemen at an altitude of more than 2000 metres above the sea level, which includes Sana'a city, are believed to be free of malaria or hypoendemic (**Figure 2.1**).

Figure 2.1 Map of Yemen, Sana'a city. The green arrow is pointing to the study area.

2.2.4 Study subjects

A total of 15 healthy adult male volunteers were selected from Sana'a University students, Yemen. The volunteers were selected depending on their willingness to participate on the study. If the participants met all the

inclusion criteria (**section 2.2.4.1**) and informed consent (**Appendix 2**) had been obtained from them, they were enrolled in the study. The volunteers were judged healthy based on the routine and standard laboratory tests (**for tests see Table 2.4**).

2.2.4.1 Inclusion criteria

- Healthy male volunteers.
- Age > 18 years.
- Malaria negative on blood film examination.

2.2.4.2 Exclusion criteria

- Malaria positive on blood film examination.
- Have abnormal results of standard laboratory tests.
- Volunteers who used anti-malaria drugs before the study commenced as treatment or as prophylactic drug.

2.2.5 Sample size calculation

All the volunteers who agreed to participate and met all the inclusion criteria were enrolled in the study. The sample size was determined as 12, but to allow for an expected follow-up loss of 10 %, the number entering the study was 15. Sample size was calculated using sample size calculation (Gore and Altman, 1982) (STATA, version 9.2). Sample size was calculated on basis of 30 % of change in the area under curve (AUC) and maximum concentration

(C_{max}) following khat chewing with a significance level of 0.05 and on a power of 80%. Values for the mean and SD of pharmacokinetic parameters were taken from Mahmoud *et al* (Mahmoud *et al.*, 1994).

2.2.6 Setting of the study

15 Healthy adult male volunteers aged 20-30 years (age mean (SD) 23.53(2.77), mean body weight 62.86 kg and mean haemoglobin (Hb) 16.2 g/dL) participated in this study. The purpose of the study was explained and consent was obtained from all the participants (**Appendix 2**). In a cross-over design with a four-week wash out period, each participant took four tablets of CQ as a single dose equivalent to 600 mg base after having eaten. The CQ tablets was kindly provided by the National Malaria Control Programme (NMCP), Batch number 35 9 2003, YEDCO, Republic of Yemen. In the first occasion, the participants chewed khat immediately after CQ was taken (the khat that was used was one of the commonest types in Yemen, known locally by Dula'ee and the khat session last for 4-5 hours). Equal amount of the same type of khat (~200 g of fresh khat leaves) were given to each subject. The water intake was not controlled during khat chewing session. In the original setting protocol, participants should take CQ without khat in the first occasion, but the reason we started with CQ co-administration with khat to encourage participation and to ensure compliance. After four weeks of the washout period, subjects received another dose of 600 mg of CQ alone after having eaten.

2.2.7 Data collection

Each volunteer was given specific numbers from 1 to 15 which was the same number in the record book, questionnaire, and blood sample to avoid mixing volunteers information. Hematology (Sysmex, Kx-21N, Kobe, Japan) and biochemistry laboratory tests (902 Automatic analyzer Roche HITACHI. Mannheim, Germany) were done for each volunteer prior of participation. Also, all participants were tested for malaria. The weight and height for each participant were taken and kept in the recorded book. Data about habitual khat use were included in the questionnaire forms (**Appendix 3).** Blood samples (3 ml) were drawn from subjects into heparinized glass at 0 min, 30 min, 1, 2, 3, 4, 6, 8, 12, and 24 h post drug administration in the two occasions i.e. with and without khat through an indwelling catheter inserted into a forearm vein and kept patent with heparinized saline. Subsequent blood samples were collected by venepunctur at 24 h after dosing. All Blood samples were transported in ice-box within 30 min of each collection to the Central Public Health Laboratory in Sana'a. All samples were centrifuged at 2000 rpm for 15 min. Plasma samples were collected after removing the buffy coat and transferred to Eppendorf tubes and stored at – 20°C for six months then stored at –70°C until analyzed. The samples were kept at –70°C for 1 year, the length of time might not be ideal, but all the samples were kept in good conditions until analysis.

2.2.8 Calibration curve and Quality Controls (QCs)

Stock solutions were prepared by dissolving CQ diphosphate salt and QND in distilled water and methanol respectively. The calibration curve was constructed by spiking blank plasma with CQ ranging from 25 ng/ml as the lower value to 1500 ng/ml as the upper value (25, 50, 100, 250, 450, 750, 900, and 1500 ng/ml). The calibration curve was also included a zero and blank sample. All the calibration standard samples were prepared in batches of 10 ml and in 150 µl aliquots and stored at –20°C. The peak-area ratio (CQ/IS) was plotted against concentration of CQ with 1/x quadratic weighing linear regression (r^2) > 0.99.

Quality controls (QCs) samples were prepared in the same manner at concentrations ranging from 75-1500 ng/ml. 25 ng/ml was selected as the LLOQ which considered as the lower limit of quantification, other QCs at concentrations of 75, 750, 1200, and 1500 ng/ml corresponding to the low quality control (LQC), middle quality control (MQC), high quality control (HQC) and high limit of quantification (HLOQ) respectively. The assay accuracy and precision was specified within 20 % for LLOQ and 15 % for the higher values.

2.2.9 Extraction procedure and sample preparation

The extraction of CQ from plasma was done as described by D.J. Bell with minor modifications (Bell et al., 2006). 150 µl of plasma sample was mixed with 100 µl of 10 µg of QND, (internal standard) in silanized Pyrex tubes and 500 µl of 0.2M hydrochloric acid was added and then incubated for 2 min followed by 1ml of 20% sodium hydroxide. CQ and QND were extracted with 5 ml hexane–tert-butyl methyl ether (1:1 v/v), tumble mixed for 40 min and centrifuged at 3,000 rpm for 10 min. The upper organic phase was transferred to a clean silanized glass tube and dried under a stream of nitrogen at 37 °C (British Gas CO, U.K). The residue was reconstituted with 100 µl of mobile phase and 50 µl was injected onto the HPLC column.

2.2.10 Assay Validation

2.2.10.1 Accuracy, intra-day and inter-day assay precision.

The accuracy and precision of the method were estimated by analysis of spiked plasma at a minimum of 3 concentrations each replicated six times and run on 4 different occasions. Three quality controls (QCs) were include, one at low QC (LQC, 75 ng/ml), one at the middle QC (MQC, 750 ng/ml) and one at high (HQC, 1200 ng/ml). These three values represent 3 times the LLOQ (25ng/ml) and 50 % and 80 % of the maximal concentration of the calibration curve respectively. Intra-day (within days) and inter-day (between days) precision were calculated and specified at a coefficient of variation (CV) of 15 %, except for LLOQ which was set at 20 % (6 at each concentration).

2.2.10.2 Linearity

Calibration curves were constructed using 9 calibration standards excluding blank samples and including zero (0, 25, 50, 100, 250, 450, 750, 900, 1500 ng/ml) in duplicate. The calibration curves were obtained by calculating the peak-area ratio of CQ to QND against the corresponding concentration. Linear calibration curves were obtained over the standard concentrations range and generated by simple linear regression analysis.

2.2.10.3 Extraction recovery

Percentage recovery was determined at 75, 750, 1200, ng/ml of CQ in plasma by comparing the peak-area of spiked extracted concentrations with the obtained by direct injecting of an equal concentration of pure standards.

2.2.11 Pharmacokinetics Analysis

The following pharmacokinetic parameters were analyzed by compartment model and trapezoidal method using kinetic computer program (Kinetica version 4.4, Thermo Electron Corporation). The following pharmacokinetic parameters were calculated for each subject and reported as the mean and standard deviation (SD).

- The maximum concentration (C_{max}), defined as the highest observed concentration between 0 and 24 hours of the healthy volunteers.
- The total area under the concentration-time curve (AUC) from time 0 to the last measurable concentration at time 24 h. were calculated using trapezoidal rule.

- The time taken to reach the peak concentration (T_{max}).
- Concentration profile post peak.

2.2.12 Statistical Analysis

The t-test for the paired values was used to compare the calculated pharmacokinetic parameters of CQ for the two occasions (with khat and without khat). A significant differences between values was considered as $P<0.05$. Values are reported as the mean and standard deviation (SD).

The study has been approved by the faculty of Medicine, Sana'a University (**Appendix 1A**), Malaria National Control Programme in the Republic of Yemen (**Appendix 1B**), and the Liverpool School of Tropical Medicine, University of Liverpool, UK (**Appendix 1C**).

2.3 RESULTS

2.3.1 Calibration curve and quality controls

The analysis of CQ in plasma was carried out as described in **section 2.2.9** by HPLC method of D.J Bell (Bell et al., 2006) with minor modification. This modification proved to give base-line separation for both CQ and QND. **Figure 2.2** shows the chromatography behaviour of spiked plasma CQ and QND with retention time (Rt) around 3.2 and 3.7 and 4.7 min respectively. QND gave two distinct peaks around 3.7 and 4.7. The area for the first QND peak was used for determination of CQ concentrations.

Before quantification of CQ in patient's plasma, a large number of calibration curves were obtained for the concentration range from 25–1500 ng/ml. Standard curve were obtained from peak areas for a series of CQ concentration (25, 50, 100, 250, 450, 750, 900, and 1500 ng/ml) in relation to the peak area of IS. Quantitation was based on the peak area ratio (the ratio between the peak area of individual CQ and the peak area of IS). Each concentration in the calibration curve was run in duplicate. All the calibration curves obtained were linear over the concentration range of 25-1500 ng/ml with correlation coefficients (r^2) > 0.99 (**Figure 2.3**). Curve fitting used a weighing (1/x) quadratic and performed obtained using Chromelion software, version Ltd. 6.7. Goodness of fit for the standard calibration curve was determined by r^2.

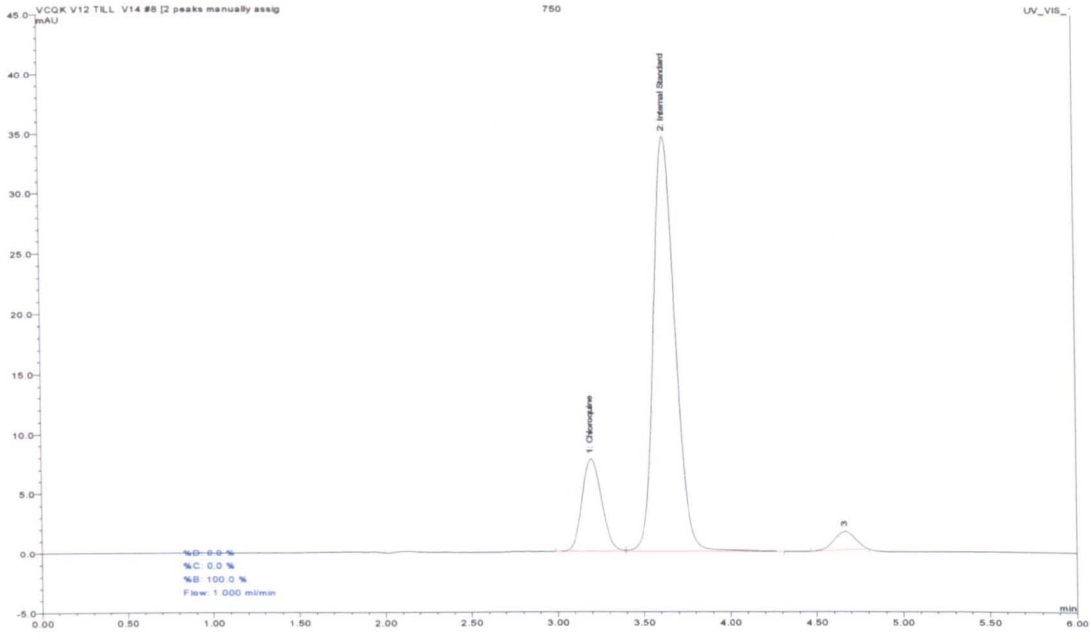
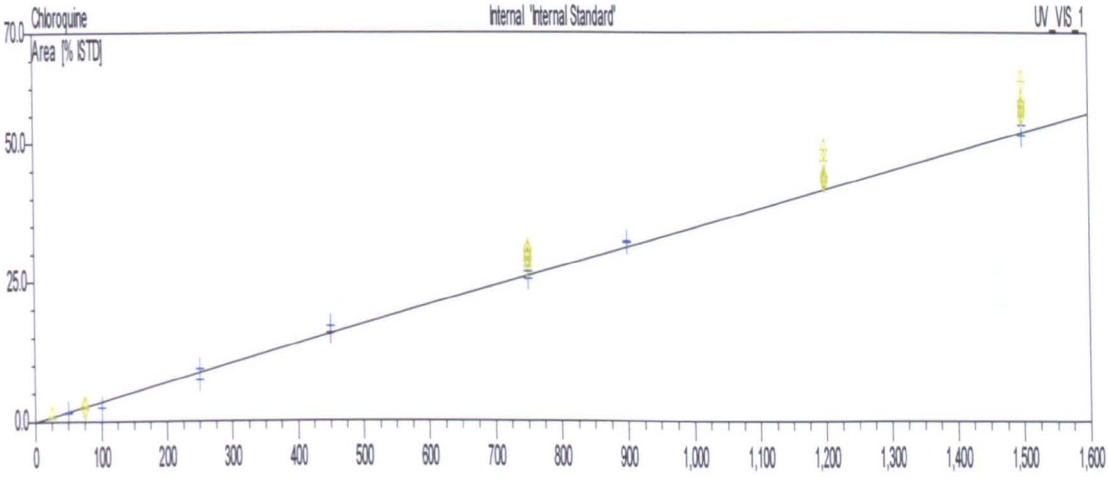


Figure 2.2 chromatogram from spiked plasma chloroquine (1) Rt= 3.2, and quinidine (2) and (3)Rt = 3.7, 4.7 respectively.



CQ concentration (ng/ml)

Figure 2.3 Standard curve of CQ.

Some endogenous compound peaks from the plasma of the subjects were seen in the chromatogram, but they appeared before the peaks of CQ and QND, thus these peaks did not interfere in the quantification of CQ **(Figure 2.4)**. In addition, there were no differences in the chromatogram peaks between khat chewers and non khat chewers i.e. there was no endogenous interfere with CQ quantification. CQ concentrations were determined using a calibration curve on the same day of analysis. No degradation was detected for QND following storage in plasma at – 20°C during the analysis.

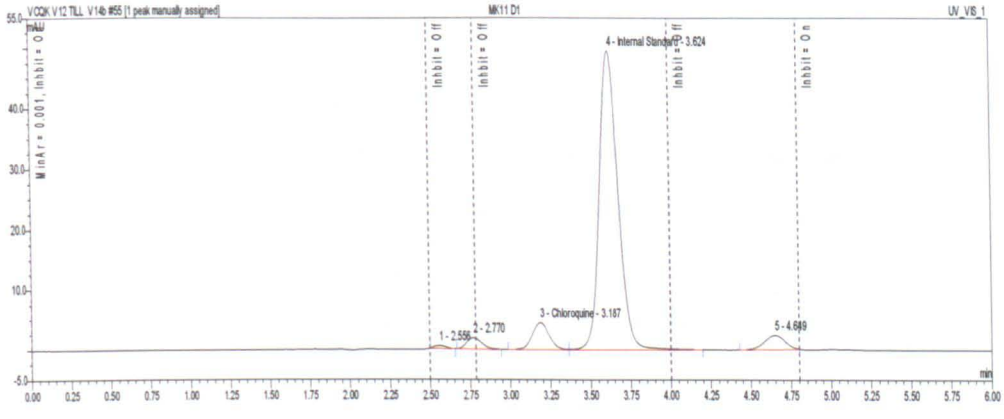


Figure 2.4 Chromatogram of plasma extract of the healthy volunteers, separation of chloroquine (1) Rt 3.2, Quinidin (2) and (3)Rt= 3.7, 4.78 respectively.

The results of the accuracy assay from QCs and standard curve samples are presented in **Table 2.1** and **Table 2.2**. Accuracy was expressed as percentage of deviation from the true value and they were within ± 20 % at LLOQ concentration and at all other concentration levels were within ± 15 %. If the values were within the acceptable limits, they were labelled with pass and if exceed, they were labelled with fail. Within-day and between-day coefficient of variation (C.V.) for CQ were 6.4% and 2.06% respectively and both are less than 15 % (**Table 2.3**). The mean extraction recovery of CQ at concentration of 92.8 %.

Table 2.1 Accuracy of standard curve values in method validation.

Sequence Name		New Conc. curve5		
Start date		03.03.07		
	Chloroquine Standards			
No.	Name	Amount Chloroquine UV_VIS_1	+/- 15%	+/- 20%
1	0	-0.141		
2	25	25.343		Pass
3	50	50.389	Pass	
4	100	87.840	Pass	
5	250	274.473	Pass	
6	450	486.201	Pass	
7	750	630.106	Fail	
8	900	913.657	Pass	
9	1500	1472.579	Pass	
1	0	0.208		
2	25	21.04		Pass
3	50	54.190	Pass	
4	100	95.181	Pass	
5	250	279.956	Pass	
6	450	400.617	Pass	
7	750	819.862	Pass	
8	900	893.063	Pass	
9	1500	1545.140	Pass	

Table 2.2 Accuracy of Quality Control values in method validation.

Sequence Name		New Conc curve5		
Start date		03.03.07		
	Chloroquine QCs			
No.	Name	Amount Chloroquine UV_VIS_1	+/- 15%	+/- 20%
21	LLOQ	26.030		Pass
22	LLOQ	26.647		Pass
23	LLOQ	26.183		Pass
24	LLOQ	26.409		Pass
25	LLOQ	25.087		Pass
26	LLOQ	30.004		Pass
28	HLOQ	1223.660	Fail	
29	HLOQ	1690.054	Pass	
30	HLOQ	1663.850	Pass	
31	HLOQ	1591.152	Pass	
32	HLOQ	1624.336	Pass	
33	HLOQ	1554.200	Pass	
35	LQC	72.623		Pass
36	LQC	66.583		Pass
37	LQC	85.634		Pass
38	LQC	66.522		Pass
39	LQC	67.271		Pass
40	LQC	69.743		Pass
42	MQC	785.442	Pass	
43	MQC	682.369	Pass	
44	MQC	790.525	Pass	
45	MQC	854.667	Pass	
46	MQC	830.745	Pass	
47	MQC	801.785	Pass	
49	HQC	1254.322	Pass	
50	HQC	1138.809	Pass	
51	HQC	1263.487	Pass	
52	HQC	1353.455	Pass	
53	HQC	1237.833	Pass	
54	HQC	1267.960	Pass	

LLOQ: lower limit of quantification, HLOQ: high limit of quantification, LQC: low quality control, MQC: Middle quality control and HQC: high quality control.

Table2.3 Within-day and between-day assay precision for CQ determination in plasma (spiked samples).

	QC Sample	Concentration (ng/ml)	n	Coefficient of variation (C.V)%
Between-day	LQC	75	6	2.908
	MQC	750	6	2.570
	HQC	1200	6	0.069
Mean (SD)				**2.06 (1.2)**
Within-day	LQC	75	6	7.278
	MQC	750	6	6.641
	HQC	1200	6	5.347
Mean (SD)				**6.4 (0.98)**

2.3.2 Effect of khat on the pharmacokinetics of CQ in healthy Volunteers

In order to study the pharmacokinetics of CQ in healthy adult Yemeni males with and without khat, the protocol in **section of 2.2.6** was followed. A single oral dose of 600 mg CQ base (CQ was supplied by the National Malaria Control Programme) was given on two occasions: with khat and without khat; with a washout period of 30 days. CQ was well tolerated in the 15 healthy adult male Yemeni subjects with no adverse event reported. The enrolment characteristics and laboratory tests are summarized in **Table 2.4.** Haemoglobin level, complete blood counts, and liver and kidney function

tests which were carried out at the start of the study were within the normal range in all subjects. In addition, blood films were negative for malaria parasite. Age ranged from 20-30 years and the mean body weight is 62.86 kg. All blood samples were collected from 0-24h interval post drug administration in both occasions and handled and stored as it is stated in **section 2.2.7.** CQ was analyzed by HPLC method. The plasma concentration and effect time data were analyzed by compartmental method using the pharmacokinetic program Kinetica Version 4.4.

Table 2.4 Characteristics and standard blood and biochemistry laboratory tests of the healthy volunteers.

Volunteer #	*Age years*	*Height cm*	*Weight kg*	*Hb g/dL*	*WBC $x10^9$/L*	*RBC $x10^{12}$/L*	*Platelet $x10^{12}$/L*	*Blood film*	*Malaria parasite*	*Liver function*	*Kidney function*
V 1	21	170	60	16.1	6.0	5.3	282	normal	Negative	normal	=
V2	21	168	57	16.6	4.5	5.3	201	=	=	=	=
V5	23	160	55	16.4	4.5	5.5	242	=	=	=	=
V6	25	170	73	16.3	6.6	5.6	250	=	=	=	=
V7	26	175	66	16.4	5.6	5.7	187	=	=	=	=
V8	26	160	74	15.0	5.3	5.3	379	=	=	=	=
V9	21	161	55	15.5	4.9	5.4	175	=	=	=	=
V10	20	167	50	15.5	4.8	5.1	234	=	=	=	=
V11	24	172	69	16.4	4.9	5.6	245	=	=	=	=
V12	21	162	70	16.1	8.6	6.3	320	=	=	=	=
V13	30	170	71	18.0	4.9	7.9	282	=	=	=	=
V14	21	164	77	17.0	9.5	6.1	299	=	=	=	=
V15	26	170	53	16.5	5.1	5.5	253	=	=	=	=
V16	24	165	60	17.5	6.9	6.0	298	=	=	=	=
V17	24	159	53	15.3	5.3	5.3	359	=	=	=	=

Liver function tests: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase, and Bilirubin (Total), kidney function tests: Urea, and Creatinine, and complete blood count: Red Blood Cells (RBC), White Blood Cells (WBC), platelets and haemoglobin (Hb).

The plasma CQ concentrations in all individual subjects with khat at various time points are shown in **Figure 2.5**. Inter–individual variation of CQ concentration was considerable with respect to peak level and area under curve (AUC) values. As it illustrated in **Figure 2.5**, the peak of CQ plasma concentration was seen at 4 hours in the majority of subjects who chewed khat. In few individuals, the peak was seen at 3 hours. The maximum CQ concentration (C_{max}) ranged from 232 to 612 ng/ml. 24 hours after CQ administration, the highest CQ plasma concentration seen was 41 ng/ml in subject number 15 and the lowest concentration seen was 13 ng/ml in subject number 17.

A similar picture was seen in the subjects who did not chew khat after a 4 week washout period. **Figure 2.6** shows that C_{max} was reached at 4 hours with a maximum value of 658 ng/ml. Few of the subjects showed the CQ peak at 3 hours. The C_{max} ranged from 310-658 ng/ml. CQ could be easily detected from 1 to 12 hours. As shown in **Figure 2.6**, CQ appeared in the plasma quickly, measurable concentrations were observed at 30 minutes after drug administration.

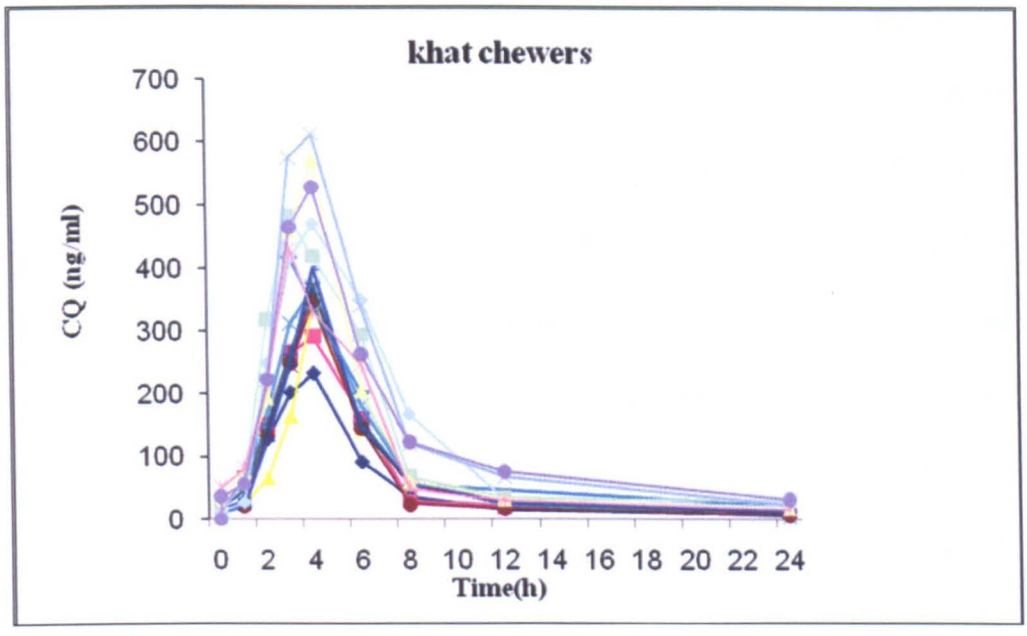


Figure 2.5 Plasma concentration of CQ versus time curve in 15 healthy male adults following an oral single dose of 600 mg base with khat.

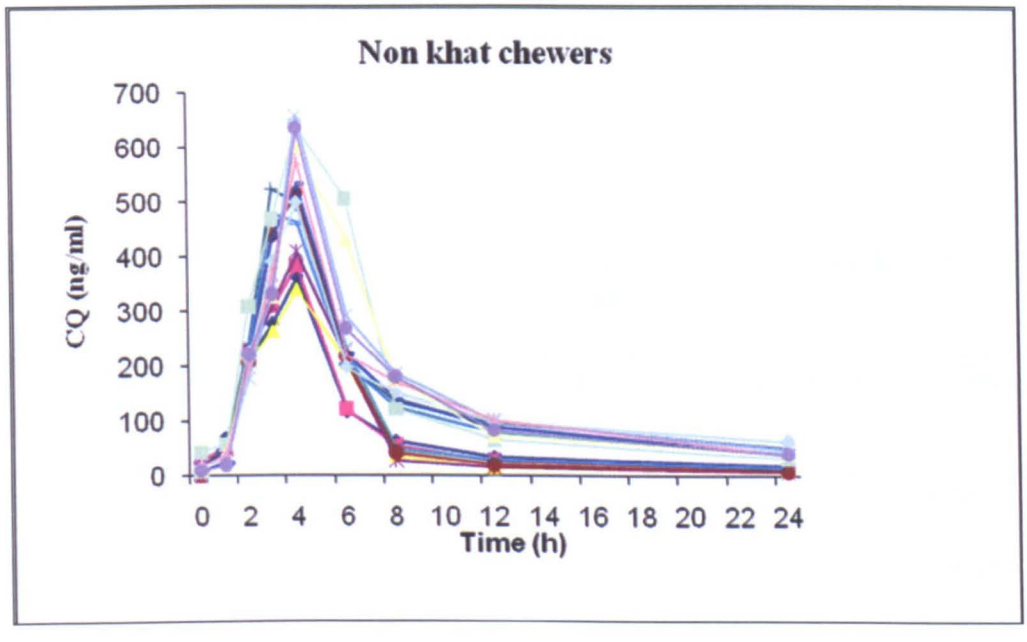


Figure 2.6 Plasma concentration of CQ versus time curve in 15 healthy male adults following an oral single dose of 600 mg base without khat.

The results of CQ given with and without khat are summarized in **Table 2.5**. In khat chewers, the mean (SD) and (range) of CQ C_{max} and the mean of area under the plasma concentration–time curve from hour 0 to 24 hours (AUC_{0-24}) were 415.6 (103.1) ng/ml (232-612 ng/ml) and 2108.9 (682.3) ng/h/ml respectively, while in non khat users they were 508.7 (106.4) ng/ml (310-658 ng/ml) and 2797.4 (845.9) ng/h/ml respectively. This indicated that subjects who chewed khat had lower C_{max} and AUC. Statistical analysis of these results showed that there were significant differences in the mean of C_{max} and AUC between the two groups, ($p = < 0.001$ and 0.002 respectively, paired t-test). Time to reach maximum concentration (T_{max}) in khat and non khat chewer subjects was attained at 3.8 (0.4) h and 3.6 (0.5) h respectively. The post peak time concentration was the same in volunteers who chewed and who did not chew.

Table 2.5 Effect of khat chewing on kinetic parameters of CQ in the plasma of healthy volunteers after a single dose of 600 mg CQ base.

Adult healthy volunteers (Mean age 23, n= 15	**Non Khat chewers**	**Khat chewers**	**P value**
AUC_{0-24} (ng/h/ml)	2797.4 (845.9)	2108.9 (682.3)	**0.002**
C_{max} (ng/ml)	508.7 (106.4)	415.6 (103.1)	**< 0.001**
T_{max} (h)	3.6 (0.5)	3.8 (0.4)	0.81

Values are Means and (SD).

Mean plasma CQ concentration in khat and non khat chewer volunteers at different times after a single oral dose of 600 mg of CQ are given in **Table 2.6.** By the time point of 2 h, the plasma CQ concentration was reduced by chewing khat and become significantly different by 4 h, with the exception of time point 6 h, the values remained significantly different (N.B. time points are not independent data and the analysis should not be over interpreted).

Table 2.6 Mean plasma CQ concentrations in khat and non-khat chewer healthy volunteers at 0-24h interval.

Time (h)	**CQ concentration (ng/ml)***		
	CQ	CQ + khat	**P-Value**
0.5	19 (9)	23 (11)	**.509**
1	39 (13)	43 (21)	**.519**
2	221 (28)	180 (62)	**.151**
3	380 (80)	345 (123)	**.336**
4	508 (102)	398 (104)	**.0001**
6	242 (102)	204 (77)	**.142**
8	109 (58)	67 (39)	**.004**
12	61 (34)	33 (17)	**.002**
24	41 (18)	21 (7)	**.002**

*Values are in means (SD).

Figure 2.7 shows the mean of the CQ concentration produced on the two occasions, i.e. with and without khat. This figure illustrated that there were significant differences in C_{max} and AUC_{0-24} between khat chewers and non khat chewers; $p = < 0.001$ and $.002$ respectively. The mean difference in the AUC was -688 ng/h/ml with 95% CI = -1086 to (-290) and in C_{max} it was -93 ng/ml with 95% CI = -125 to (-61). These results established a reduction of

approximately 25 % in AUC of CQ in khat chewers when compared to those who did not chew khat and C_{max} was reduced by 20 % when the drug was co-administered with khat. T_{max} and concentration post peak were comparable in both occasions when the drug was taken with and without khat, $p > 0.05$.

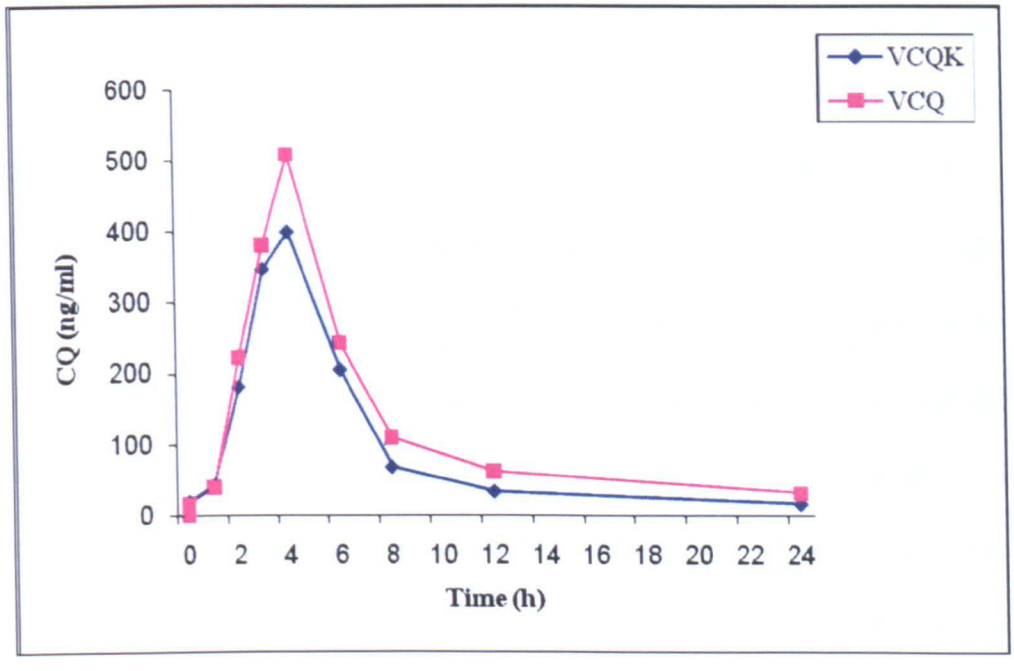


Figure 2.7 Mean plasma concentration-time curves of CQ during co-administration with khat (VCQK) and without khat (VCQ) in 15 healthy male volunteers.

2.4 DISCUSSION

Khat chewing is probably more extensively used in Yemen than anywhere else in the world. Co-administration of khat with therapeutic drugs is very common; therefore, khat-drug interaction is a real possibility. CQ is used for malaria treatment and still the first line drug, therefore study of khat and its effect on CQ pharmacokinetics is needed. We have employed the HPLC assay by D.J Bell (2006) with minor modification which was a reproducible and specific analytical method. Adjusted the pH after adding triethylamine solvent instead before adding triethylamine and pumped the mobile phase at 1 ml/min instead of 3 ml/min has proved to give base-line separation for both CQ and QND. The inter-day and intra-day assay precision were excellent with low coefficient variation (C.V) values less than 15 % at all levels of the QCs and 20% at the LLOQ concentration as recommended in method validation guidelines (USFD, 2001). This makes the method with high reproducibility. In addition, accuracy of the method was within the acceptable deviation from the true value. Linearity is likewise excellent across the relevant range of concentration with correlation coefficient of $r^2 > 0.99$.

The peaks and retention time obtained from chromatogram of samples from the study subject corresponded to those observed in the spiked samples. No interference was detected in the chromatograms with peaks of other endogenous substances. We did not run the assay with other potentially interfering drugs, but any peaks that did appear were before the CQ and QND peaks (**Figure 2.4**). One of the advantages of this method is that the assay

required only small amounts of blood or plasma and so it makes the method applicable in studies involving children and also allows repeated measurements on individual samples. Also, the run time of the samples in HPLC analysis (approximately 8 min) is advantageous in comparison to the already published methods.

The results in the present study suggest that khat affects the absorption of CQ, as judged both by C_{max} and the total AUC. AUC and C_{max} were considerably lower when CQ was given in combination with khat ($p= 0.002$, and < 0.001 respectively). The AUC and C_{max} were reduced by approximately 25 % and 20 % respectively. These findings provide evidence of khat–CQ interaction. Ette (1989) have reported that individuals with longer T_{max} values had lower C_{max} which was due to slow absorption of the drug (Ette et al., 1989). Similar results were seen in our study in khat chewers who have longer T_{max} resulted in lower C_{max} due to the slow absorption when CQ was co-administered with khat, although there was no significant differences in T_{max} between khat and non khat chewers *(P= 0.81)*. These significant statistical differences in pharmacokinetic parameters between khat and non khat chewer subjects are the first indication for a possible drug-herb interaction. The mechanisms underlying interaction are unknown, but the following are some of the hypothesized mechanisms which may be involved in the reduction of CQ bioavailability during khat chewing.

The first mechanisms relates to the involvement of interaction between CQ and ingredients of khat leaves. Chewing khat has been found to interfere with the absorption of some orally administered antibiotics, particularly ampicillin and amoxicillin (Attef, 1997) resulting in low bioavailability of these antibiotics. Investigations of the chemical and biological effects of tannins (Haslam, 1981) indicate that, tannins compounds, which are one of the khat components, are most likely to be responsible for the observed effects on antibiotic absorption. In general, β-Lactam antibiotics are nitrogenous compounds and would be expected to combine with tannins to form insoluble or poorly absorbed complexes. The reported effects of khat chewing on ampicillin and, to a lesser extent amoxicillin may be as a result of tannins in complexing the antibiotics and converting them to insoluble, non-absorbable compounds (direct effect) and, as a result of tannins interfering with gut absorption processes (indirect effect). The components of khat are numerous and co-administration of khat may result in forming insoluble substance with CQ, which consequently delays the absorption of CQ.

Another possible mechanism influence on CQ absorption is due to the changes in gastric content and gastric motility when co-administered with khat. Previous studies have shown that food facilitates CQ absorption, the AUC and peak plasma level were significantly higher when CQ was given with food. The enhancement of CQ absorption with food could be due to a change in the pH of the gastric contents might result in decreased the ionisation of basic drug such as CQ and facilitate absorption (Bates, 1974,

Lagrave, 1985, Tulpule, 1982). However, some beverages indigenous to Sudan including lemon squash decrease the absorption of CQ significantly (Mahmoud *et al.*, 1994). These beverages enhanced acidity and lead to decreased absorption and increased renal excretion. In addition, an increase in gut acidity would lead to increases in ionization of CQ and then resulted in slow absorption of CQ. However, in renal tubules the acidification of urine resulted in reduction of CQ reabsorption, CQ excretion consequently increases in urine (Mahmoud *et al.*, 1994). Another factor may affect drug absorption is delayed gastric empting time when taking with food. It has been reported that khat chewing delayed gastric emptying of a semi-solid meal, therefore, co-administration of CQ with khat would delay gastric emptying, prolonging the stay of CQ in the stomach and resulting consequently in slow absorption of CQ.

Finally, the increase in diuresis, which is often reported among khat chewers, appears to be due to the fluid intake during khat sessions. (Halbach, 1972). This could lead to an increase in excretion and washout of CQ in urine. In addition, a high prevalence of urinary problems has been also reported among khat users which may affect drug excretion (Kennedy *et al.*, 1983).

Studies of cathinone pharmacokinetics showed that blood levels of cathinone start to rise within 1 hour and reach maximum level was within 1.5-3.5 hours (Halket, 1995). From the data in our study we found that CQ levels were reduced markedly at time points coinciding with the peak level of cathinone.

This might result in an interaction between CQ and cathinone forming insoluble or a poorly absorbed compound, a conceivable mechanism being an impairment of gastrointestinal absorption which could be responsible for the reduction in plasma CQ concentration.

Because this is the first study on khat and its effect on CQ pharmacokinetics, we could not compare our results to previous studies and to the best of our knowledge, there is no published information about khat and its effect on pharmacokinetics of CQ when co-administered with khat or even estimation of CQ concentration in Yemeni individuals. Thus, these results may work as reference for future studies. The levels of CQ in the studied subjects were lower than we expected, storage conditions and the length of storage may had slight impact on the measured level of CQ. In another studies after a single dose of 600 mg base, C_{max} and AUC in healthy adult male were 739.5 (139) ng/ml and 6410 (1234) ng.h./ml respectively (Tulpule *et al.*, 1982). In Yemen as well as in many developing countries, drugs may be of variable quality, partially or completely ineffective. A study on the quality of anti-malarial drugs available in Yemen showed high and low failures in ingredient content for CQ tablets and CQ syrup. Also it was found that there was some dissolution failure for CQ tablets, and high sulfadoxine/pyrimethamine tablets dissolution failures (Abdo-Rabbo, 2005). Therefore, reduction of the drug concentration due to the quality of CQ tablets cannot be excluded. However, in the present study, assay of CQ tablets has not been done.

2.5 CONCLUSION

This HPLC method assay has the required sensitivity to measure CQ in 150 µl of plasma. The CQ was detectable in plasma within 30 min and was measurable at LLOQ. Also there was no interference with the CQ and QND peaks. The co-administration of CQ with khat significantly decreased C_{max} and AUC_{0-24} of CQ. The reduction in CQ concentration is an evidence of a pharmacokinetic interactions with khat. This indicated that CQ bioavailability was affected by khat chewing. However, the mechanism underlying such decrease is not clear, but a possibility of impaired absorption of CQ when co-administered with khat cannot be excluded and further investigation are required to determine which components of khat are responsible for the interaction and what the mechanism of interaction.

CHAPTER 3

EFFECT OF KHAT ON PLASMA CQ CONCENTRATION IN *PLASMODIUM FALCIPARUM* MALARIA PATIENTS

3.1 INTRODUCTION

Malaria is a serious health problem in the world, it is estimated that malaria is responsible for the death of over 1 million people each year, mainly in children (Greenwood, 2002) and for 300-660 million clinical cases of *Plasmodium falciparum* in 2002 (Snow *et al.*, 2005). In Yemen, malaria still one of the major health problems, around 60 % of the population are exposed to malaria and more than 90% of malaria is caused by *P. falciparum.* The annual malaria cases are estimated to be 700,000-800,000 with 0.9 % mortality (NMCP, 2006b).

The use of herbal preparation in the treatment of malaria is common in many endemic countries in Africa and Asia and the possibility of concurrent administration of these preparations and anti-malarial drugs is likely to be taking place with possibility of interaction between both agents. Plant-drug interactions have been extensively studied and *in vitro* and *in vivo* studies indicate that some plants lower plasma drug concentrations and this may be attributable to the, impairment in the drug absorption, in addition the induction (or inhibition) of hepatic and intestinal drug-metabolizing enzymes, rendering the drug more or less effective (Zhou *et al.*, 2003, Zhou, 2004).

The chewing of khat leaves is widespread habit in Yemen and it is used as a recreational drug. The epidemic of khat use has induced researchers to investigate its pharmacology and its effect on human health. Chewing of khat is commonly used even during sickness as a first resort to treat fever in the

belief that khat makes users feel better although khat is not known as a traditional anti-malarial drug. Concurrent use of khat with standard medicine is common practice; the possibility of khat interactions with anti-malarial drugs must not be ignored.

To date there are no studies have been found in the literature about khat and anti-malarial drug interaction. Our studies (**chapter 2**) have shown reduction in plasma CQ levels in healthy adult male. Reduction in plasma drug concentration in malaria patients might affect parasite clearance and consequently lead to therapeutic failure. In addition to self–medication and inadequate dosing, subtherapeutic levels in blood are believed to be important factors that contribute to CQ resistance to *P. falciparum* (Mockenhaupt *et al.*, 2000).

The low cost and easy availability makes CQ the drug of choice for most patients in Yemen and still the first line drug. Khat and CQ interaction may represent a potential risk of affecting CQ efficacy, therefore, the present study attempted to evaluate the effect of khat chewing on CQ concentrations in malaria patients during treatment, extending our studies on volunteers (**chapter 2**) to a more complex group, malaria patients in the field.

3.2 MATERIALS AND METHODS

3.2.1 Choices of study area

The environment and the capacity to conduct field research are very limited in Yemen. Lack of financial support, supplies, and equipment, compliance of participants, illiteracy, humidity and electricity supply problems were the main factors that hindered the research work in this study. This is illustrated by the fact that the study area had to be changed two times during the field work. The first one was in Taiz city which belong to Taiz governorate, where the prevalence of malaria is very high. A pilot study on 10% of the sample size has been done to ensure the accuracy of the chosen method of sample collection, storage, and transportation. Due to poor commitment and compliances of the patients, the study in this area was cancelled. The next two districts that were chosen were recommended by National malaria Control Malaria (NMCP), Al-Heemah in Sana'a governorate and Bajil in Tihama region which belong to Al-Hudaydah governorate. Unfortunately, the study was terminated in Al-Heemah city due to two reasons: first, compliances were very poor because patients live very far from the Malaria Health Centre and it was very hard for the patients to come back due to the limited and high cost of transportation there. Second, the health clinics were not suitable and technicians were not qualified in diagnosis of malaria. The NMCP was informed verbally about the situation in that centre. The third study area was in Bajil city which belongs to Al-Hudaydah governorate. The indicators of malaria cases were high and the compliance was good. Therefore, we decide to conduct the fieldwork in Bajil city. We provided the centre with all

requirements and supplies for the research. In addition, as incentives, the patients were given free over the counter drugs (OTC drugs) and transportation costs to come in the scheduled follow-up visits because most of patients live out of the city. If patients could not come back, the work team went to their homes for follow-up visits. The overtime paid to technicians and most of the expenses of the study were funded by the main investigator.

3.2.2 Study area

The study was conducted from January–April 2006 at the centre of NMCP in Bajil, which is one of the six sentinel sites of NMCP, and Al-Marawa'a districts which belong to Al-Hudaydah—a major governorate in the west of Yemen on the coastal plain (**Figure 3.1**). About 25 % of total population lives in this area, in which the prevalence of malaria is very high and considered to be meso to hyperendemic. Temperature in this region is 25- 40 °C and humidity percentage is 70-85 %. The transmission season is during the winter from October-April and the predominant species is *P. falciparum* and the predominant vector is *An. arabiensis* (NMCP, 2005b). The slide positive rate (SPR) was 9.6 % in 2006 (NMCP, 2006).

The population in Bajil and Al-Marawa'a districts is 172600 and 129247 respectively and most of the people work as farmers. In Bajil district, there is only one public hospital, 1 family clinic and 7 health units. While in Al-Marawa'a district, there is one public hospital, 1 family clinic, and 6 health

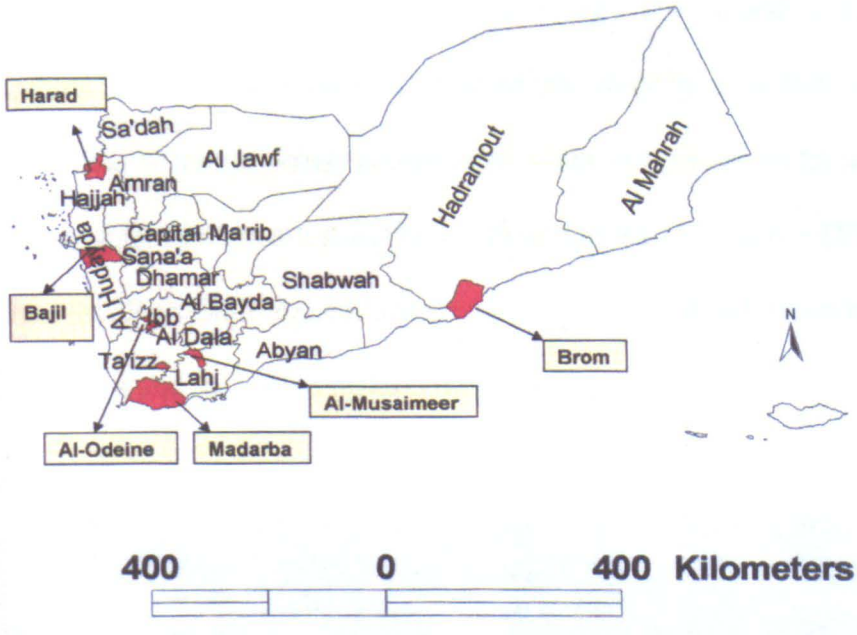


Figure 3.1 Map of Yemen, showing study area; Bajil (source: National Malaria Control Program, 2005).

units. However, there are deficiencies in the supplies and equipment; therefore, they are not able to provide the required health care service. The private sectors are taking part in the development of the health sector by providing health services to people. There are 3 private hospitals in Bajil and one private hospital in Al-Marawa'a, but most of people cannot afford the cost of the service and the quality of service is not much better than the public sector (NMCP, 2006). Due to the limited resources of health care and the poverty and low level of education, some diseases including tuberculosis, diarrhoeal diseases, malnutrition, and malaria are common in this area. The

results of the survey that was conducted in this study regarding the education level showed that of 132 participants about 78.6 % were illiterate, 16 % attended elementary school, 5% attended high school and 1 % attended diploma level. In the rural area, most people work in agriculture and due to the limited access to water, people store water in open tanks for a long time period which provides a suitable breeding site for mosquitoes (**Figure 3.2**). The NMCP in Bajil and residents of the houses were informed about these open tanks and the risk of malaria transmission.

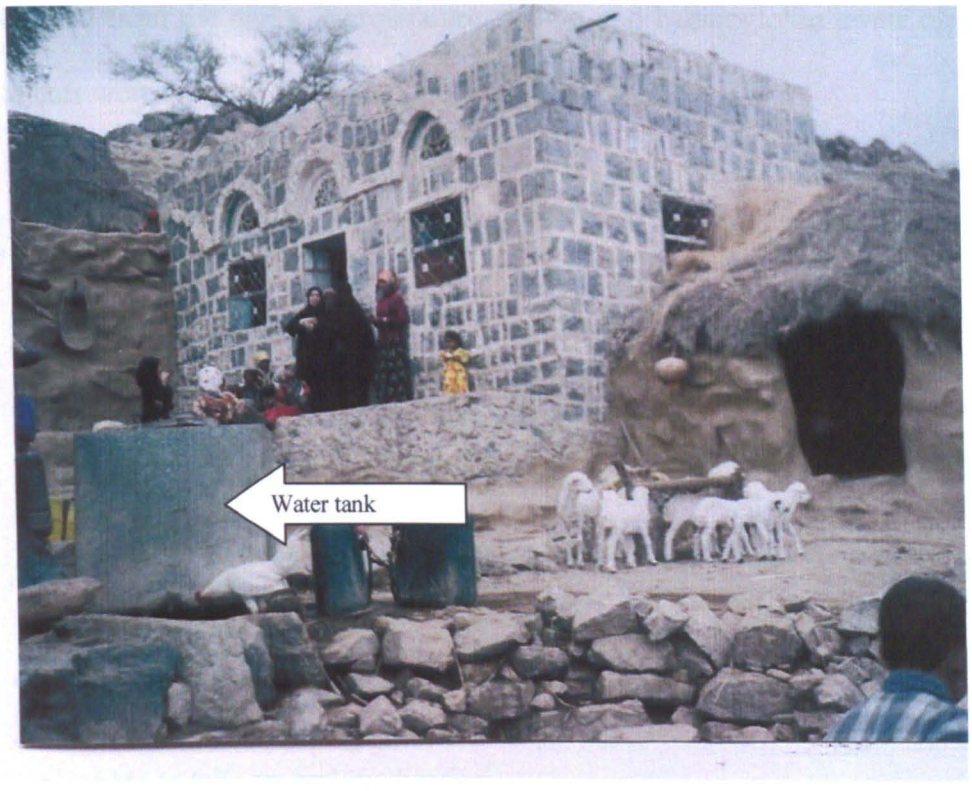


Figure 3.2 House of malaria patient in Al-Marawah district in Al-Hudayada governorate. Water tank potential source of malaria transmission.

3.2.3 Study subjects

Patients who came to the NMCP centre in Bajil city, Al-Hodaydah governorate, for malaria diagnosis and treatment and were positive for malaria parasites on screening blood film examination, and also met the inclusion criteria and were willing to participate were entered in the study. A total of 132 adult male and female malaria patients were slightly more than required from the sample size calculated (**section 3.2.4**). Patients with danger signs or signs of severe malaria, patients who did not complete the study and patients who were confirmed negative for *P. falciparum* **(section 4.3)** were excluded from the study. Temperature, weight and haemoglobin levels of all subjects were measured (**Table 3.1**).

3.2.3.1 Inclusion criteria

- Positive for *P. falciparum* malaria parasite.
- Axillary temperature ≥ 37.5 °C or history of fever during the last 24 hours.
- Absence of general danger signs or signs of severe and complicated *P. falciparum* malaria.
- Age > 18 years.
- Able to take an oral medication.
- Able to come for follow-up to the Malaria Centre.
- Informed consents by the patients.

3.2.3.2 Exclusion criteria

- Pregnancy.
- Patients who use any anti-malaria drug before the study began.
- Patients with complicated malaria were identified by the following signs:
    - Not be able to drink or feed.
    - Repeated vomiting.
    - Convulsions during the present illness.
    - Lethargic or unconscious.
    - Unable to sit or stand up.
    - Haemoglobin less than 7 g /dl.

3.2.4 Sample size calculation

Sample size was calculated using sample size and power calculation (*STATA, Version 9.2*) on basis of 30 % of change in plasma CQ level following khat chewing and on a confidence interval of 95 % and on statistical power of 80 %. Values for the mean and SD of CQ levels in plasma are taken from (Bustos *et al.*, 2002). The sample size required 50 patients in each group and allowing for withdrawal and false positive, 60 patients in each group were required.

3.2.5 Setting of the study

All patients who met inclusion criteria and who were willing to be included in

the study were entered into the study. After the selection, informed consent was obtained from all participants (**Appendix 4**). The study is based on the use of CQ, which still the first line treatment of the NMCP. All patients received CQ tablets using the recommended treatment regimen of a total dose of 25mg CQ base/kg body weight over three days. The CQ was given as an initial dose of 10 mg/kg in the first day (day 0), 10 mg/kg in the following day (day 1) and 5 mg/kg in day 2. The drug was given orally under direct supervision of the study team; patients who had not had breakfast were supplied with free sandwiches to avoid upset stomach and vomiting. The patients were observed for 30 minute after drug intake and if vomiting occurred, the dose was readministered. In addition, the patients were asked to complete the questionnaire regarding khat habit, previous use of CQ or other anti-malarial drugs and the use of khat during the treatment (**Appendix 5 and 7**). The amount, type of khat and time of khat session were not controlled in khat chewer malaria patients.

3.2.6 Data and sample collection

Each patient was given a consecutive study number in the record book; this number was the same for blood samples, slides, case record form (**Appendix 6**) and questionnaire. Patients were weighted on calibrated scale to guide the treatment doses (**Figure 3.4**). Body temperature, taken as a marker of illness or clinical cure, was recorded on days 0, 1, 2, and 3 with a reliable standard thermometer (**Figure 3.5**) and parasitaemia was also assessed on each visit.

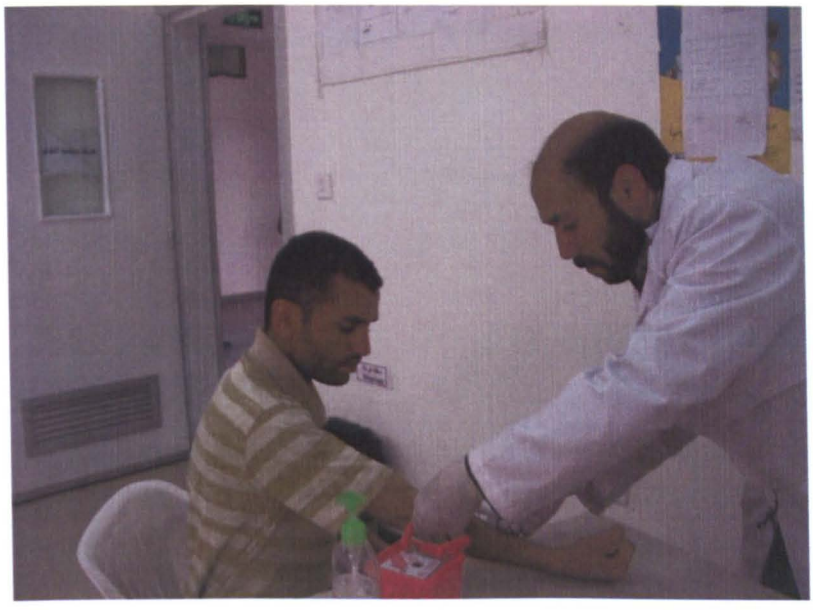


Figure 3.3 Collections of blood samples from malaria patients.

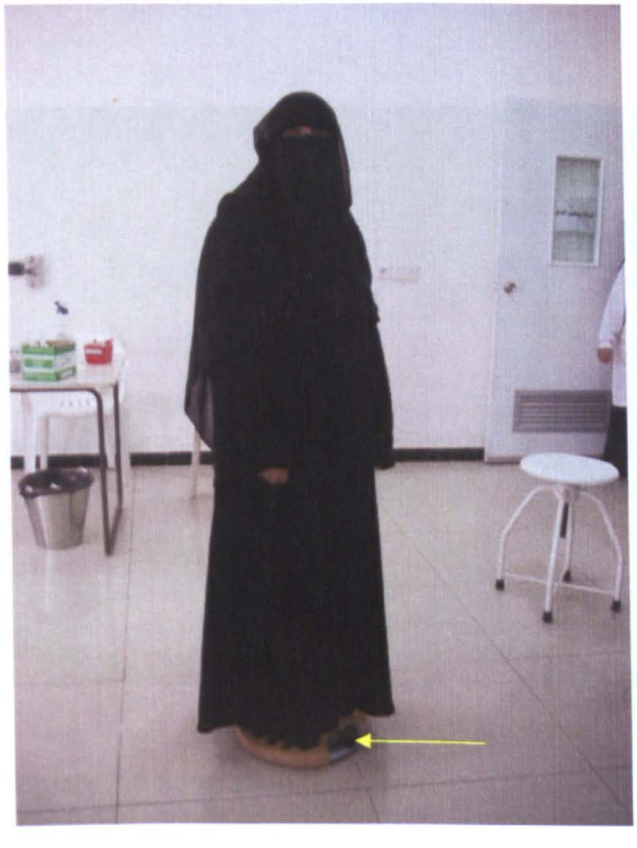


Figure 3.4 Weighing patients to guide treatment dose. Yellow arrow indicates the scale.

Figure 3.5 Taking axillary temperature to guide clinical assessment of malaria patients.

Figure 3.6 Separation of plasma.

The patients were asked by the investigator to come back on the days 1, 2, and 3 of treatment for follow-up. Those who did not return were followed up at their homes. In addition, 3 ml of blood was drawn from each patient on each visit at 24 hour intervals and blood sample were transferred to heparinized tubes and centrifuged at 2000 rpm for 15 min **(Figure 3.3 and Figure 3.6)**. The plasma then was transferred to Eppendorf tubes and stored at –20°C prior to transport to Central Health Laboratory in Sana'a in an ice box, where they were kept at –70°C. The samples were brought by the investigator to the UK and stored at –20°C until analyzed.

3.2.7 CQ measurement in malaria patients

CQ concentrations were measured in plasma in days 0, 1, 2, and 3 using HPLC method by D.J. Bell (2006) **(sections 2.2.9).**

3.2.8 Statistical analysis

Before choosing the right statistical test, a histogram was drawn and a normality test was carried out. The *P value* of the normality test was close to 0.05 (D (103) = 0.043) which means only a small deviation from normality, also the mean and the median were comparable, therefore we used the original data for the statistical analysis. However, the use of the original data or the transformed data produced the same conclusion in the test of significance. Values were reported as mean and SD. Differences between khat chewers and non khat chewers were tested for significance with Student's t-test and one way ANOVA repeated measurements when applicable. A value

of $P<0.05$ was considered statistically significant.

The study has been approved by the faculty of Medicine, Sana'a University (**Appendix 1A**), Malaria National Control Programme in the Republic of Yemen (**Appendix 1B**), and the Liverpool School of Tropical Medicine, University of Liverpool, UK (**Appendix 1C**).

3.3 RESULTS

One hundred and three *P. falciparum* malaria patients , 57 khat chewers, mean age (SD) 27.9 (8.2) years; weight 48.7 (7.0) kg and 46 non khat chewers mean age (SD) 25.4 (7.4) years; weight 45.9 (4.7) kg participated in the study, which was conducted at the National Malaria Control Program Centre in Bajil city. Male mean age was 26.8 (7.3) years and mean weight was 52.7 (7.3) kg and female mean age 24.9 (6.9) years and the mean weight was 45.6 (3.7) kg. The mean haemoglobin levels in female and male who chewed khat were 9.8 and 13.9 g/dl respectively, while the mean haemoglobin levels in patients who did not chew khat were 10.6 and 12.7 g /dl (**Table 3.1**). Patients were treated with CQ, 25mg/kg over three days. CQ concentration was measured by HPLC method (D.J. Bell, 2006). All subjects with fever > 37.5°C prior to treatment had normal axillary temperature by day 2 of treatment.

Table 3.1 Baseline characteristics of recruited *P. falciparum* malaria patients.

Characteristic	**Mean (SD)**
Khat chewers (n = 57)	
Age (in years)	27.9 (8.2)
Body weight (kg)	48.7 (7.0)
Haemoglobin (female n= 20)	9.8 (1.5) g /dl
(male n= 37)	13.9 (1.2) g/dl
Enrolment axillary temperature (°C)	37.5 (0.54)
Non khat chewers (n = 46)	
Age (in years)	25.4 (7.4)
Body weight (kg)	45.9 (4.7)
Haemoglobin (female n= 29)	10.6 (1.4) g/dl
(male n= 17)	12.7 (.91) g/dl
Enrolment axillary temperature (°C)	37.4 (0.66)

Plasma CQ concentration-time profiles after three days of treatment in *P. falciparum* malaria patients who chewed khat and who did not chew khat during treatment are shown in **Table 3.2**. During co-administration of khat the plasma CQ level was significantly reduced ($p < 0.001$) in khat chewers compared to non khat chewers. The mean and (SD) plasma CQ concentrations on the Days 1, 2, and 3 in khat chewers were 145.4 (73.8), 202.7 (130.1), and 266.4 (164.3) ng/ml while in non khat chewers they were 216.5 (94.8), 313.3 (123.4), and 427.5 (125.6) ng/ml respectively. **Figure 3.7** illustrates that subjects who chewed khat during treatment had a lower mean CQ level than those who did not chew khat throughout the 3-day treatment. The differences in the mean of CQ between both groups were significant ($p= <0.001$. The mean difference in CQ levels was 114 with 95 % CI = 72.5 to 156.1 ng/ml **Figure 3.8**.

Table 3.2 Effect of khat chewing on plasma CQ concentration of malaria patients after 3 days of treatment.

Study group	**Plasma CQ concentration (ng/ml)**		
	Day 1	Day2	Day3
Khat chewers (n= 57)	145.4 (73.8)*	202.7 (130.1)*	266.4 (164.3)*
Non khat chewers (n= 46)	216.5 (94.8)	313.3 (123.4)	427.5 (125.6)

Values are Mean and (SD).
*$P= <0.001$.

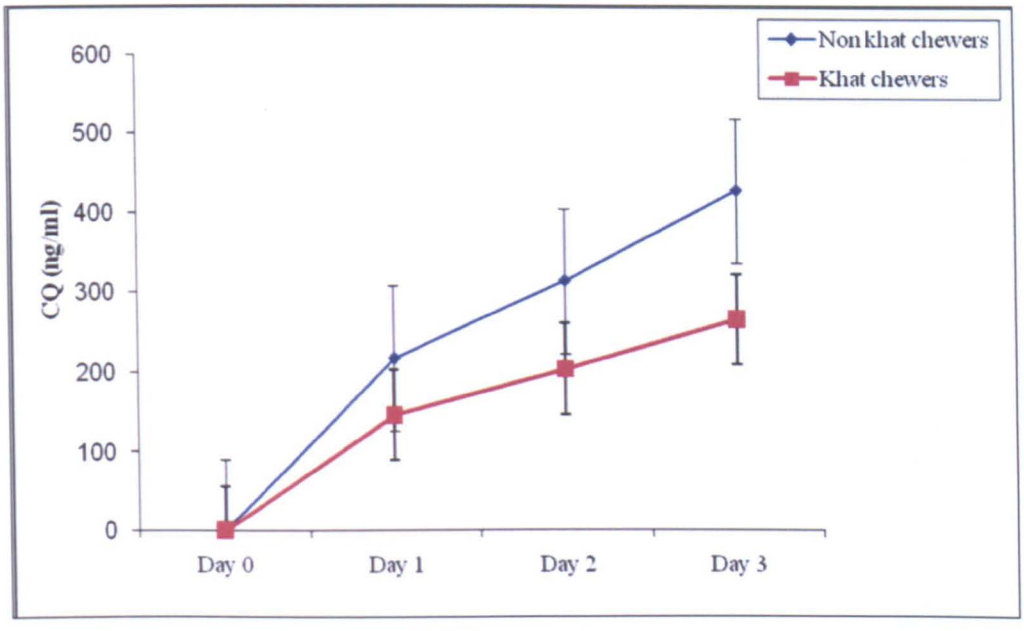


Figure 3.7 Mean of plasma CQ concentrations in khat chewers and non khat chewers infected with *P. falciparum* malaria.

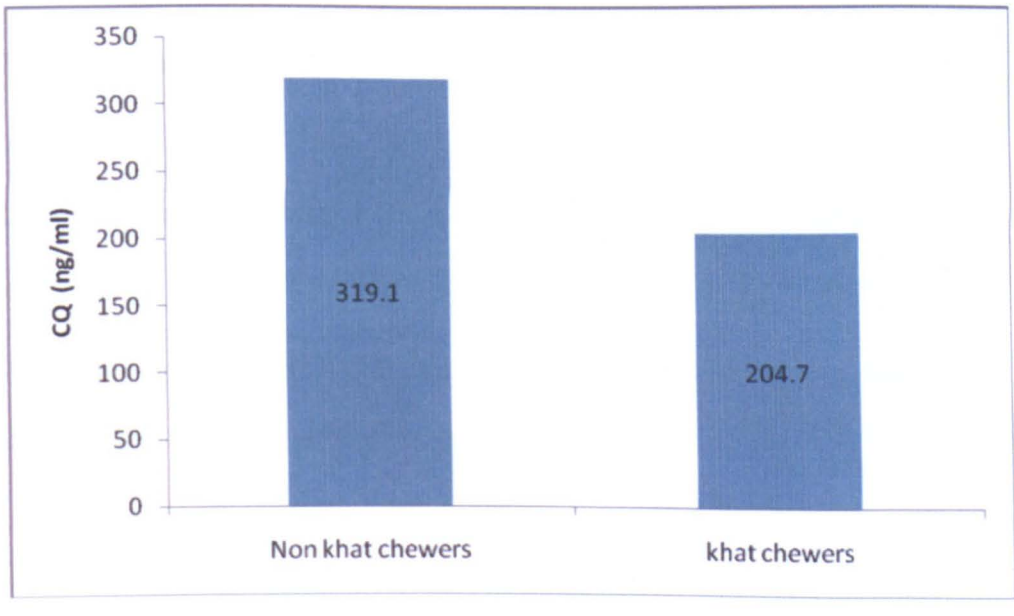


Figure 3.8 Mean of plasma CQ concentrations in khat chewers and non khat chewers infected with *P. falciparum* malaria after 3 days of treatment with CQ.

In univariate analysis, sex found to be not significantly associated with CQ concentration level reduction within the study groups. **Table 3.3** shows the mean plasma concentration in male and female after 3 days of treatment. The mean plasma CQ concentrations were comparable between male and female in malaria patients irrespective of the treatment groups, p = *0.76*. In addition, there was no statistical significant difference in CQ level between male and female in non khat users. The values were 326 (106) and 314 (99) ng/ml in male and female respectively (*p= 0.72*). However, the difference between male and female in khat chewers was statistically significant. The mean (SD) plasma CQ concentrations in males and females were 228 (114) and 161 (87) ng/ml respectively (*p = .028*) (**Figure 3.9**).

When comparing females who chewed khat *vs* females who did not chew khat as well as males who chewed khat and males who did not chew, the differences in CQ levels were highly significant (*p<0.001* and *p=0.004* respectively) **(Figure 3.9)**. The difference in CQ levels between females in khat and non khat chewers was larger than the difference between males. This indicates that females are more susceptible to the effect of khat chewing.

Table 3.3 Comparison of mean plasma CQ concentrations between male and female in khat and non khat chewers.

Study group		CQ plasma concentration (ng/ml)	P-value
Khat chewers		319 (101)	**<0.001**
Non khat chewers		204 (110)	
Male		259 (120)	**.76**
Female		252 (120)	
Non khat chewers	M (17)	326 (106)	**.72**
	F (29)	314 (99)	
Khat chewer	M (37)	228 (114)	**.028**
	F (20)	161 (87)	

Values are in mean (SD). M= male, and F= female

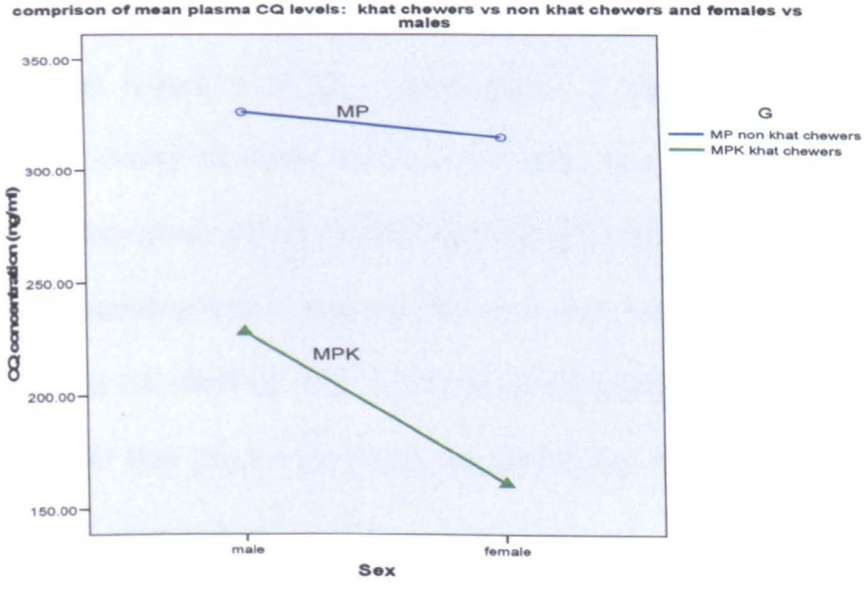


Figure 3.9 Summery of plasma CQ concentration in khat and non khat chewers (male and female).

Based on the habitual use of khat, no statistical differences in the mean of CQ levels were detected between heavy (chew khat on daily basis) and light khat chewers (chew khat once a week) ($P > 0.05$). In heavy khat chewers, the mean of plasma CQ concentration was 204 (103) ng/ml, while in light khat chewers it was 207 (133) ng/ml **(Table 3.4).**

Table 3.4 Plasma CQ concentrations in heavy and light khat users.

Khat habit	**CQ plasma concentration (ng/ml)**			
	Day1	**Day2**	**Day3**	**Mean**
Heavy (n= 43)	152 (75)	204 (124)	255 (146)	**204 (103)**
Light(n=14)	124 (65)*	196 (151)*	299 (212)*	**207 (133) ***

Values are in mean and (SD).
*P > 0.05, t-test (2-tailed).

The significant reduction of CQ concentrations in malaria patients who chewed khat during treatment indicate that khat had an effect on CQ absorption, although no certain mechanism is known. The results established the CQ concentrations were comparable between male and female suggesting that gender has no effect on CQ concentration. In addition, level of CQ in heavy and light khat users were similar indicating that habitual of chewing khat had no significant impact on CQ concentrations.

3.4 DISCUSSION

CQ concentration has not been studied in malaria patients or in different conditions in Yemen. In addition, there are no previous reports of pharmacokinetic interaction of CQ co-administration with khat.

The results showed that the bioavailability of CQ was significantly reduced when administered with khat ($p<0.001$) when comparing to those who did not chew khat. These results were similar to those previously reported for the effect of khat on the bioavailability of some antibiotics which was determined using a urinary excretion method (Attef, 1997). The extent and rate of ampicillin bioavailability were reduced significantly by khat chewing except when administered 2 h after the khat chewing session. The mechanisms of this interaction are not certain. However, it has been proposed that ampicillin combines with khat tannins to form an insoluble and poorly absorbed complex.

The work presented **in Chapter 2** compared healthy volunteers with and without khat and showed plasma CQ concentration was reduced significantly in subjects who chewed khat. The reduction in CQ levels was even more significant in patients infected with *P. falciparum* malaria who chewed khat during the treatment (approximately 33%). This may associated with the general health of the study group, such as malnutrition, anaemia, and parasitic infestation. Tulpule (1983a) found faster clearance of CQ in undernourished subjects (Tulpule, 1983a). From personal observation and conversation with

the patients, they seldom eat a further significant meal after lunch, and their meals are mostly very poor in nutrition value. In general, khat chewers have poor appetite; anorexia and malnutrition which are one of the khat-chewing induced symptoms (Kalix, 1988). Reduction in CQ levels may be attributed to khat-CQ interaction and to other factors such as malnutrition and disease status which made the reduction of CQ level more profound in malaria patients. The mechanisms of interaction are not clear but suggested mechanisms were detailed in **chapter 2.**

Clinically there are marked inter-individual variations in the pharmacokinetics of CQ following single or multiple doses (Ette et al., 1989, Gustafsson et al., 1983b, Hellgren, 1995, Wetsteyn et al., 1995). In this study there was also a large inter-individual variability of CQ concentration between subjects. This marked inter-individual difference in CQ levels and the potential effect of khat on CQ in khat chewer patients may affect the therapeutic level of CQ in malaria patients and consequently CQ efficacy might be impaired. The inter-individual variation also can be also attributed to age, sex, weight and variation in metabolism of drugs.

Drugs in general use are given by mouth, since it is the most convenient method of drug administration. CQ as an anti-malarial is usually given orally. In Thai *P. vivax* malaria patients, CQ blood concentration and AUC were significantly higher than in healthy Thai (Na-Bangchang et al., 1994), but there was no difference between Thai controls and malaria patients following

intravenous administration (Edwards et al., 1988). Therefore it was concluded that malaria induced absorption changes contributed to the increased systemic exposure to CQ, but this was not the case in Yemeni malaria patients when compared to the healthy volunteers (**chapter 2**) after an oral administration of CQ with khat, where the systemic exposure to CQ was reduced. The reduction of CQ in malaria patients was higher (33 %). This reduction could be related to the effect of khat on CQ absorption suggesting impaired gastrointestinal absorption.

Gastric emptying time and an intestinal transit rate are important factors in drug absorption (Heymann *et al.*, 1995). These two factors were delayed in khat chewers which results in CQ remaining longer in an acidic environment might affect absorption by increase ionization of basic drugs. Mahmoud (1994) reported that CQ level was reduced when co-administered with lemon and other local beverages in Sudan due to the changes in the pH of the gut which increases the ionization of CQ and resulted in delaying CQ absorption (Mahmoud *et al.*, 1994).

Although gender differences in pharmacokinetics of numerous drugs have been identified, the differences are generally only slight. Physiological and molecular differences such as drug transporters and drug-metabolizing enzymes between genders can cause sex-related differences in the pharmacokinetics of drugs. For example, females tend to have lower body weight, a greater percentage of body fat and higher CYP3A4 activity than

males (Meibohm, 2002). Although the presented results provided no significant evidence for sex-related differences in the concentration of CQ in overall total patients, however, there was a significant difference in the mean plasma CQ concentrations at all the time points between males and females in khat chewers ($p < 0.05$), but no such significant differences was found between males and females in non khat chewers. This indicates that khat induces the reduction of CQ in khat chewers. In addition the results revealed that females are more affected by khat chewing than males.

The reduction in plasma CQ concentrations could have clinical relevant if the CQ reduction in the system falls below the minimum therapeutic level needed for the parasite clearance during malaria treatment. Delayed absorption of CQ due to the potential effect of khat is likely to have a major relevance on the clinical outcome of CQ treatment in khat chewers if more than one possible factor is involved such as inter-individuals variation, malnutrition, anaemia and tablets quality. However, an adequate plasma CQ concentration was reached throughout the study period (3 days) of treatment in all study groups and exceeded the target therapeutic concentration of > 16 μg/L which is considered an adequate level for the suppression of sensitive of *P.falciparum* (Wetsteyn *et al.*, 1995) although in our study not all of the malaria patients were sensitive to CQ, about 26.2% of the cases were with resistant parasite (**chapter 4**). In this study, malaria patients were only followed up for 3 days and this might underestimate late treatment failure in the 28 day-follow-up.

3.5 CONCLUSION

On the basis of the present results we found preliminary evidence that chewing of khat reduced plasma CQ concentration significantly in malaria patients when compared to those who did not chew khat during treatment, therefore, khat should not be taken during treatment to avoid parasitological failure and treatment failure due to inadequate plasma CQ level in malaria patients. However, in this study, the reduction of CQ level was not below the therapeutic level for sensitive strain of *P. falciparum* but khat chewing may affect other drugs with low therapeutic levels.

This is the first study describing the effect of khat chewing on CQ concentration in malaria patients. The mechanism of khat-drug interaction is not fully understood. Clearly more studies and further investigations are needed to fully assess the mechanism of interaction and other factors involved in this interaction.

CHAPTER 4

EFFECT OF KHAT ON PARASITAEMIA AND PARASITE CLEARANCE OF UNCOMPLICATED *P.FALCIPARUM* MALARIA PATIENTS

4.1 INTRODUCTION

Malaria infection is usually associated with fever. One of the basic approaches to the alleviation of fever has been the use of some crude extracts from various medicinal plants that are presumed to be antipyretics and analgesics (Kambu *et al.*, 1990b, Kambu *et al.*, 1990a, Kerharo, 1974, Mukherjee, 1991, Oliver-Bever, 1986). These extracts have been screened *in vitro* and/or *in vivo* to evaluate their potential for inhibition of malaria parasite growth (Benoıˆt *et al.*, 1996, Gbeassor *et al.*, 1990, Gbeassor *et al.*, 1989, Gessler *et al.*, 1995b, Gessler *et al.*, 1994, Jurg, 1991, Khalid, 1986, Weenen *et al.*, 1990). A number of the extracts that showed promising antiplasmodial activity have been selected and extensively studied with the isolation and characterization of their active constituents determined. (Ang, 1995, Bray *et al.*, 1990, Bray *et al.*, 1987, Chan *et al.*, 1986, Franc¸ois *et al.*, 1994, Kardono *et al.*, 1991, Khalid, 1989, Koumaglo *et al.*, 1992, Likhitwitayawuid *et al.*, 1993a, Likhitwitayawuid *et al.*, 1993b, Ratsimamanga-Urverg *et al.*, 1992, Thebtaranonth *et al.*, 1995, Wright, 1992, Wright *et al.*, 1991). This approach is in line with the discovery of some of the well known antimalarial drugs derived from plants such as quinine and artemesenin.

Khat is widely used as a recreational drug, but it is also used as a first resort to manage fever (Al-Haborri, 2002), and it is possible that khat components could possess anti-malarial activity. In addition, in **chapter 3** of this study, the plasma level of CQ was assessed in malaria patients and it was found that chewing khat significantly reduced the CQ levels in khat chewers when

compared to the patients who did not chew khat. Therefore, we thought it would be important to study khat and its potential effect on parasitaemia and parasite clearance in uncomplicated *P. falciparum* malaria patients which have not been studied before.

4.2 MATERIALS AND METHODS

4.2.1 Microscopic blood examination

Thick blood films were prepared on microscope slides and dry films were fixed with methanol and stained with 10 % Giemsa to assess the parasitaemia. A total 103 patients were confirmed positive for *P. falciparum*, fulfilled all the inclusion criteria (**section 3.2.3.1**), and were enrolled in the study. Thick and thin blood films were prepared on the same slide for each patient and stained with 3 % Giemsa on days 0, 1, 2, and 3 in duplicate, a total of 1056 slides were examined to assess parasitaemia (**Figure 4.1**). All the slides were examined by technicians in Bajil malaria centre. Subsequently, all the positive slides were then sent to the Central Public Health Laboratory to be reviewed and re-examined by a senior microscopist to rule out all the false-positive results and to count parasites. Parasitaemia were determined by counting the number of asexual parasites against at least 200 white blood cells (WBCs) in thick films. In case of hyperparasitaemia, x100 fields were counted by experienced laboratory technicians at the NMCP. A smear was considered negative when no parasites were seen after viewing 100 high-powered fields. Results were recorded as parasites/µl blood assuming a mean of WBC count of 8,000/µl of blood. If the parasite count drops below 10 asexual parasites/200 WBCs, the count was made against 500 WBCs. The parasitaemia was calculated using the following formula:

Parasites (per µl) = number of parasites x 8,000 / number of WBC.

Axillary temperatures were taken on days 0, 1, 2, and 3 and whenever a patient presented, each patient was asked about the previous use of anti-malarial drugs and about the habit of chewing of khat on the previous day (**Appendix 7**). Patients who did not return for follow-up on days 1, 2, or 3 were visited at home on the same day.

Figure 4.1 microscopic examinations of malaria cases.

4.2.2 Definition of outcomes

Due to the barriers that were found in the field, a 7-day observation period was not possible, so patients were followed for 3 days instead of 7 or 14 days. Elimination of asexual parasites was taken as the criterion for treatment success. Responses to treatment were classified in terms of 1) parasitological response and 2) clinical response.

1. Parasitological response (using WHO 1973 protocol).

a) Sensitive = clearance of parasites by day 2.

b) RII (moderate grad resistance) = reduction in parasitaemia to ≤ 25% of day 0 level by day 2 but no complete clearance of parasitaemia.

c) RIII (high grad resistance) = no reduction of parasitaemia or parasite density ≥ 25% on day 2 of the day 0 parasite density.

2. Clinical and Parasitological response (using the WHO 1996 protocol):

    a) Treatment successes = clearance of parasites by day 3.

    b) Early treatment failure = any of the following:

1. Danger signs or severe malaria on days 1, 2, or 3 with parasitaemia
2. Axillary temperature ≥ 37.5 °C on day 3 with parasitaemia
3. Parasite density on day 3 > 25% of day 0 parasite density.

As defined in the 1996 protocol requires follow-up observation in week two (WHO, 1996) which could not be carried out in this study. In this study, treatment success will be overestimated and late treatment failure will be underestimated because of short follow-up period.

In this study we have used day 2 and the parasitological response was interpreted according to the old WHO protocol using S, RII and RIII classification system. We have used also day 3 and the therapeutic response was measured according to a more recent protocol, WHO 1996 in which clinical response is taken into account in the outcome classification. We prefer to use day 3 results for two reasons: 1) the therapeutic end point for

parasitaemia determinations is on day 3; 2) the last dose of CQ was given on day 2, so assessing parasitaemia on day 3 will be more appropriate than day 2 and since assessing parasitaemia on day 2 would result in a high rate of false-positive early treatment failure/RIII designations and unnecessary re-treatment with second line drugs as we observed many cases with parasitaemia on day 2 were cleared completely by day 3 of the treatment. Patients who classified as treatment and parasitological failure were given the recommended dose of the second line drug treatment in Yemen (sulfadoxine/pyrimethamine, S/P, Fansidar). Patients with gametocytaemia were treated with primaquine.

4.2.3 Statistical analysis

Data analysis was carried out by SPSS Version 15 for descriptive and analytical statistics. For non-normally distributed values, the data was log transformed prior to analysis. Geometric mean was used to summarize parasite densities and Student's t-test was used for comparison of means. Parasite clearance and association with khat use were analyzed with Fisher's exact tests for two-sided significance. P-values less than 0.05 were accepted to imply significant differences between study groups.

4.3 RESULTS

The effect of khat chewing on parasitaemia and parasite clearance was investigated using same samples as in **chapter 3** and considerations of sample size remain the same. Each patients who had fever or had history of fever within the last 24 h was referred for screening and if positive with *P. falciparum* malaria and willing to participate was enrolled in the study until a total number of 132 was reached. This number was based on the calculated sample size with additional of 10 % to allow for the drop out cases. 103 cases were enrolled in the study while 29 were excluded for different reasons. Slides from the 132 cases were re-examined by quality control microscopists in Central Heath Laboratory, 10 cases were reclassified as negative because the slides were unsatisfactory and could not be accurately assessed; 6 cases missed the dose treatment on day1; 6 cases used self-treatment with other anti-malarial drugs and 7 patients were lost to follow-up. These cases were excluded from the study and 103 patients with completed follow-up were taken into account for the analysis in this study. Parasitaemia were determined by counting the number of asexual parasites against at least 200 WBC in thick blood films (**section 4.2.1).** All the patients' characteristics have been presented in **Table 3.1**, in day 0, 11 patients (12 %) had temperature > 37.5 °C and 88 % had history of fever within the previous 24 h. The patients were followed for 3 days instead of 7 days because most patients refuse to participate if requested to come back on day 7 of treatment due to living far away from study location.

4.3.1 Parasitaemia and parasite clearance

The parasite densities in chewing and non chewing subjects are summarized in **Table 4.1** and **4.2)**. In khat chewing subjects, hyperparasitaemia (parasite density > 100,000 /μL blood) appeared in one patient at day 0 and on day 2, three patients had higher parasite density than at day 0, while in non khat chewing subjects, two patients had a parasite density > 200,000 /μL blood at enrolment, and on day 2, seven patients had higher parasite density than at day 0, however, none of these patients had symptoms of sever malaria and danger signs and all fulfilled the inclusion criteria.

Table 4.1 The level of parasitaemia in khat chewer patients on day 0 and day 3 of treatment with CQ.

Level of parasitaemia (parasites/μL blood)	**No. and (%) of patients**
Day-0 Values	
1-400	15 (26.3)
401-1000	8 (14.1)
1001-4000	15 (26.3)
4000-100,000	18 (31.5)
>100,000	1(1.8)
Parasite density range 32-111,760 (per *μl*	
Day-1 > Day-0 Values	6 (10.5)
Day-2 > Day-0 Values	3 (5.3)
Day-3 Values	
0	43 (75.4)
1-400	12 (21)
401-1000	1(1.8)
1001-4000	0
>4000	1(1.8)

Table 4.2 The level of parasitaemia in non khat chewer patients on days 0 and day 3 of treatment with CQ.

Level of parasitaemia (parasites/µl blood)	No. and (%) of patients
Day-0 Values	
1-400	11 (23.7)
401-1000	8 (17.3)
1001-4000	13 (28.2)
4000-200,000	12 (26.6)
> 200,000	2 (4.2)
Parasite density range 48-256,800 (per µl)	
Day-1 > Day-0 Values	9 (19.6)
Day-2 > Day-0 Values	7 (15.2)
Day-3 Values	
0	33 (71.7)
1-400	11 (23.9)
401-1000	0
1001-4000	0
>4000	2 (4.4)

The parasite density at enrolment ranged from 48-256,800 and 32-111,760 asexual parasites /µl blood in non khat and khat users respectively. No danger signs of sever malaria was recorded for all subjects. Geometric mean of asexual parasites densities in khat and non khat chewing subjects were determined for each day. The geometric mean parasite densities at enrolment were 1494 (95% CI (771-3837)) and 1861(902-2890) /µl of blood and were 3 (1.7-5.2)/ µl and 4 (1.9-7.9)/µl of blood on day 3 for khat and non khat users respectively (**Table 4.3**). Statistically, there was no significant difference in the mean of parasites density and in the axillary temperature between khat and non khat chewers before treatment and on day 3 ($p = 0.65, 0.59$).

Table 4.3 Parasite density and axillary temperature at enrolment and on day 3 of treatment in khat and non khat chewer malaria patients.

Study group	Geometric mean parasitaemia/µl blood		Mean axillary temperature (°C)	
	Day 0	Day3	Day 0	Day3
Khat chewers (n= 57)	1494 (771-3837)	3 (1.7-5.2)	37.5 (0.54)	37
Non khat chewers (n= 46)	1861 (902-2890)	4 (1.9-7.9)	37.4 (0.66)	37
	P= 0.65	*P=0.56*		

N.B: parasite densities using the geometric means (95 % CI), no statistically significant differences were observed on days 0 and 3 (t-test, p = 0.65 and 0.59 respectively).

There was also no statistical significant differences in parasite clearance between long term khat users (more than 2 years) and short term khat users (less than 2 years) (*p= 1.0*). 74 .4 % and 78.6% of long term users and short term users were cleared of parasites by day 3 respectively (**Table 4.4**). The association between parasite clearance and chewing of khat was not statistically significant (r = 0.042, p = 1.0).

Table 4.4 The effect of khat habit and history of chewing khat on parasite clearance.

Khat habit	**Parasite clearance**	
	cleared	Not cleared
Chew khat (n= 57)	43 (75.4%)	14 (24.6%)
Not chew khat (n=46)	33$^{\square}$ (71.7%)	13 $^{\square}$(28.3%)
Long term users (n=43	32 (74.4%)	11(25.6%)
Short term users (n=14)	11*(78.6%)	3*(21.4%)

Long terms users = more than 2 years, short term users less than 2 years.
Fisher's exact test, $^{\square}$p= 0.82., *p= 1.0

The results from this investigation indicated that neither based on chewing of khat during treatment nor a history of using khat had significant effect on parasite clearance ($p = 0.82$ and 1.0 respectively) although khat chewers had lower parasitaemia at the enrolment and had higher level of parasite clearance by day 3 than non khat chewers (**Table 4.3 and 4.4**).

4.3.2 Response to chloroquine (CQ)

CQ effected a general reduction in the counts of asexual parasite in most of the cases. To examine the therapeutic response of CQ with and without khat, treatment response was interpreted by two ways. First, outcome treatment was classified according to 7-day standard WHO protocol (WHO, 1973). The responses to treatment are summarized in **Table 4.5**. Of 103 microscopically positive cases of *P. falciparum* malaria, 61 (59.2%) (parasitaemia was cleared completely by day 2 of treatment) was classified as sensitive/RI. Using parasitological definition of resistance, the remaining 42 patients (40.8 %) showed parasite resistant of CQ therapy for uncomplicated *P. falciparum*. In non khat chewers, 27 (58.7 %) patients were completely cleared on day 2, while 19 (41.3 %) showed parasitological failures. However, in khat chewers, CQ was effective in 34 (59.6 %) of 57 cases, and 23 (40.4%) were not cleared. This indicated that the CQ was more effective in khat chewers than in non khat chewers, but this difference was not statistically significant (fisher's Exact test = 2.0, p = 0.39). Of the 46 non khat chewer patients, resistance was detected in 19 patients; 10 (21.7 %) moderate grad (RII); and 9 (19.6 %) high grad (RIII). While of 57 khat chewers, RII was detected in 17 cases (29.8 %)

and RIII was 6 (10.6 %) respectively. Statistical analysis of these results showed that there was no significant difference in level of the overall resistance between the two groups (p = 0.39) (**Table 4.5**). RII resistance level is higher than RIII resistance level in both groups. Second, according to WHO 1996 protocol classification, therapeutic responses are shown in **Table 4.6.** All subjects had no symptoms of fever or sever malaria and danger signs. However, 26.2 % did not clearer parasites by day 3 and considered as early treatment failure (ETF).

Table 4.5 *In vivo Plasmodium falciparum* response to CQ in khat and non khat chewers using WHO 1973 protocol.

Study group	**Parasitological outcome patient No. (%)**			
	Sensitive/RI	RII	RIII	**Resistant**
Khat chewers (n = 57)	34 (59.6)	17 (29.8)	6 (10.6)	**23 (40.4)**
Non khat chewers (n = 46)	27 (58.7)	10 (21.7)	9 (19.6)	**19 (41.3)**
Total (n = 103)	**61 (59.2)**	27 (26.2)	7 (14.6)	**42 (40.8)**

R= resistance level Fisher's Exact test = 2.0, p = 0.39, r = 0.06, p= 0.5.

Table 4.6 *In vivo Plasmodium falciparum* response to CQ in khat and non khat chewers using WHO 1996 protocol.

Therapeutic Response	**Khat chewers n= 57 (%)**	**Non khat chewers n= 46 (%)**	**Total**
Day 3			
Treatment Success/ LTF	43 (75.4)	33 (71.7)	**76 (73.8)**
Early treatment Failure	14 (24.6)	13 (28.3)	**27 (26.2)**

Fisher's Exact test (2-sided) = 0.18, p = 0. 82

Figure 4.2 and Figure 4.3 show the mean of the daily parasite density, plasma CQ concentration and parasitological response to CQ respect to time in khat and non khat users. Based on the initial parasitaemia on day 0, the parasite density in most of the patients was cleared by more than 75 % by day-2 or day-3 in both study groups. In some of the subjects, parasites were not cleared completely by day 3 of treatment although the mean CQ plasma level was above the therapeutic level for *P. falciparum* suppression (≥ 16 μg/L) (Wetsteyn *et al.*, 1995). In **chapter 3**, patients who did not chew khat had the higher mean plasma CQ concentration, but at the same time they had higher parasitological failure. However, malaria patients who chewed khat during treatment showed a reduction in CQ levels when compared to patients who did not chew but had lower parasitological failure.

The results in this investigation indicated that CQ efficacy in patients who chewed khat was not significantly different from those who did not chew khat during treatment although khat chewers showed higher proportion of sensitivity and treatment success and lower mean parasite density than non khat chewers.

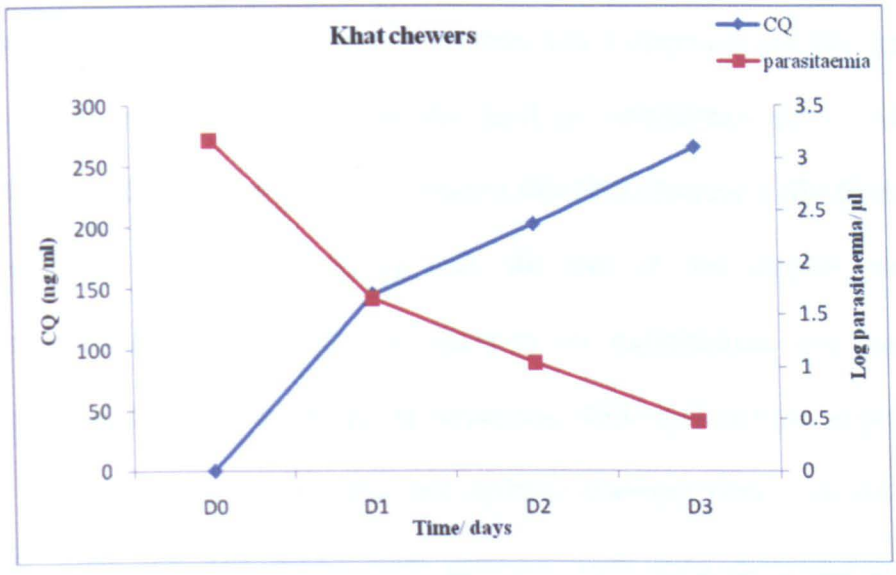


Figure 4.2 Mean parasitaemia and mean of plasma CQ concentrations in relation to time (days) in khat chewer malaria patients.

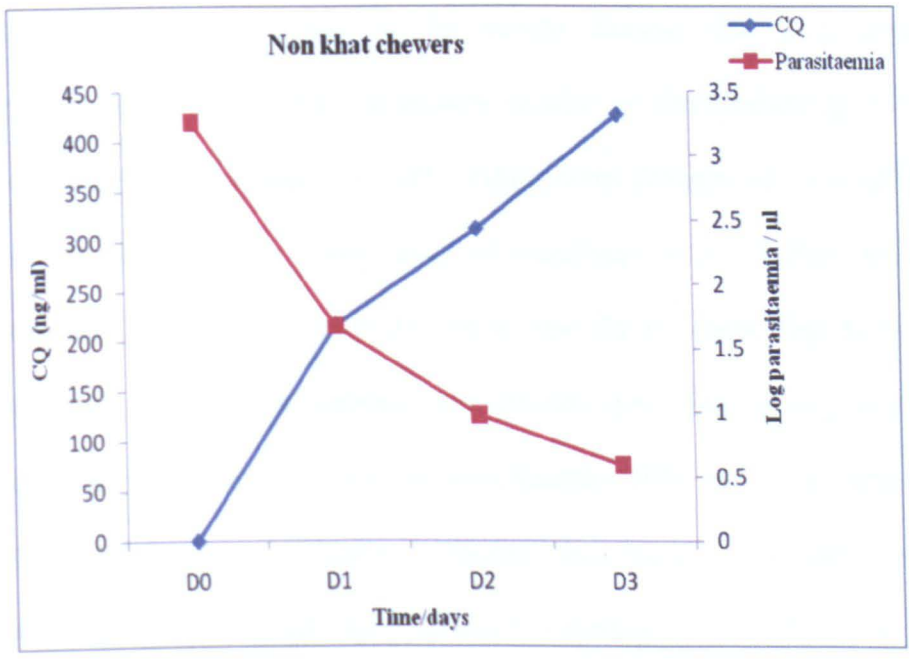


Figure 4.3 Mean parasitaemia and mean of plasma CQ concentrations in relation to time (days) in non khat chewer malaria patients.

4.4 DISCUSSION

Chewing of khat during sickness or treatment is a common practice among khat users because khat is commonly used as antipyretics and to relieve fatigue. In addition, most khat users believe that khat chewing make them feel better during sickness. Based on that, the aim of this chapter was to investigate the influence of khat chewing on parasitaemia and parasite clearance in malaria patients during treatment. This work compared patients who were treated with CQ with and without chewing khat. The standard WHO 7-day test and WHO 1996 protocol were used in evaluating the treatment outcome.

Using either of these protocols, the results showed that there was no significant differences neither on parasite density at ennoblement ($p = 0.59$), nor on therapeutic outcome ($p = 0.82$), although the patients who chewed khat during treatment had a lower level of resistance and a higher level of treatment success when compared to those who did not chew khat. However, plasma CQ levels in the patients who did not chew khat during treatment were higher than those who chewed khat (**section 3.3**), but at the same time the treatment failure was found to be higher (28.3 %) when compared to khat chewers (24.6 %) although not statistically significant ($p = 0.82$). This may indicate that khat has a potential effect on parasite clearance, however, immunity, drug resistance, fluctuation in parasite density as well as high density of parasitaemia may contribute to this treatment failure (Mockenhaupt *et al.*, 2000). The parasitaemia is extremely variable among the study subjects

(range from 79-287,000/µl of blood) and it would be expected that the drug more easily eradicates small numbers rather than large number of parasites. In addition, *in vivo* studies are affected by many factors related to host, such as absorption deficit due to the malnutrition (Tulpule, 1983a) or drug interactions (Mahmoud *et al.*, 1994).

Khat has many chemical compounds which contain variety of alkaloids flavonoids, and tannins. Several studies reported that some plants contain alkaloids as one of their constituents showed anti-malarial activity and antimicrobial activity (Francois *et al.*, 1995, Garavito *et al.*, 2006). Large number of studies examined alkaloids, flavonoids, and tannins for their anti-malarial activity *in vivo* and *in vitro* against CQ-sensitive and resistant strains of *P. falciparum*. These activities were considerable and comparable with CQ activity (Garavito *et al.*, 2006, Muregi *et al.*, 2003, Tona *et al.*, 1999). This means, khat leaves might possess a mild anti-plasmodial activity as we observed differences in treatment outcome between khat and non khat chewers although not significant. The results given in **chapter 5** suggest that some of khat extracts may have a very mild anti-malarial activity against *P. falciparum* though it is not clear that an effect will be seen *in vivo*. In addition, one of the studies on antimicrobial effects of khat showed that two isolated compounds from khat leaves, which were identified as 22 ß-hudroxytingenone and tingenone have shown to possess antimicrobial activity against certain bacteria species (Al-Hebshi, 2005a).

The study was designed to determine the effect of khat on parasitaemia and the response to CQ with and without khat. The response was classified as cleared or not cleared on the third day of treatment. Additionally, our objective was not to assess the therapeutic efficacy of CQ and the study was not designed to determine the frequency of therapeutic failure to CQ or CQ resistance. However, during data analysis we found useful information to determine the level of CQ-resistant parasites although we could not apply the follow-up observation period recommended in these WHO protocols (1973 and 1996).

The standard WHO 7-day test (WHO, 1973) may be more appropriate to use in this study because the short period of follow-up observation. This standardized protocol has been used for many years to monitor CQ resistance by *in vivo* trials, which depends on parasitological treatment outcomes only. The resistance in malaria was classified as S (sensitive), RI, RII, and RIII (resistance level) based on the reduction of initial parasitaemia or clearance on day 2. Low grade, RI resistance level (clearance of parasite on or after day 7 followed by recrudescence) was not determined because patients were followed for 3 days, therefore S/RI outcome were combined into one category. This protocol was modified and replaced in 1996 by WHO which based on clinical and /or parasitological outcome. We have used these two protocols to evaluate the effectiveness of CQ based on the available data.

Based on WHO 1973 classification of resistance to anti-malarial drugs, our results showed high proportion of resistance to CQ (RII and RIII), 40.8 % in Bajil city in Al-Hudaydah governorate in the west of Yemen (where the study was carried out) where malaria is endemic and seasonal. Comparing the results obtained from this study with results of previous studies is difficult because of the variability in the period of follow-up, type of subjects recruited and definition of the outcome. However, our results were similar to the previous reported results on CQ resistance that was carried out in Bajil city in 2002 with treatment failure of 43% (NMCP, 2005).

The alternative protocol, has been used, in which treatment outcomes includes both clinical and parasitological criteria (WHO, 1996). In this protocol, the parasitological resistance does not necessarily imply therapeutic failure. The clinical success was in 73.8 % of the cases. This indicate that clinical success was high but parasitaemia still evident in 26.2 % of the cases by day 3 (classified as early treatment failure (ETF)) indicating resistance to CQ. Our results were lower than the recent reported incidence of resistance of *P. falciparum* malaria to CQ in Al-Musaimeer district (Mubjer, 2006, NMCP 2003). However, in our study, treatment success was overestimated and late treatment failure was underestimated due to 3-day observation period instead of 7, 14 or 28 days. This is one of the limitations of the study; therefore a study should be conducted in which patients are followed up for 28 days.

The problem of CQ resistance in Yemen is gradually worsening and has increased both in prevalence and grade (Abdel-Hameed, 2003). Few studies have investigated CQ resistance. Most of the studies on anti-malarial drug efficacy conducted *in-vivo* have been non-systematic and used a test protocol developed only to evaluate the parasite response to treatment rather than evaluating the therapeutic outcome (WHO, 1999). These studies were based on the standard WHO 7-day test. The detection of first indigenous cases of *P. falciparum* resistance to CQ was in 1989 (Mamser, 1989). Several studies have been conducted in different areas in the country and demonstrated variable degree of resistance. 3 % early RI/RII, 17% early RI/RII and 12.9 % R/ RII were detected in Taiz (1987), Al-Hudaydah (1991), and Lahj (1995) respectively (NMC, 2002). A recent study on 122 patients in Al-Musaimeer district, which is considered as a sentinel site in the country, reported 56.1% CQ treatment failure (NMCP, 2003). Another two studies were conducted in Bajil city and Al-Odein. In Bajil city, the treatment failure was 43 % (2002) and in Al-Odein was 46 % (2003) (NMCP, 2005b). Recently, an assessment of the therapeutic effect of chloroquine for uncomplicated *P. falciparum* malaria in Al-Musaimeer district in Lahj governorate in the south of Yemen found a high rate of CQ treatment failure, 60.7 %, which confirms the serious extent of CQ resistance in Yemen (Mubjer, 2006).

4.5 CONCLUSION

The work presented in this chapter has showed that the treatment failure (poor response to CQ) was not associated with khat chewing. Also using khat during treatment showed no significant affect on parasitaemia ($p > 0.05$), but khat chewers had a lower parasite density and lower parasitological failure. This indicates that khat might have some effect on parasite clearance but this cannot be simply explained. The quantitative effect of khat on parasitaemia and parasite clearance is not known and is not predictable at an individual level. Because of this uncertainty and the lack of information on the amount that each person has chewed, it would be prudent to advice people not to chew khat during treatment as we found significant evidence of its effect on reducing CQ concentration in both volunteers and in malaria patients ($p = < 0.05$ (**chapters 2 and 3**). Therefore, the results in this investigation provide the basis for further investigation on khat-CQ combination effect on malaria parasites.

The WHO threshold for treatment failure was classified as: grace (0-5%), alert (6-14 %), action (15-24%) and change (≥ 25%) in order to guide the anti-malarial drug policy. Our findings showed that the overall treatment failure was higher than 25 %, a level considered unacceptable by WHO (WHO, 2002a). Based on these results and the previous review on CQ resistance in Yemen, the frequencies of parasitological failures following CQ treatment are indicative of the continuous emergence of CQ resistance strains of *P. falciparum.* Thus, the need for action to be taken to address the problem

in this region and most likely in other parts of the country has become very urgent.

CHAPTER 5

IN VITRO TESTING OF ANTI-MALARIAL ACTIVITY OF KHAT EXTRACTS

5.1 INTRODUCTION

Despite all the efforts to eradicate malaria, the disease is remains one of the most important health problems facing the tropical and subtropical regions. *P. falciparum*, the most virulent species of human malaria is becoming increasingly resistant to cheap standard anti-malarial drugs (Plowe *et al.*, 2001). Therefore, there is need for continuous effort to search for new anti-malarial drugs. Plants-derived medicines continue to play essential role in health care. Although little is known about the treatment seeking behaviour of patients with malaria, WHO estimates that 80% of people in developing countries rely on traditional medicines (WHO, 2002).

The use of herbal preparations in the treatment of malaria is popular in many parts of Africa and some of other developing countries where malaria infestation is endemic. Plants have formed an important and a rich source of drugs against malaria. Amongst the major anti-malarial drugs in use today are quinine and artemisinine were either originate from plants or developed using their chemical structures as templates (Gessler *et al.*, 1994, Muregi *et al.*, 2003). In addition, various phytochemical constituents have been isolated from plants and demonstrated to possess anti-malarial properties. Some of these include alkaloids, flavonoids such as quercetin and rutin (Nokov, 1982), limonoid from *Khaya senegalensis* and gedunin from *Azadirachta indica*, which have been reported to be as effective as quinine in malaria infected cell cultures (Khalid, 1986, Khalid, 1989).

Khat is a plant widely used in Yemen as a recreational drug and it is also used as antipyretics. Despite the overwhelming number of studies on the anti-malarial activities and anti- microbial activities of plants and natural products derivatives, there is some information on the ethnopharmacological use of khat and its anti-microbial activity but non about its anti-malarial activity. In addition, an *in vivo* study is relevant to the results obtained in **chapter 4**, in which khat chewers showed a lower parasitaemia and a lower level of resistance to treatment than non khat chewers ($p > 0.05$). Therefore, this study was carried out to investigate the potential anti-malarial activity of khat extracts against CQ-sensitive and resistant strains of *P. falciparum in vitro.*

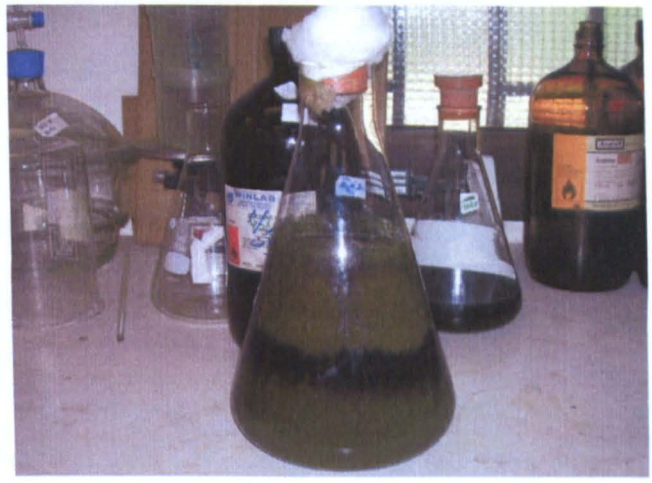
5.2 MATERIALS AND METHODS

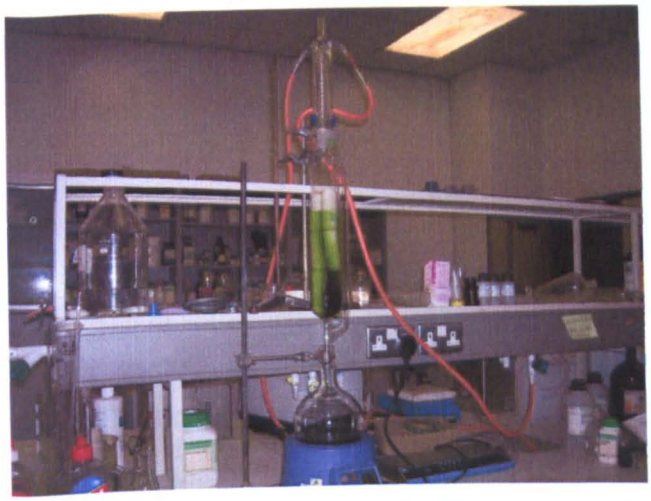
5.2.1 Preparation of khat extracts

The khat used in this study was one of the commonest types in Yemen, known locally by Dula'ee (cathinone concentration 255.3 mg/ 100 g and tannic acid 7.46 mg/100 g in fresh khat leaves) (Al-Motarreb, 2002a). Khat leaves were obtained from the local market and it was extracted with the following five solvents using the Soxhlet method; 1) petroleum ether (Boiling point (B.P) 30-40 C, BDH, Poole, England); 2) chloroform (B.P. 61 °C, GRG, Reagent Fine UK.); 3) acetone (B.P. 55-56 °C, Analar, Uk.); 4) ethanol (B.P. 78-79 °C Fluka-Chemika Swiss Fedral AC); and 5) distilled water (**Figure 5.1**).

A total weight of 1kg of freshly picked shoots, soft and small branches of khat leaves were dried at room temperature for 1 week and then grounded in a laboratory blender (Waring Commercial Products, New Hartford, Conn. USA). The resultant powder, (289.76g, 72% water) was soaked in petroleum ether for 24 hours. The solution was filtered through Whatman filter paper (Whatman No. 24 cm, Hardened 54) and the residue was added to the thimble of the Soxhlet apparatus. The extraction was carried out sequentially with 2000 ml of solvent for 12 hours starting with the petroleum ether used for soaking followed by chloroform, acetone, ethanol and distilled water. The extraction process was repeated until clear extract was obtained. Each extraction was dried by rotary evaporation under vacuum at 37 °C. After evaporation of the solvents in the vacuo, 1 kg of khat leaves gave 4.85g, 2g,

13.09g, 45.69g and 48.45g of petroleum ether, chloroform, acetone, ethanol, and distilled water respectively and the dried residue extracts were collected by dissolving it in the representative solvents and left in a dark place to evaporate at room temperature. All the extracts were freeze dried to ensure dryness of the compounds and stored at −20 °C until required.





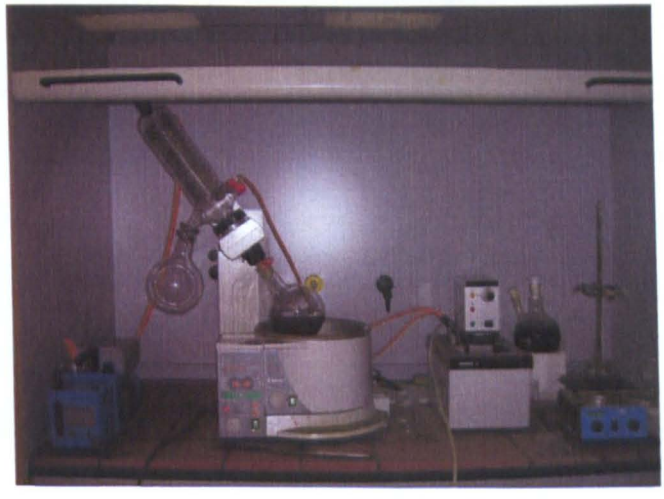


Figure 5.1 khat extraction process using Soxhlet method.

5.2.2 Malaria cell culture

Antiplasmodial activity of the khat extracts was determined against CQ-sensitive strain (3D7) and CQ-resistance strains (Dd2 andV1/S), kindly donated by Professor Steve Ward, Department of Molecular and Biochemical Parasitology, Liverpool School of Tropical Medicine. Khat extracts were prepared at different concentration and tested for anti-malarial activity.

5.2.2.1 Cultivation of malaria parasites, *P. falciparum*

Malaria strains were cultivated and maintained in complete media (CM) according to the method of Trager and Jensen with minor modification (Trager & Jensen, 1976). Asexual cultures were grown in fresh group O-positive human erythrocytes which suspended in RPMI 1640 containing 25 mM HEPES, 0.02 mg/ml gentamicin, (Sigma®) and 10 % v/v human serum in T 75 cm^2 culture flask (Nunc™). Cultures were gassed for 1 min with a gas mixture of 3 % O_2, 4 % CO_2, and 93% N_2, (British Oxygen Special Gases, U.K.) and incubated for 24 h at 37°C. The serum used in CM was supplied by Royal Liverpool Hospital, UK and thawed out for 15 min in 37 °C water bath.

At least two full life cycles (96 h) were completed before the parasites were used for the assays. The level of parasitaemia of the culture was measured by light microscopy following 10 % Giemsa staining. In addition, the malaria parasites were sub-cultured to keep them within the wanted limits. After determining the parasitaemia via blood film, parasite suspension was centrifuged at 2000 rpm for 5 min. Then the supernatant was discarded and

pellets were diluted accordingly with uninfected red blood cells (UIRBCs) to yield the desired hematocrit then the culture was maintained as above.

5.2.2.2 Synchronization of parasites

Synchronization was performed in 5 % w/v sorbitol (Lambros & Vanderberg, 1979) to generate cells in the ring stage of asexual life cycle. The culture was centrifuged at 2000 rpm for 5 min, and the supernatant was removed and 5ml of 5 % w/v sorbitol was added, the culture incubated for 20 min at room temperature. The culture was then centrifuged at 2000 rpm for 5 min, supernatant was removed and the pellets were re-suspended in 5 ml of CM followed by another centrifugation. After removing the supernatant, the culture was re-suspended with 35 ml of CM and parasites were cultured as detailed in **section 5.2.2.1.**

5.2.2.3 Preparation of parasite inoculums

The cultures were diluted in CM containing UIRBCs to yield a final hematocrit of 1 % and 2% parasitaemia before using in IC_{50} assay. Parasite inoculums was prepared by adding 100 µl of infected red blood cells (IRBCs) and URBCs to 10 ml of CM by using the following the formula to get 2% of parasitaemia and 1 % hematocrit.

IRBCs = desired parasitaemia / actual parasitaemia x volume required

UIRBCs = 100- IRBCs

5.2.2.4 Preparation of uninfected Red Blood Cells (UIRBCs)

Group O-positive human blood was supplied by the regional Blood Transfusion Centre, Liverpool U.K. and it was tested against infectious deceases. The RBCs was centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the buffy coat layer was removed. RBCs was resuspended in 30 ml of RPMI 1640 medium (Sigma®) and washed two times in the same media and centrifugation conditions and stored at 4°C and used within one week.

5.2.2.4 Preparation drug stock solutions.

Stock solutions of 10 mM of artemether (Novartis, Switzerland, from Dr. Paul O' Neil, University of Liverpool) (MW 298.38) and 10 mM of CQ (Sigma®) (MW 515.9) were made in dimethylsulfoxide (DMSO) (Sigma®) and 50: 50 ethanol and water respectively. The artemether was used to establish the base line of complete inhibition of parasite growth and 10 mM CQ was used as a standard in the IC_{50} test. Working drug solutions were prepared by further dilutions of stock solution in CM to obtain 200 μM. All khat extracts except the water extract were dissolved in DMSO at the concentration of 10 mg/ml. The water extract was dissolved in 50:50 water and ethanol. Each extract was further diluted in CM to obtain a stock solution of 2 mg/ml. The solvent concentrations were < 1 %. All the stock drug solutions were stored at −20°C until used.

5.2.2.5 Plate set up

The following 3-fold serial dilutions to make 8 concentrations range of CQ and artemether (1000nM to 0.4123 nM) and khat extracts (60 to 0.028 μg/ml) were prepared. 50 μl each of the diluted drug concentration were dispensed in triplicate into 96-well microtiter plates (Microwell, Nunc, U.K.), followed with 50μl of the parasite inoculum (2% parasitaemia, 1 % hematocrit). Artemether was used at high concentration (1000 nM) to ensure complete parasite death and served as negative control in well number 1. 50 μl of CM was placed into a column of wells number 6 and 11 with no drugs and served as positive control. The plates were mixed with agitation and placed in a humidified incubation chamber (Flow, UK); gassed for 3 min and incubated at 37°C for 48 h.

5.2.2.6 Determination of IC_{50}

After 48 h of the parasites growth, 100 μl of fluorescence dye SYBR Green (0.2 μl of SYBR Green I /ml of lysis buffer. The lysis buffer contained Tris (20 mM, pH 7.5); EDTA (5 mM); Saponin (0.008 % (w/v); Triton X-100 (0.08% v/v) (Bennett *et al.*, 2004) was added to each well and the contents were gently mixed and then incubated at room temperature in the dark for 1 hour. After incubation, the plates were read at 485 nm excitation, 535 nm emissions in Varioskan fluorescence reader (Thermo Electron Corporation, Finland) equipped with a dispenser running SkanIt®. Fluorescence values were calculated and averaged using Excel programme and values were expressed in relative fluorescence units. IC_{50} values (Concentration of CQ and

khat extract required to inhibit growth by 50%) were determined by plotting concentration versus percentage inhibition using Gra-FIT Computer Programme version 3.0 (Kent.UK). Experiments were conducted with at least 3 replicate on more than three independent occasions.

5.3 RESULTS

Khat extracts were prepared from fresh khat leaves and extracted with 5 different solvents. All extracts were screened for *in vitro* anti-plasmodial activity against the CQ-sensitive *P. falciparum* strains (3D7) and resistance strains (Dd2 CQ resistance and V1S, multi-drug resistance).

5.3.1 Antiplasmodial activity against sensitive strains

All the extracts, petroleum ether, chloroform, acetone, methanol, and water, were first tested at a concentration of 10, and then 20 μ g /ml. All the extracts showed no activity at these two concentrations. We also used a higher concentration of 60 μ g /ml. All khat extracts showed insignificant activity against CQ-sensitive *P. falciparum* strain (3D7), except with chloroform extract and water extract. **Figure 5.2** and **5.3** show the inhibition effect of chloroform and water extract on parasites growth. The chloroform extract showed mild activity against 3D7 strain with an IC_{50} of 21.5 (9.8) μg/ml and water extract showed a comparatively IC_{50} of 37.1 (10.1) μg/ml (**Table 5.1**). This indicates that chloroform and water extracts have some effect on parasite growth.

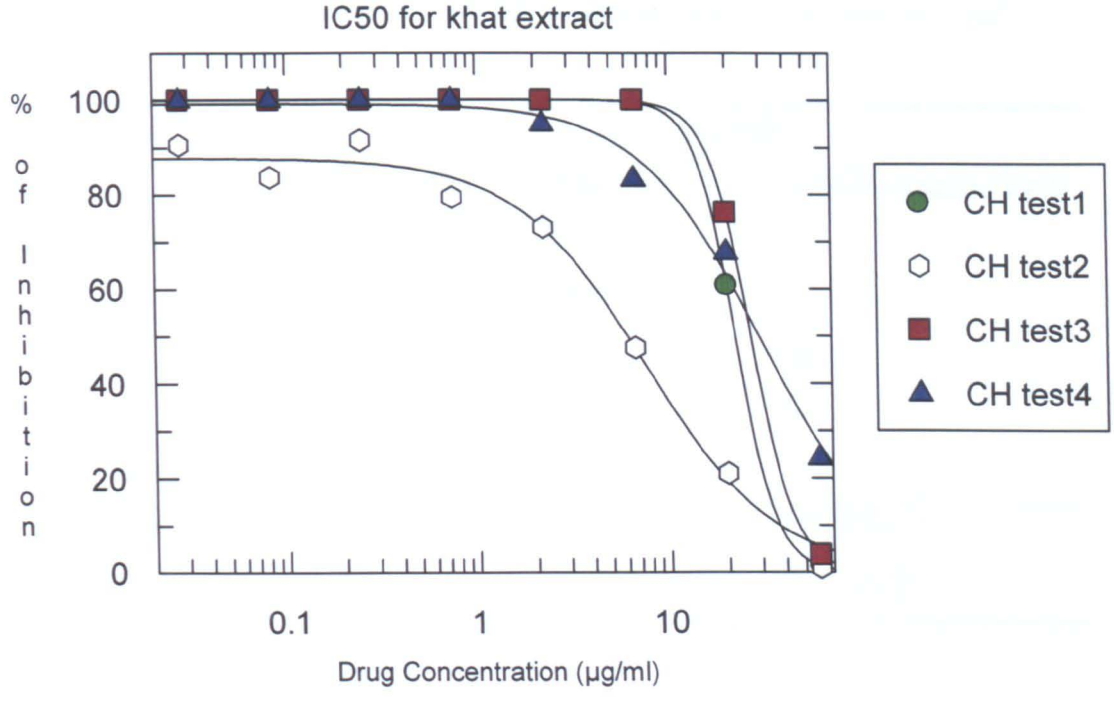


Figure 5.2 Effect of khat chloroform extract on growth inhibition for CQ-sensitive strain 3D7 in four different tests.

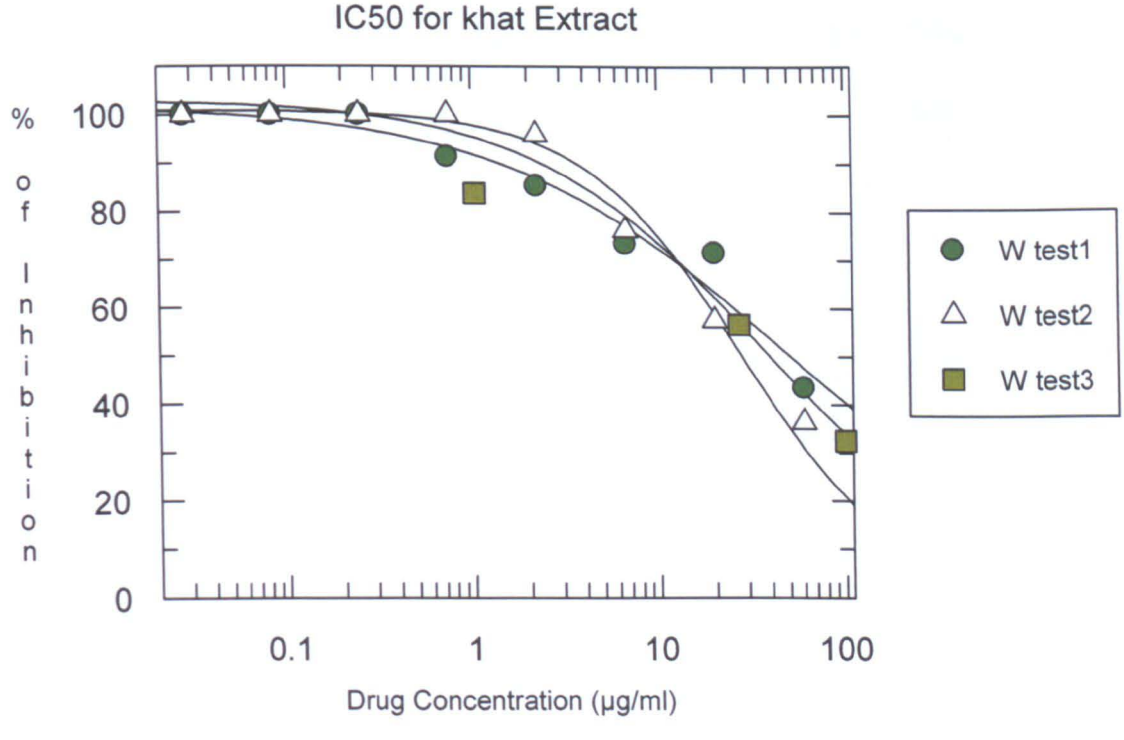


Figure 5.3 Effect of khat water extract on growth inhibition for CQ-sensitive strain 3D7 in 3 different tests.

Table 5.1 *In vitro* the mean IC_{50} for khat extracts against CQ-sensitive and CQ-resistance strains.

Khat extract	**IC_{50} (µg/ml)**		
	CQS 3D7	**CQR Dd2**	**Multi-resistance V1/S**
Petroleum ether	> 60	ND	ND
Chloroform	21.5 (9.8)	> 60	> 60
Acetone	> 60	ND	ND
Ethanol	> 60	ND	ND
Water + ethanol	37.1 (10.1)	> 60	> 60
Standards CQ (nM)	11.6 (2.2)	163.4 (20.7)	509.3 (16.1)
CQ (µg/ml)	6 (1.2)	84.3 (29.2)	262.6 (8.2)

IC_{50} = 50 % inhibition of concentration, values are in mean (SD).
CQ was included as positive control.
CQS= CQ sensitive strain, CQR= CQ resistant strain.
ND = not done

CQ functioned well as positive control, it had IC_{50} value of 11.6 (2.2), nM against CQ-sensitive strain (3D7) **(Figure 5.4)**, and 163.4 (20.7), 509.3 (20.7) nM against CQ-resistance strains (Dd2 and V1S) of *P. falciparum* respectively **(Table 5.1)**.

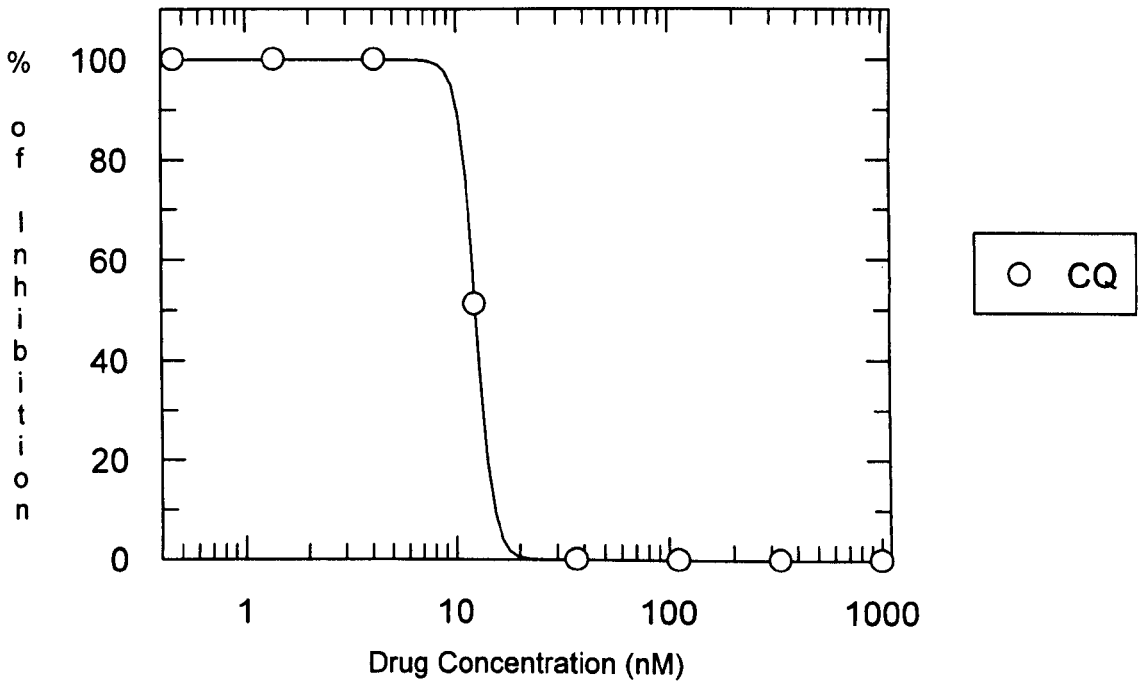


Figure 5.4 Growth inhibitions for CQ-sensitive strain 3D7.

5.3.2 Antiplasmodial activity against resistant strains

In the preliminary results, from all khat extracts tested against 3D7, a CQ-sensitive *P. falciparum*, chloroform and water extract showed mild activity with an IC_{50} of 21.5 and 37.1 µg/ml respectively. These extracts were further assayed against Dd2 and V1/S, CQ-resistance strains. None of the plant extracts showed any appreciable activity (IC_{50} > 60 µg/ml) **(Table 5.1)**.

The results from these experiments indicated that chloroform and water extract have mild anti-malarial activity against the sensitive strain of *P. falciparum* (3D7) but no activity was found against the resistance strains (Dd2 and V1/S). Further investigation is needed to isolate and purified these substances and tested for their anti-malarial activity independently.

5.4 DISCUSSION

The aim of this study was carried out to investigate the potential effect of leaf extract of khat on drug sensitive-and resistance strains of *P. falciparum*. *In vitro* study was conducted as a way of elucidating the anti-malarial activity of khat extracts in different solvents. Results presented in this work showed some crude extract possess constituents capable of inhibiting to different degrees, *P. falciparum* growth *in vitro*. This mild antiplasmodial activity of water and chloroform extracts is worth exploration *in vitro* for further study. This may have relevance to the result seen in khat chewers who had lower levels of resistance when compared to non khat chewers (**chapter 4**).

Khat extracted showed no anti-malarial activity when tested at concentration of 10 or 20μg/ml, while at a concentration of 60 μg/ml, chloroform and water extracts had mild and weak potency against CQ-sensitive strain 3D7 respectively. The concentration of the extracts used was very high, it was not possible to increase the concentration further. The question arises whether this effect was from the effect of khat components or from the solvents toxicity effect since DMSO concentration exceeded 1 % at the final concentration. In order to address this question and rule out the interference or contribution of DMSO in the growth inhibition, 60 μg/ml of DMSO was tested against 3D7 strains along with the two extracts. The result showed that there was no effect of DMSO on the parasite growth at this concentration.

This is the first study on the anti-malarial activity of khat; therefore, it is not possible to compare the results obtained from here with other studies. However, in some plant extracts, like alkaloids, tannins and flavonoid compounds have been shown to possess, at different levels, antiplasmodial activity in both *in vitro* and *in vivo* tests (Likhitwitayawuid *et al.*, 1993a, Likhitwitayawuid *et al.*, 1993b, Ratsimamanga-Urverg *et al.*, 1992, Wright *et al.*, 1991). Therefore, it is plausible that the antiplasmodial activity displayed by chloroform and water extracts could be related to the presence of these compounds which are found in khat leaves (Al-Motarreb, 2002a). Isolation and characterization of these compounds followed by purification should be further investigated to identify their antiplasmodial activity.

The question may arise as to whether khat leaves are used as a remedy, for example, in traditional medicine as an anti-malarial plant. In the early Arab literature, khat was mainly used for alleviating the sensations of hunger and fatigue among soldiers. In the beginning of this century, several attempts were made to introduce tonic preparations based on khat, and a patent has been granted on a khat extract designed for medical and alimentary us (Kalix, 1990a). In the countries where khat is cultivated and widely used, the leaf is used in folk medicine, for example as a remedy for asthma and as an antipyretic (AL-Meshal *et al.*, 1985a, Kalix, 1990a) an indication that may suggest the sympathomimetic properties of the leaf. Nonetheless, khat is not mentioned in a compendium of Yemen traditional remedies that was published some years ago (Kalix, 1990a, Schopen, 1983). In addition to

these purposes, nowadays, khat is commonly used by people who engage in strenuous physical work and as a recreational drug (Kalix, 1990b). Chewing of khat has a stimulating effect, and has been shown to induce a state of mild euphoria and excitation (Kalix, 1992). Although there is no information in the literature that khat has ever used as an anti-malarial agent, the chloroform and water extracts are of special interest for further investigation of their anti-malarial activity.

Using khat leaves as anti-malarial compound, we have to consider the amount of khat that can be consumed by a person. The intake of khat is self-limiting because of its bulkiness, and due to the fact that the leaves are not a standardized material, the potency of which may vary considerably from batch to batch and from type to type. In addition, there are certainly large inter-individual variations in the mastication efficiency and in the absorption of the active ingredients and there is probably also a limit to blood levels that can be attained during chewing, since the active compounds may be metabolized rather rapidly while its absorption is delayed by the mastication process. It is difficult to relate the *in vitro* studies to the situation *in vivo*; however, the following considerations are interesting. From the extraction process with water, 1 kg of leaves yield about 48.45 g of dried material, so individuals might consume 10 g of water extract which might be enough to inhibit parasitaemia as indicated in **chapter 4.** However, we have no information regarding the volume of distribution of these compounds. Therefore, it would be ironic if khat, which is considered a plant of abuse,

turned out to be plant with effective medical properties. However, we cannot relate the concentration used in *vitro* to the amount of khat consumed by person or in *vivo*.

5.5 CONCLUSION

Growth inhibition of *P. falciparum* chloroquine-sensitive strains *in vitro* was found using water and chloroform extracts of khat with IC_{50} ~ 20-40 μg/ml. Chloroform extract was found to be the more potent khat extract against *P. falciparum* (3D7) CQ-sensitive with the water extract demonstrating the least potency of activity. However, both khat extracts showed no anti-plasmodial activity against CQ-resistance strains (IC_{50} > 60μg/ml). This mild potent effect of khat on CQ-sensitive strain may not qualify the use of this plant as a traditional anti-malarial plant and its antiplasmodial activity *in vitro* may not necessarily have the same effect *in vivo* since khat plant undergoes metabolic changes, also plant extracts may display *in vitro* activity which is not seen *in vivo* (Gessler, 1995c) or vice versa. The two khat extracts seem to be of particular interest for further investigations focus on separation and identification of the major active component for anti-malarial activity *in vitro* and in animal models to support the preliminary results in this study.

CHAPTER 6

SUMMARY AND RECOMMENDATIONS

6.1 SUMMARY AND RECOMMENDATIONS

Khat (*Catha edulis* Forssk, Celastraceae) is a plant widely used in Yemen and in East Africa. The fresh young leaves of this plant are commonly chewed for several hours to produce stimulating amphetamine like effects, alleviate hunger, relieve fatigue, improve performance, and to increase work capacity. The habitual use of khat is a common practice even during sickness and is associated with social, economic and medical problems. Malaria is also a major public health in Yemen; approximately 60 % of the population is at risk of malaria (NMCP, 2005). Chloroquine (CQ) is still the first line drug for the treatment of uncomplicated *Plasmodium falciparum* malaria.

Many studies have focused on the pharmacology of khat and on the effects associated with the khat chewing habit. However there is no information in the literature concerning khat–drug interactions. Drug-interaction occurs when one drug either alters the concentration (pharmacokinetic interactions) or the biological effect of another drug (pharmacodynamic interactions). This interaction occurs by administration of food, herbs and other drugs. Pharmacokinetic interactions can occur at the level of absorption, distribution, or clearance of the affected drug (Leucuta, 2006).

The consumption of khat and its effect on drugs concentration and its effect on malaria parasites has not been investigated. Therefore, the overall aim of this thesis was to examine the effect of khat on anti-malaria chemotherapy. To address this aim, the study was conducted in four parts. First, to evaluate

CQ pharmacokinetic parameters in healthy volunteers and secondly in malaria patients. Thirdly, to investigate the potential effects of khat on parasitaemia and parasite clearance in *P. falciparum* malaria patients. Finally, to examine the effect of khat extracts *in vitro* on drug sensitive-and-resistant strains of *P. falciparum.*

All the objectives of the study were achieved and since this is the first study of CQ pharmacokinetic and anti-malarial effect of khat, these novel results may be used as reference for future studies of CQ and drug interaction in Yemeni individuals. The focus of **chapter 2** was to elucidate the pharmacokinetics of CQ following a single oral dose with and without khat in fifteen healthy adult males using HPLC method. These results established a reduction of approximately 25 % and 20 % in AUC and C_{max} respectively when CQ was co-administered with khat. Similar reduction in the bioavailability of some antibiotics was seen after 2 hours of chewing khat (Attef *et al*, 1997). In our study, the results showed that khat is accounted for the CQ reduction. This raises important questions about the complicated interaction with khat and the clinical consequences of this drug interaction. Further studies into the interaction may provide an understanding of the mechanism of interaction between CQ and khat components which khat constituents are involved and other possible factors that could be involved in this interaction.

In chapter 3, CQ concentration was measured in malaria patients over the three days of CQ treatment with and without khat. The CQ levels were also reduced significantly in khat chewers (33%), which was more pronounced and highly significant when compared to the healthy study groups (25%). This may be associated with the general health of the patients. However, the results presented in **chapter 4** showed 73.8 % of malaria patients showed adequate response (parasites were completely cleared by day 3 of treatment) and 26.2 % of the cases were parasitological failures regardless of the treatment group. In addition, khat chewers had lower parasite density and higher proportion of treatment successes by day 3 when compared to non khat chewers although these differences is not significant ($p > 0.05$). This seems to be in consistent with *in vitro* investigation of khat extracts on CQ-sensitive of *P. falciparum* (3D7) in **chapter 5** which revealed weak anti-malarial activity (IC_{50} = 21.47 (9.83) μg/ml for chloroform and 37.06 (10.13) μg/ml for water extract), suggesting the possible positive side of khat. This mild effect of khat on a CQ-sensitive strain may not qualify the use of this plant as a traditional anti-malarial until further investigation for anti malarial activity *in vitro* and in animal models to support the preliminary results in **chapters 4.**

Gender showed no influence on CQ concentration as we found no significant differences in CQ concentration between males and females regardless of treatment group. However, it is likely that females are more susceptible to the effect of khat chewing as we found significant differences in plasma CQ levels between males and females who chewed khat during treatment. In

addition, no statistical differences in the mean CQ levels were detected between heavy (chewed khat on daily basis) and light khat chewers (chewed khat once a week).

In this study, CQ efficacy was overestimated and the rate of treatment failure was underestimated due to the limitation in the follow-up period. However, based on the study findings, the level of parasitological resistance (26.2%) was higher than 25 %; a level considered unacceptable by WHO (WHO, 2002a) for the level of CQ resistance in any endemic areas. This is indicative of continuous emergence of CQ resistance strains of *P. falciparum* in Yemen and specifically in the study area.

In conclusion, the study revealed novel important findings. CQ was reduced significantly by khat chewing, khat had no significant influence on parasites clearance, and the rate of CQ resistance was above the WHO acceptable phase. Therefore, based on these preliminary results, the following actions are recommended:

- It is advisable not to chew khat during CQ treatment or during other chemotherapy. Alternatively, we recommend that CQ be administered at least 3 hours before khat chewing.
- The government should increase public awareness of chewing khat and its impact on public health and integrate education about khat into the curricula of primary and secondary schools.

- Since most khat users believe that khat makes them feel better and reduce their temperature during sickness, public education becomes more difficult. Therefore we suggest that treatment guidelines should be given before taking medicine and those health workers (private and public) and manufacturers should be educated about the potential risk of co-administration of khat with other drugs.
- Action to be taken to address the problem of CQ resistance, particularly in the study area and most likely in other parts of the country, it has now become very urgent to review and change malaria treatment policy in Yemen.
- NMCP should monitor the water tank storage in some areas which has become possibly the source of malaria transmission and increase awareness of the influence of cultivation practices on malaria transmission and other diseases.

6.2 FUTURE STUDIES

- To study the pharmacokinetics of CQ and parasite clearance in khat and non khat chewer malaria patients by following them for 28 days.
- To separate and identify chloroform and water extracts to and investigate their effect on CQS and CQR strains and their effect in combination with CQ.
- To investigate the mechanism of khat-CQ interactions.

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APPENDICES

Appendix 1A

Republic of Yemen
Sana'a University
Faculty of Medicine & Health Sciences
Dean Office

Date/
Our Ref/
FileNo/

بسم الله الرحمن الرحيم

الجمهورية اليمنية
جامعة صنعاء
كلية الطب والعلوم الصحية
مكتب عميد الكلية

التاريخ / ٢٠٠٤/٨/٢٦
الإشارة رقم /
رقم الملف /

الى من يهمه الامر

تهديكم كلية الطب والعلوم الصحية بجامعة صنعاء أطيب تحياتها وتود الإحاطة بان الأخت / فائزة حمود عيسى – المدرس بقسم الكيمياء الحيوية وطالبة الدراسات العليا لنيل درجة الدكتوراه في البرنامج المشترك بين جامعة صنعاء وليفربول برجاء التكرم بتقديم التسهيلات والمساعدة لانجاز الجانب البحثي من رسالتها.

أعطيت لها هذه الإفادة لتقديمها الى من يهمه الأمر .

القائم باعمال عميد كلية الطب والعلوم الصحية

أ.د. طلال أحمد حيدر

P.O.Box :13078 Sana'a
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ص.ب:١٣٠٧٨ صنعاء
تلفون :٣٧٥٥٣٥ / ٣٧٠١٨٩ - فاكس :٣٧٠١٨٩

Appendix 1B

بسم الله الرحمن الرحيم

Republic of Yemen
Ministry of Public Health & Population
National Programme
For Roll Back Malaria

الجمهورية اليمنية
وزارة الصحة العامة والسكان
البرنامج الوطني لمكافحة وحصر الملاريا

No. : ..... الرقم : .....
Date : ..... التاريخ : .....

صادر
الرقم: ٦٨٤
التاريخ: ٢٠٠٦/٧/١٧
التوقيع:

الأخ / مدير محور تهامة **المحترم**

تحية طيبة وبعد،،،،،

يهديكم البرنامج الوطني لمكافحة الملا ريا أطيب التحيات ونود إفادتكم بأن الأخت الدكتورة فايزه حمود عيسى أحد أعضاء هيئة التدريس بكلية الطب وتحضر حاليا لنيل درجة الدكتوراة في الكيمياء الحيوية في جامعة ليفربول ببريطانيا وعنوان الرسالة " تأثير القات على علاج الملاريا (الكلوركوين)" كجزء من متطلبات الدراسة وهي تقوم حاليا بالبحث الميداني في اليمن وجمع عينات الدراسة ، نرجو تسهيل مهمتها والتعاون معها

ولكم جزيل الشكر والتقدير

د . جمال عمران
مدير عام البرنامج

الأخوة شركة الوحدات للمقاولات

الجمهورية اليمنية - صنعاء - شارع الستين (مازدا سابقاً) - هاتف: ٥٦٢١١٢-١-٩٦٧ فاكس ١٦: ٥٦٢١١٦-١-٩٦٧ ص.ب: (١٦٤٥٦)
Republic of Yemen / Sana'a - Hamama St. Mazdaj. - Tel: 967-1-562112 Fax:967-1-562116 - P.O.Box: (16456)

Appendix 1C

LIVERPOOL
SCHOOL OF
TROPICAL
MEDICINE (Affiliated to the University of Liverpool)

Pembroke Place
Liverpool L3 5QA
Telephone: 0151-708 9393
Fax: 0151-705 3370
http://www.liv.ac.uk/lstm/lstm.html

5 October 2004

Ms Fayza Hamood Zeed Eyssa
C/o Dr M Chance

Dear Ms Eyssa

The research protocol **Khat (Catha Edulis Forsk) and its effect on anti-malaria chemotherapy**Reference No **04.47** was considered by the Research Ethics Committee on **9 September 2004.**

Thank you for your letter of **5 October 2004** with information requested by the committee. The protocol now has formal Ethical Approval from the LSTM Research Ethics Committee.

Conditions of Approval

- The approval is for a fixed period of three years or for the duration of the grant, renewable annually thereafter.
- The committee may suspend or withdraw ethical approval where it is felt appropriate.
- In accordance with International Committee on Harmonisation of Good Clinical Practice (ICH GCP) Guidelines, annual update must be provided to the committee. Failure to do so could result in suspension of the study without further notice.
- A copy of the final report should be sent to the committee
- Any serious adverse events must be reported to the committee.

Any proposed amendments to the protocols must be notified to the LSTM Research Ethics Committee for approval before implementation. (Full application is not necessary at this stage)

The Research Support Office (RSO) maintains a Database of Local Research Committees in the countries where collaborative work is being carried out. Could you, therefore, feed back to me (via Sharda Mistry in the RSO) as much information as possible on the local Committees/Review Bodies that will review (or have reviewed) this protocol. The following details would be much appreciated:

- Name
- Address
- Contact numbers or individuals (tel / fax / e-mail)
- A copy of the appropriate form or some details on the submission mechanism (including charges)
- Any details you are able to obtain on
  a) number on the committee
  b) how many lay representatives sit on the committee?

Yours sincerely

Tom Blanchard

Dr T Blanchard
Acting-Chair, Research Ethics Committee

Appendix 2

INFORMED CONSENT

(Healthy volunteers)

The aim of this study is to find out the effect of khat chewing on the anti-malarial drug chloroquine. I will study if the amount of drug in the body is changed if you chew khat. To do this, the researcher is conducted a study in which a group of healthy volunteers will be given a single dose of chloroquine for two occasions. Finding of the study will contribute in reconsideration of using khat as stimulant and as social drug during treatment.

This is not mandatory and you have the right to participate or withdraw without any obligation. If you agree to participate in the study, you will take a single dose of 600 mg of CQ in two occasions, and you will be asked to chew khat in one occasion, and each time a blood sample of 5 ml will be withdrawn 10 times using indwelling catheter on back of your hand. Blood samples will be taken from you at 0 min, 30, 1h, 2, 3, 4, 6, 8, 12, and 24h post drug administration in the two occasions. CQ is given as a prophylactic agent, and the dose is given to you is the normal dose prescribed by doctors. In addition, you will fill out a questionnaire about the use of khat. It is very important that you participate in the two occasions, if you are not able to do so, please let us to know now.

You participation is completely voluntary and you may withdraw from the study any time and for any reason. The gathered data will be used confidentially only for the research purpose and will be destroyed after the study has been completed. We expect no harm or health complication for you as a result of the use of these procedures in this study.

I have been read and understood the information above and I have the right to ask any question and get an answer which satisfies me. I agree to participate in this study and I understand I have the right to withdraw at any time and if I did so this will not affect my treatment in any way.

Name of participant: .....Signature..... Date.....

(Arabic translation of the informed consent for volunteers)
Translated by Fayza Eyssa

موافقة للاشتراك في دراسة تأثير القات علي فعالية علاج الملا ريا (الكلوروكوين) من 6 مارس إلى 6 ابريل2005)

عزيزي المتطوع:

تقوم الباحثة بأجراء بحث ميداني ضمن الأعداد لأطروحة الدكتوراه بعنوان تأثير القات على علاج الملا ريا وتهدف الدراسة إلى دراسة تأثير تناول القات على الكلوروكوين ومعرفة ما إذا كانت كمية الدواء في الجسم تتغير عند تناول القات. سيكون لمشاركتكم الفضل في إتمام هذه الدراسة وكذلك الإسهام في مراعاة عدم استخدام العلاج اثنا تناول القات إذا ما أثبتت الدراسة أن هناك تأثير على العلاج.

مشاركتكم ليست إجبارية ولكم الحق في المشاركة أو الانسحاب دون أية التزامات. في حالة الموافقة على المشاركة في الدراسة سوف تتناول 600 مليجرام من الكلوركوين مرتين. في المرة الأولى ستأخذون العلاج فقط وفي المرة الثانية سوف تأخذون العلاج مع تناول القات. سيسحب 5 مل من الدم مباشرة بعد استخدام العلاج ثم بعد نصف ساعة، ساعة ونصف، ساعتين، ثلاث ساعات، أربع ساعات، ثمان ساعات، اثنى عشر ساعة، وأربعة وعشرون ساعة من استخدام العلاج. سوف يستخدم في سحب الدم القصيبة (الكانيولا) التي ستوضع على ظهر اليد في المرتين. يستخدم الكلوروكوين كعلاج وكجرعة وقاية ضد الملا ريا والتي توصف عادة من قبل الأطباء فليس هناك أي مضاعفات. وسوف تقومون أيضا بالإجابة على الاستبيان الخاص بعادة تناول القات.

مشاركتكم طوعية كاملة ويمكنكم الانسحاب من المشاركة في اي وقت ولأي سبب ترونه. العينات التي ستسحب ستكون لغرض هذه الدراسة فقط وسوف تتلف بعد انتهاء التحاليل. لا نتوقع اى أضرار صحية أو أية مضاعفات اثنا إجراء التجربة

شكرا لمشاركتكم وتعاونكم

الاسم التوقيع التاريخ

Appendix 3

Khat and its effect on anti-malaria chemotherapy

(Questionnaire form: volunteers participants)

Record number:

Date:

Place of the study: Town District

Sociodemographic Information:

Name: age: sex: Male Female

Weight (kg) Height (Cm):

Occupation Education level:

Home address:

Place of work:

1- Do you have a habit of khat chewing?

Yes No

If yes, how often

(1) Every day (2) 2-3 days/week

(2) Once a week (4) occasionally

2- How long have you chewed khat?

(1) Less than 1 year (2) 1-3 years (3) more than 4 years

3- How long do you chew khat at each session?

(1) Less than 3 hrs (2) 3-4 hrs (3) more than 4 hrs

4- Have you ever been infected with malaria?

Yes No

If yes, when?

5- Are you taking any anti-malaria drugs?

Yes No

If yes, what type? When did you take it?

6- Do you have a history of kidney failure disease?

Appendix 4

INFORMED CONSENT

(Malaria patients)

I am doing a study to find out the effect of khat chewing on the anti-malarial drug chloroquine. I will study if the amount of drug in the body is changed if you chew khat and also if there is any change in the number of parasites that are in your blood which make you feel sick. To do this, the researcher is conducting a study in which a group of patients with malaria will be treated and are followed for 3 days. Findings of the study will contribute in reconsideration of using khat as stimulant and as social drug.

This is not mandatory and you have the right to participate or withdraw without any obligation. If you agree to participate in the study, you will come to the Malaria Programme Centre 3 times at each time you will receive chloroquine as part of your normal treatment. CQ will be given as initial dose of 4 tablets in the first day, 4 tables in the second day, and 2 tablets in the third day. 3 ml of blood will be withdrawn each time to measure CQ concentration in your blood and also to see if you still have the malaria parasite. In addition, you are required to answer a questionnaire, which will take you approximately 10 minutes. The questionnaire will be about the use of khat and previous use of other anti-malarial drugs. It is very important that you come back to see you on these days, if you are not able to return, please lets us to know now.

You participation is completely voluntary and you may withdraw from the study any time and for any reason. If you do not want to participate, this is will not modify your relationship with the doctors, nurses, and staff of the Malaria Programme Centre and your treatment will not be affected.

The gathered data will be used confidentially only for the research purpose and will be destroyed after the study has been completed. We expect no harm or health complication for you as a result of the use of these procedures in this study. If you continue to suffer from malaria, you will receive alternative treatment from the Centre

I have been read and understood the information above, or have been read for me. I have the right to ask any question and get an answer which satisfies me. I agree to participate in this study and I understand I have the right to withdraw any time and if I did so this will not affect my medical care in the centre.

Name of participant: .....Signature.....Date.....

(Arabic translation of the informed consent for malaria patients)
Translated by Fayza Eyssa

موافقة المريض للاشتراك في دراسة تأثير القات علي فعالية علاج الملا ريا (الكلوروكوين) من 12 يناير إلى 3 ابريل2006

عزيزي المريض:
تقوم الباحثة بأجراء بحث ميداني يهتم بدراسة تأثير القات على علاج الملا ريا وتهدف الدراسة إلى دراسة تأثير تناول القات على الكلوروكوين ومعرفة ما إذا كانت كمية الدواء في الجسم تتغير عند تناول القات وكذلك طفيل الملا ريا والذي يسبب الحالة المرضية

في حالة الموافقة على المشاركة في الدراسة سوف يعطى المريض 1500 مليجرام من الكلوركوين تعطى منها 4 أقراص في اليوم الأول ثم 4 أقراص في اليوم الثاني ثم 2 أقراص في اليوم الثالث. سيسحب 3مل من الدم كل يوم خلال فترة العلاج وسيتطلب مجيئكم ثلاث مرات بعد الفحص لأول.

مشاركتكم ليست إجبارية ولكم الحق في المشاركة أو الانسحاب دون أية التزامات. إذا وافقتم على المشاركة سوف تأتون إلى مركز مشروع الملا ريا ثلاث مرات وسوف تستلمون العلاج المعتاد في المركز. ستسحب 3مل من الدم في كل مرة لمعرفة تركيز الكلوروكوين وكذلك عمل شرائح لفحص وإذا كنتم طفيل الملا ريا. وسوف تقومون أيضا بالإجابة على الاستبيان الخاص بعادة تناول القات. ترون عدم القدرة على العودة ارجوا إبلاغنا من البداية

مشاركتكم طوعية كاملة ويمكنكم الانسحاب من المشاركة في اي وقت ولأي سبب ترونه وفي حالة عدم المشاركة لن تتأثر حقوقك من الخدمات الصحية في المركز وسوف تستلمون علاجكم بالكامل كالمعتاد.

العينات التي سوف تأخذ لغرض هذه الدراسة فقط وسوف تتلف بعد انتهاء التحاليل. المشاركة في الدراسة ليست إجبارية ولكم الحق في الانسحاب في أي وقت دون أي التزامات. لا نتوقع أي ضرر أو أي مضاعفات جراء عمل الدراسة.

سيكون لمشاركتكم الفضل في إتمام هذه الدراسة وكذلك الإسهام في مراعاة عدم استخدام العلاج اثنا تناول القات إذا ما أثبتت الدراسة أن هناك تأثير على العلاج.

شكرا لمشاركتكم

الاسم: التاريخ التوقيع

Appendix 5

Khat and its effect on anti-malaria chemotherapy
(Questionnaire form: malaria patients)

Record Number:
Place of the study: Gov: District Province

Sociodemographic Information:

Name: age: sex: Female Male

Occupation:

Education level: (1) illiterate (2) elementary (3) secondary
(4) University (5) others

Telephone Number:

Home address:

Place of work:

7- Do you have a habit of khat chewing?

Yes No

If Yes, how often

(1) Every day (2) 2-3 days/week
(3) Once a weak (4) occasionally

2- How long have you chewed khat?

(1) Less than 1 year (2) 1-3 year (3) more than 4 years

3- How long do you chew khat at each session?

(1) Less than 3 hrs (2) 3-4 hrs (3) more than 4 hrs

4- Have you ever been infected with malaria?

Yes No

If yes, when?

5- Are you taking any anti-malaria drugs now?

Yes No

If yes, specify

Drug: Dose: unknown

6- When did you take it?

(Arabic translation of the questionnaire)
Translated by Fayza Eyssa

القات وتأثيره على الملا ريا (الكلوروكوين)

استبيان خاص بإمراض الملا ريا

الرجاء كتابة البيانات المطلوبة:

الاسم: العمر:

الجنس: ذكر( ) انثى( )

المهنة: محل العمل:

المؤهل: رقم الهاتف:

1- هل تتعاط القات؟ نعم لا

إذا كانت الإجابة بنعم كم مرة

(1) مرة في الأسبوع (2) مرتين في الأسبوع

(3) ثلاث مرات في الأسبوع (4) الأسبوع كامل (5) نادرا

(2) منذ متى بدأت تعاط القات؟

(1) اقل من سنة (2) سنة إلى ثلاث سنوات (3) أربع سنوات فأكثر

3- كم ساعة تتعاط القات؟

(1) اقل من ثلاث ساعات (2) 3-4 ساعات (3) 5 ساعات فأكثر

3- هل سبق وان أصبت بالملا ريا؟

نعم لا

إذا كانت الإجابة بنعم

4- هل تتعاط أي علاج للملا ريا؟

نعم لا

إذا كانت الإجابة بنعم

5- ما هو العلاج؟

6- منذ متى بدأت استخدام العلاج؟

Appendix 6

Khat and its effect on anti-malaria chemotherapy

Case report form

Date:

Name:

Age (years):

Sex: Female Male

Health Facility Name: Town:

Hb mg/dl

Contact address:
Tel. #:

Blood film Result:

Malaria parasite species:

Parasite Count: /μl

Day0	Day1	Day2	Day 3

Axillary temperature:

Day0	Day1	Day2	Day 3

Danger signs:

Drug treatment:
Total CQ does: 25 mg base/kg
Batch number:
Expiry dates:
Manufacturer:

Appendix 7

Follow-up khat use during treatment

Date	Patient #	# of chewing days					Total days of chewing of khat
		-1	0	1	2	3	