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The Effect of Chewing Khat on Drug Absorption: A Case Study of Pharmacokinetic Profiles of Ciprofloxacin 500 Mg Tablets

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ABSTRACT

Background Khat (*Catha edulis* Foskal) is an evergreen plant, and about 50% of the population chew khat in most of their activities; e.g. political, marriages, merchant, working etc. The literatures reported that the *Catha edulis* plant contains mainly alkaloids: cathine and cathinone besides polyphenolic compounds (tannins) in an amount that ranges between 7–27.4%. Moreover, the essential components are chemically active and decrease drug absorption. Cross-over design for the study was conducted and eight healthy adult Yemeni volunteers participated in the study. The medicine ciprofloxacin 500 mg tablet was administered under fasting conditions and after chewing the khat, the essential precautions of the study were taken. **The purposes** of this study are to evaluate the pharmacokinetics parameters of ciprofloxacin 500 mg tablets under fasting condition and after chewing the khat. **The method** of analysis was carried out by HPLC instrument at 278 nm by UV detection. The method of analysis was valid and achieved reproducibility, accuracy, and linearity. The correlation coefficient was close to 1 (0.994). The relative standard deviation percent (RSD%) was 1.54%. **The results** of the study show that decrease Cmax, and AUC significantly to 38% and 52% respectively, whereas Tmax, delay time increase to 2.94 fold. **The conclusion** of this study agrees with our hypothesis expectation that chewing the khat with concurrent medicines can lead to lack of effectiveness of medicine. So the investigator advises the authority of health to make regulations that prohibit chewing khat during the course treatment medicine.

INTRODUCTION

Khat is the name generally used for *Catha edulis*, a **dicotyledonous** evergreen shrub of the family Celastraceae (also spelled kat, qat, cat or ghat); the amharas call it 'tchat'. The tree khat has a slender bole and white bark, in Yemen, the trees range from 1 to 10 m in height, while in Ethiopian **highlands** they can reach in height 18 m; however, they can **prune** annually to keep their height to 5 m [1]. Moreover, in Yemen, we can distinguish between two situations: Before 1950 the khat was **cultivated** in a very little area which is characterized as poor production and keeps the enriched land to produce crops such as wheat, sorghum and corn, while after 1950 the Yemeni farmers start to **cultivate** the khat in the enriched lands and replaced the khat trees by their food production to a degree that the cultivated khat area constitutes more than 20% from the enriched area. And consequently, the khat has become **a terrible problem** that effects (celebrate) on every matter such as health system, social economic, political and the community. Most reports mention that Khat originated in Ethiopia and spread through Yemen, Kenya, Djibouti, Eritrea, Somalia, and South Saudi Arabia and chewing habit is particularly more prevalent in Yemen and horn African countries [2, 3].

The first historical reference, as far as could be determined from the literature consulted, occurs in a medieval Arab manuscript (ms.143 Bibliotheque rationale, Paris) [4] where it is stated that the king of Ifat, Sabr-addin decided to plant khat in the town of Marad (the period of question seems to be in the first half of the fourteenth century). According to Rochet, khat was introduced from Ethiopia into Yemen in 1424 by **Sheikh Abu Zerbin**. Another reference to its cultivation in the fourteenth century in the region of Aden and in Yemen is found in the sixteenth-century Arab chronicler, Abdul-kader. Furthermore, after two centuries by classification of khat as ***Catha edulis*** that was available in ***Flora-Aegyptiaco-Arabia*** by the Swedish botanist Peter Forskal, who died in **Arabia Felix** (now, Yemen; Yerim city) in 1763 [5].

Khat Chemistry

During the period of the Swedish botanist, Peter Forskal who classified the khat as *Catha edulis*; the family Celastraceae, there was a long series of chemical studies that focus on the identification of khat's active principles (Table 1). The studies have determined that khat leaves contain more than 40 different compounds; contain 3 phenylalkylamine alkaloids

(identified as cathinone, cathine and norephedrine); and have stimulant, euphoric, and sympathomimetic effects among others [6]. The chemical structure of cathinone, cathine which is amphetamine like (Fig. 1). In addition, Cathinone is the most stimulant active ingredient in the khat [3]. It is also estimated to be 7-10 times more potent than cathine due to a ketone group that is less polar than hydroxyl that is available in cathine and facilitates to penetrate the cathinone to cross blood brain barrier but to a less extent than amphetamine, whereas cathine is responsible for **unwanted systemic effects** [2].

Furthermore, there are highly variations that are available in the literature reviews about the amount of the active ingredients of cathinone, cathine, and norephedrine ranged between 78 - 343, 83 – 120, 8 – 47 mg/100 gm., respectively [7, 8]. Unrelated to alkaloids, khat leaves contain another group of bioactive compounds known as **tannins**/ **or Polyphenols** which possess astringent effects and ranges from 7 – 27.4% [9, 10, 6].

Previous Studies

There are many studies that covered most of the human beings systems which can be manifested in the following: Khat chewing has potentially **several adverse systemic health effects** including cardiovascular disorders[7, 11, 8], liver and gastrointestinal disturbances [12, 8, 3], pulmonary edema [13], renal toxicity, and psychosis [14, 15, 8, 16].

Pharmacological Action of the Khat (cathinone-cathine) and tannins on the Gastrointestinal Tract (GIT):

In addition to the adverse effects that are mentioned above, a hypotonic and atonic stomach probably due to the sympathomimetic action of **khatamines**, which inhibits the peristaltic movement and delays the emptying time of the stomach [9,15,17]. And in the same pattern, **Tannins** (polyphenols) have the ability to form insoluble complexes with proteins by their flagship ability in addition, **they produce reaction with different non protein organic N compounds** [18]. Moreover, **tannins** ranges in molecular weight from 500-3000 Daltons[10, 18].

Pharmacokinetics of cathinone (norephedrone) and cathine

Maximal plasma concentration (C_{max}) was reached (t_{max}) on an average after 2.3 hrs. for cathinone, 2.6 hrs. for cathine, and 2.8 hrs. for norephedrine. The elimination half-life of cathinone was 1.5 ± 0.8hr. and that of cathine was 5.2 ± 3.4 h.[19]. And in the same pattern,

Wilder *et al*, 2011[20] mentioned that elimination half-life for cathinone was much longer mean half-life 4.3 ± 1.7 hrs.

Khat contents are summarized in the below **Table No. 1**:

1	Tannins	7– 27.7%	5	Water contents in twigs	60 -66
2	Cathine	Variable quantities	5	Water contents in leaves	8.4-9.2%
3	Cathinone	Variable quantities	6	Minerals	11%
4	Ascorbic acid	136-324/100 g			

It is very clear from the previous studies that there is a **lack of information** upon the chewing khat persons and at the same time administered medicines; how the khat contents such as **tannins** and **cathinone** can decrease bioavailability of the medicine, especially, if we know that adult Yemeni people who chew khat exceed 60% of the population. Hence, the investigator decided to pave away in the researches in this way *i.e.*, **lack of knowledge in concurrent chewing khat while taking medicines.**

The previous reasons justify to check the bioavailability of ciprofloxacin500 mg tablet among Yemeni people who chew khat and to ensure a desired level of efficacy, safety and quality in this pivotal situation.

The general objective of the study is to determine the effect of khat contents mainly: cathinone, cathine and tannins on drug absorption.

Specific objectives

1. To determine the pharmacokinetic parameters such as C max, T max, AUC, half-life, elimination rate constant, and MRT for ciprofloxacin tablet under fasting conditions.

2. To measure the changes in pharmacokinetic parameters after chewing the khat.

3. To evaluate the deficiency in bioavailability in chewer people.

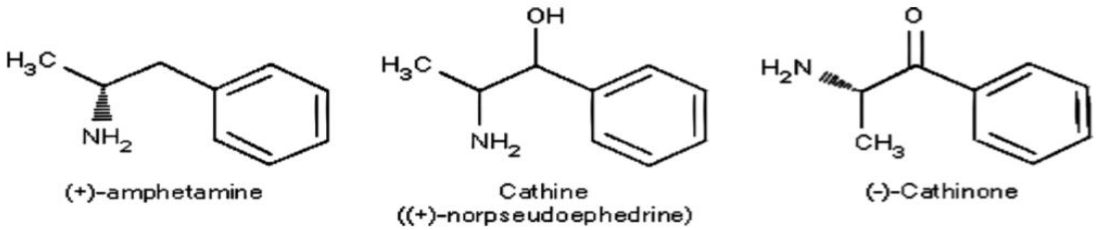

Figure 1. *Chemical structures of amphetamine, cathine and cathinone.*

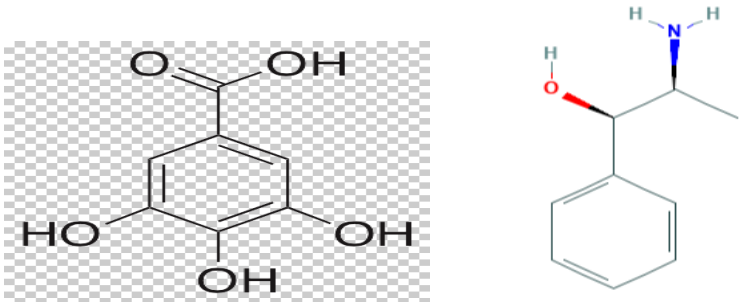

Figure No. 2: Chemical structure of phenolic tannins (Gallic acid) and norephedrine

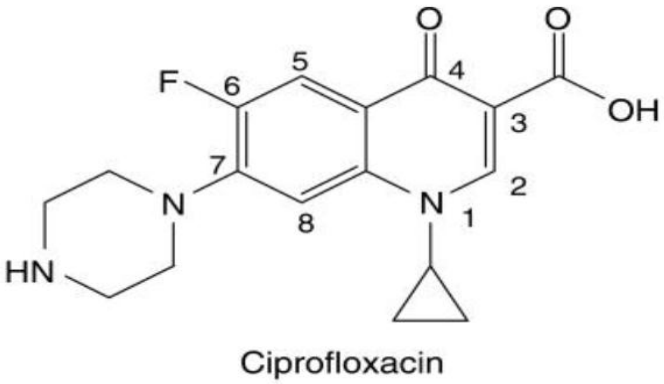

Figure No. 3: Chemical structure of ciprofloxacin

MATERIALS AND METHODS

The ciprofloxacin reference product (ciproby 500 mg tablets) are purchased from the local market and khat was collected from the Dhahban area (north Sana'a capital city).

Chemicals:

Ciprofloxacin and norfloxacin raw materials powders (external and internal standard) are obtained from drug quality control (DQC) [21]. Phosphoric acid, acetonitrile, methanol and triethylamine (HPLC grade; Merck, company, West Germany) were supplied also from DQC.

Clinical trials:

Eight healthy adult Yemeni male volunteers of comparable age and weight participated in the trials. Each volunteer was given a full explanation of the purpose of the study and his approval was recorded in a written consent form. Two periods, two sequences crossover design was adopted. Ciproby 500 mg tablet was used.

The trials plan followed the order; administered of ciprofloxacin under fasting conditions in period 1, and the effect of the khat on ciprofloxacin bioavailability in period 2. The products were administered as a single dose of 500 mg tablets on empty stomach preceded by an overnight fast, with 250 ml of drinking water. Breakfast was allowed 3 hrs. post dosing and in case of khat, the drug was given to the volunteers immediately when they started chewing khat. Cigarettes and beverages containing xanthine were not allowed. Uniformity of meals was ensured throughout of the trials. A washout period of one week was ensured between the trials. No drugs were taken during the trial period. Blood samples (5 ml) were collected at 0.0 hr. (before dosing), 0.5, 1.0, 2.0, 3.0, 4.0, 8.0 and 12 hrs. post the dosing. The blood samples collected in heparinized test tubes centrifuged immediately at 4000 rpm for 10 min and the plasma was transferred by master pipettes to plain plastic test tubes and kept in deep freezing at –25 ºC until analysis [22].

Validation of the HPLC method used for determination of ciprofloxacin in plasma:

Chromatographic conditions:

Column	: Symmetry C18 (4.6 mm ID x 25 cm) connected to a guard column (ODS, 4 cm).
Mobile phase	: 0.245% w/v phosphoric acid and acetonitrile (86:14). Adjust the pH to 3 by triethylamine.
Detection UV	: λ 278 nm.
Flow rate	: 1.5 ml/min.

Temperature	: 40 ºC
Injection volume	: 100 µl

Direct extraction of the drug from plasma (Present study):

In this type of extraction, add to Eppendorf test tube 200 µl of plasma, 100 µl of norfloxacin (2 µg/ml) and 300 µl of perchloric acid (5%), then vortex for 1 min, after that centrifuge at 5500 rpm for 7 min. Make decantation to a new Eppendorf tube, inject onto the column (C 18) and measure using UV detection at 278 nm.

Description of method:

A 100 µl of the internal standard working solution (norfloxacin, 2 µg/ml) was added to 200µl plasma sample (standard sample or volunteer sample) 300µl of perchloric acid (5%) was added and vortex for 60 second, centrifuged for 7 minutes at speed 5500 rpm. Decantation to another Eppendorf test tube was performed then 100µl aliquot samples were injected onto a C18 (5 µm, 4.6 mm ID x 25 cm) HPLC column connected to ODS guard column (4 cm) where norfloxacin (internal standard) and ciprofloxacin separated from endogenous plasma substances, respectively. Our method is a simple one-step protein precipitation procedure.

In the present study, the amount of ciprofloxacin in the plasma was measured by HPLC coupled with UV detection at wavelength 278 nm. Peak area ratios of drug/ internal standard were determined and plotted against their relative concentrations. Regression analysis was performed using the following equation y = a+ bx. Calibration factor was determined from the slope and was used for calculation of drug concentration in the samples. Five points calibration curve of 500, 1000, 2000, 4000, and 5000 ng/ml were constructed on every day of analysis.

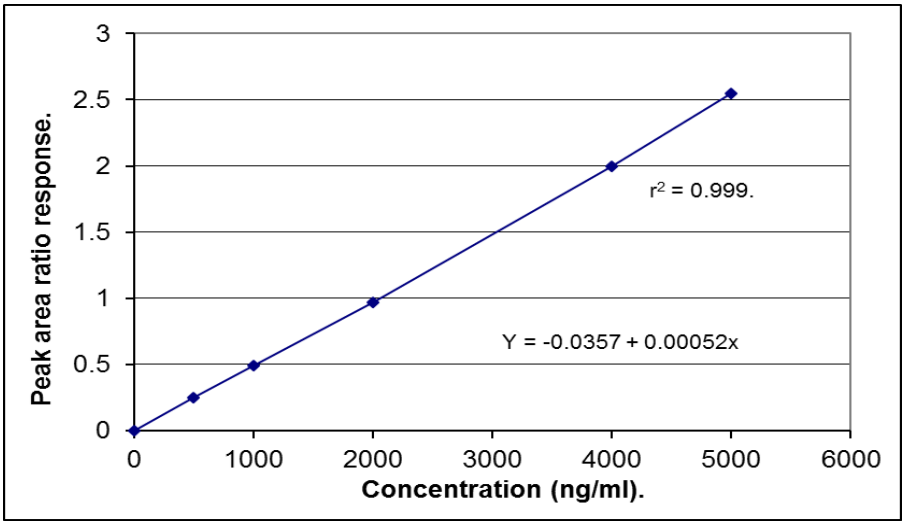

Figure No. 4: Calibration curve of ciprofloxacin in plasma by HPLC analysis.

The WinNonLin program was used to calculated parameters, while the statistical analysis carried out by SAS program.

RESULTS

The present study designed to evaluate the effect of khat on drug absorption. Eight male adults healthy volunteers participated in the study and given full explanation about the study. Ciprofloxacin tablets 500 mg (ciproby®,) were selected for this study. The method of analysis was valid (Fig. 4) and carried out by using HPLC instrument (see methodology). The results in Table 2 show that ciprofloxacin product was well tolerated in all subjects following oral administration of 500 mg tablet ciproby® under fasting conditions and also after chewing khat in the second period (Phase II). Again, the results in Table 2 illustrate that the rate which represented by maximum blood concentration (C_{max}), T_{max} (the time required to occur C_{max}) and extent (the area under the plasma concentration curve; AUC); the amount of ciprofloxacin that reached into the blood (bioavailable) in case of under fasting conditions were comparable with the previously reported in the literature reviews as reported under discussion. **Moreover**, there are significantly reductions after chewing the khat Table No. 2. In the $AUC_{0-\infty}$ after chewing khat and C_{max} decrease to 52%, and 38% compared with under fasting conditions (Fig. 5, Fig. 6) respectively, whereas the T_{max} increases to 2.94 fold. The statistical analysis showed that the p values of AUC, C_{max} and T_{max} under fasting condition and after chewing the khat were 0.001, 0.0001 and 0.0001 respectively. The other pharmacokinetic parameters such as half-life, elimination rate constant (Ke) and mean residence (MRT) were **comparable** under fasting condition and after chewing the khat as

shown also in Table 2 and Fig. 5. The statistical analysis represents that p-values were 0.639, 0.774 and 0.175 respectively Table 2.

DISCUSSION

Table 2 shows the results of the average of pharmacokinetic parameters under fasting condition of AUC $_{0-\infty}$, C $_{max}$, T $_{max}$, half-life, elimination rate constant (Ke) and MRT were 12355± 1579 (10388 – 14183), 2899 ± 673 (1872 – 3357), 1.19 ± 0.53 (0.5 – 2), 4.02 ± 0.1.25 0.194 ± 0.057 and 5.57 ± 2.09 (3.20 – 9.69), respectively. The Semi logarithmic profiles of the plasma concentration versus time for eight volunteers, (Fig. 5). Moreover, Fig. 5 showed a fairly evident α absorption, β distribution and σ terminal excretion phase. Our findings are consistent with data reported in the literatures *i. e*. Lettieri *et al*, 1992,[23] mentioned the mean value of $AUC_{0-\infty}$ was 10740 ± 2440 ng.h/ml (range 7500-14400). Again the second study that was carried out by Daniel *et al*, 1996[24], reported the mean value of $AUC_{0-\infty}$ was 13400 ± 8320 ng.h/ml. The ratio of our AUC $_{0-\infty}$ of reference to both of $AUC_{0-\infty}$that reported above were 115% and 92%, respectively. These ratios are agreeable with the FDA[25] acceptance 2001, where the limit ranged between 80-120%.

The mean values of C_{max} and T_{max} in our results are essentially comparable with those reported in the literatures by Lettieri *et al*, 1992[23], Daniel *et al*, 1996[24], and Robert *al*, 1997[26], the mean values were 2700 ± 800 ng/ml, 2590 ±1240 ng/ml and 2940 ± 510 ng/ml, 1.38 ± 0.43 h and 1.25 ± 0.55 h respectively. The statistical analysis at 90% confidence interval showed that the p- values were 0.566 and 0.305.

The mean values of the Ke, half-life and MRT were 0.194 ± 0.059 (range 0.085-0.269), 4.02 ±1.80 h (range 2.61-8.16) and 5.57 ± 2.09 h (range 3.98-9.69) for our findings which were in agreement with the following studies; the mean values of the Ke, half-life and MRT that were reported by (Thorsteinsson *et al*, 1987, Rambout *et al*, 1994, Neuman, 1988, Davis *et al*, 1985 and Galicia *et al*, 1998) [27, 28, 29, 30, 31] were 0.229 ± 0.057 h^{-1} and 0.169 ± 0.030 h^{-1}, 3-5 h and 4.82 ± 0.65 h, respectively, whereas Anneto et al (2000) [32] reported that MRT equals 5.8 ± 0.94. Moreover, the statistical analysis of our results and that reported above in Ke and half-life, and MRT at 90% confidence interval were 0.055, 0.465, 0.respectively.

Table 2 again represents the results of pharmacokinetic parameters **after chewing the khat**; the average of pharmacokinetic parameters for AUC $_{0-\infty}$, C_{max}, T_{max}, half-life, the Ke and MRT were 6577± 828 (5350 – 8028), 1108 ± 435 (498 – 1915), 3.50 ± 2.88 (1 – 8), 3.82 ±

1.20 (1.71 – 5.89), 0.203 ± 0.087 (0.118 – 0.404) and 7.09 ± 1.90 (5.50 – 10.43), respectively. **Moreover**, the results after chewing khat that mentioned above illustrated that AUC $_{0-\infty}$ and C_{max}, decrease significantly to 52% and 38% respectively (Fig. 5), whereas delay the gastric emptying leads to increase(T_{max}) to 2.94 fold (Fig. 6) due to decrease peristaltic motility of GIT. **In contrast**, the half-life ($T_{1/2}$), elimination rate constant (Ke) and mean residence time (MRT) represented insignificant change between under fasting conditions and after chewing the khat. The p values were 0.774, 0.639 and 0.175 respectively. The significant reduction in AUC $_{0-\infty}$, and C_{max}, and also delay time to occur T_{max} can be attributed to different reasons **first**, due to the sympathomimetic action of khat amines (mainly cathinone), which inhibits the peristaltic movement and delays the emptying time of the stomach as mentioned previously [9, 13, 17]. **Secondly**, the chemical reactions that occur throughout GIT bowel between the **polyphenols tannins** that available in khat contents are in highly amount that ranged between 7 – 27.4% as mentioned previously [9, 10, 6, 18] have ability to react chemically with soluble proteins in the site of absorption and form insoluble precipitate complexes that blocked partially the pores in the site of absorption mainly in small intestine. **Thirdly**, the excess of **polyphenols compounds; tannins (Fig. 2)** again can also **react** with medicine (Fig. 3) **as chelating agents** and arise highly molecular weight complexes that lead to slowly and retard the processes of drug absorption. **Fourthly**, the change in pH throughout the GIT bowel also leads to decrease the process of absorption and hence all the aforementioned reasons comprised **too much synergism** and the outcomes of this synergism leads to highly decrease in **bioavailability of the medicine** to a degree that the amount of drug absorbed lack its effectiveness. The final conclusion that is created from this situation: very serious cases may arise e.g. exacerbation in health of the patients, resistant of pathogen microbes for medicines, irrational use of the drugs, and economic loss without any benefits.

According to the available literature reviews that were mentioned under introduction [19, 20], the average of half-life of cathinone and cathine were 4.3 ± 1.7 and 5.2 ± 3.8 hrs. respectively. And hence according to the bases of pharmacokinetic parameters the effect of cathinone and cathine; that decrease GIT motility bowel is still to the time that ranged between 43 – 52 hrs. (the average 47. 5) and hence this means that **drug interactions of khat content** may be still to more than 24 hours. Our conclusion agreed with that reported by Al Habori (2005) [33] and Guesh, (2014) [2] that reported, the effect of cathinone and cathine were detect within 24 hrs. Hence our findings with the previous studies necessitate that the authority of health should

convince the chewers to stop chewing khat during the course of the patient treatment. **On the other hand,** the three pharmacokinetic parameters *i.e.* the half-life, the Ke and mean residence time (MRT) after chewing khat were comparable with essential pharmacokinetic parameters that occurred under fasting conditions (control) as represented in (Fig. 5). This means that tannins make its effects in GIT bowel by different mechanisms as mentioned previously and did not absorbed into the blood and this ensured by the statistical analysis that showed the p values were 0.774, 0.639 and 0.175 respectively. Furthermore, the excess amount of polyphenols tannins that are available in GIT mainly in the large intestine again can react with soluble protein and formed insoluble precipitate that lead to absorbed the water in GIT bowel and lead to hardness stool and finally lead to constipation that formed **hemorrhoids and anal fissures** in chewers of khat. Most of the experienced people complain from this problem and again these are comparable with the findings available in data reported as mentioned under the introduction [12, 8, 3].

CONCLUSION

Our findings indicate that the rate and extent of bioavailability of ciprofloxacin tablet that represented by C_{max} and AUC decrease to 38% and 52% respectively, whereas, T_{max} increases 2.94 fold compared with under fasting conditions. These results are comparable with the hypothesis of the study that predict cathinone and the polyphenols (tannins) that available in khat contents can affect drug absorption when administered concurrent in chewing khat people and lead to **drug-khat interactions to a degree that lack effectiveness of the medicines.** The other pharmacokinetic parameters that were illustrated by Ke, half- life and MRT did not affect by the human being. Moreover, the tannins and cathinone still make their effects on GIT bowel to at least 24 hrs. In addition, the decrease of absorption to the aforementioned values mean failure of the treatment and may lead to resistant of the antibiotics or failure the purposes of the medicines in case of other medicines and finally may lead to death or exacerbation of the patients. It could be concluded that chewing khat during the treatment course is **contraindication**. The investigator advises the authority of health to extend the research on these types of studies and extend the training on the risks of khat contents between the chewers upon the health care system. Moreover, the disasters of chewing khat while taking ciprofloxacin 500 mg tablets are more dangerous than that occurred with the pregnant women that used thalidomide world-wide between the fifties and mid-sixties of the last century.

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Table No. 2: Mean (± SD) bioequivalence parameters of ciprofloxacin (ciproby) after chewing the khat and under fasting conditions.

Parameter	Chewing the khat	Under fasting condition	P value
AUC $_{0\text{-}12}$ hr.(ng.hr/ml)	5563±1048	11208±3066	
AUC $_{0\text{-}\infty}$(ng.hr/ml)	6577±828	12839±3247	0.001*
Cmax (ng/ml)	1108±435	2899±754	0.0001*
T max (hr.)	3.5±2.88	1.19±0.53	0.055*
Ke (h^{-1})	0.203 ±0.087	0.183±0.043	0.639
T1/2 (hr.)	3.82 ±1.20	4.02 ±1.25	0.774
MRT	7.09 ± 1.90	5.57 ±2.09	0.175

* = Significant difference (≤p 0.05).

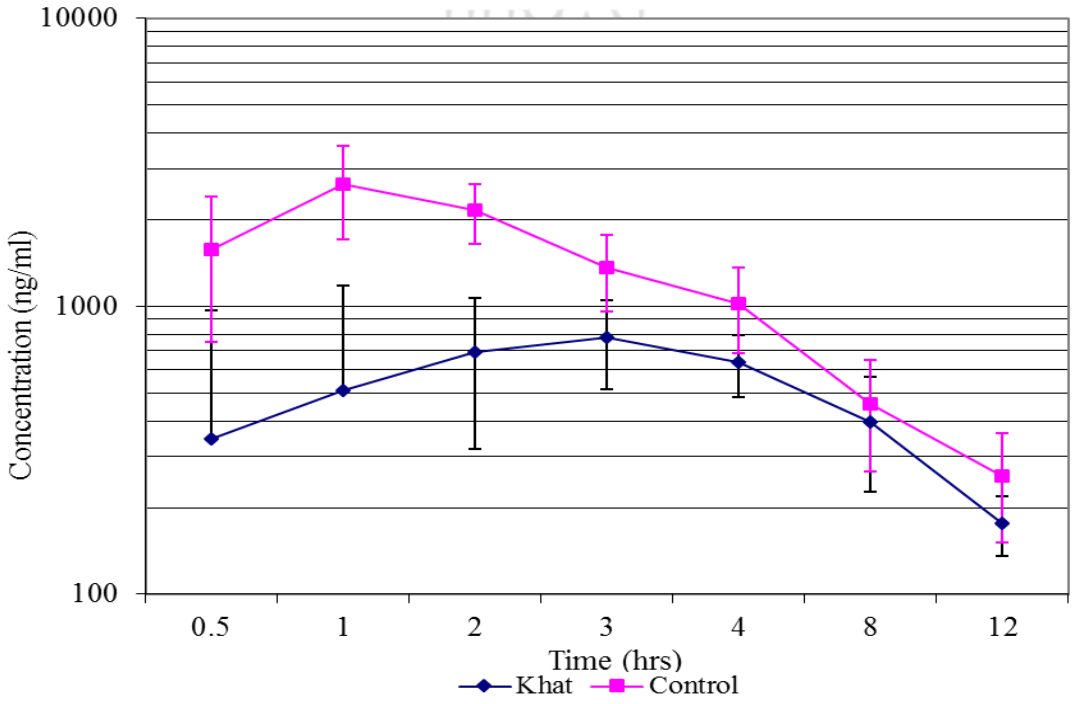

Figure No. 5: Mean ± SD plasma concentrations versus-time profiles of ciprofloxacin (ciproby) after administration of 500 mg tablet to eight volunteers under fasting conditions and after chewing the hat.

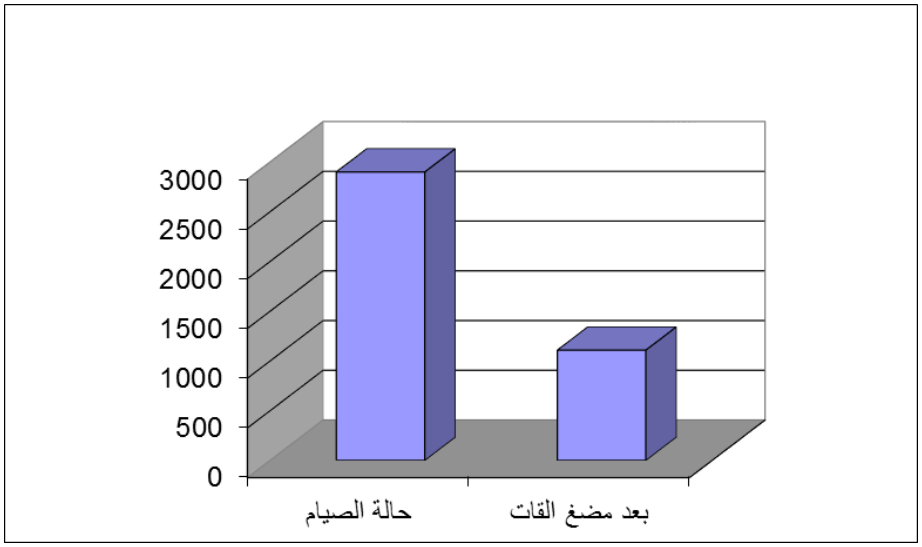

Figure No. 6: Represents Cmax under fasting conditions and after chewing khat

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