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EFFECTS OF CRUDE EXTRACT OF KHAT (*CATHA EDULIS*) ON LIVER FUNCTION IN RATS

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ABSTRACT: Introduction: Khat is chewed traditionally in Ethiopia, Somalia, Yemen and Kenya. In Ethiopia, khat chewing is now becoming an every-day substance of abuse for the general population regardless of its adverse effects on human health. Therefore, this study was conducted to investigate the effects of crude extract of khat fresh leaves and soft twigs on liver function in rats. **Methods:** Adult male Wistar rats (N = 35) aged between 14 to 16 weeks were randomly allocated into five groups of 7 animals each. Rats in group I were given 1mL of distilled water, and served as control. Rats in group II, III and VI were treated with crude extract of 150, 250, 350 and 450 mg/kg doses of khat respectively. At the end of the experiment, blood samples were collected and serum level of liver enzymes, total protein and bilirubin were determined. **Results:** Serum level of AST, ALT and ALP were significantly elevated while the total protein was significantly decreased ($P < 0.05$). The total and direct bilirubin were also significantly elevated ($P < 0.05$), except in rats treated with 150 mg/kg dose of khat. Increase in dose of crude extract of khat has shown strong linear relationship with the serum level of the liver function biomarkers. **Conclusions:** Crude extract of khat fresh leaves and soft twigs had toxic effects on the liver in rats as evidenced by alterations in biochemical indices of liver function which was dose related.

INTRODUCTION: Khat (*Catha edulis*) is an evergreen shrub, which is cultivated as a bush or small tree. The plant grows in a variety of climates and soils [1]. Catha edulis is known with different vernacular names: Khat in English and in Arabic and Jimaa in Afaan Oromoo (language of Oromo people) [2].

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The buds and leaves of khat contain a psycho-stimulant alkaloids (mainly cathinone, cathine and norephedrine), terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals [2] and are chewed in a fresh or dried condition as a stimulant [3].

Fresh leaves and soft twigs of khat (*Catha edulis*) are chewed to attain a state of euphoria [1]. Khat chewing is traditionally a habit in some African countries such as Ethiopia, Somalia and Kenya, as well as in Yemen. However, the habit is now being introduced into different countries of the world [4]. In Ethiopia, khat chewing is more prevalent in ethnic

communities with a tradition of khat chewing but it is now becoming an every-day drug of abuse for the general population regardless of its adverse effects on human health [5, 6].

It is now well documented that khat is potentially harmful to human health either as primarily producing damages to human body, or by aggravating the already existing disease conditions. Some of these adverse health effects of khat include hypertension, gastritis, depression, insomnia, urinary retention, sexual impotence, and obstetric effects like low birth weight, impaired lactation, and others [5]. Despite all these adverse effects of khat revealed by different studies, chewing of khat in the city of Addis Ababa and other parts of the country is becoming very common regardless of profession, race, religion, sex, and age [4, 6].

However, limited data are available regarding the liver toxicity of khat. Even, the findings of the available research works are controversial. Some literatures reported that khat has liver toxicity effect in experimental animals [7, 8, 9]. In contrast, there is also a report indicating that khat has no liver toxicity effect in experimental animals [10]. This controversy indicates that there is need for further investigation in this area.

On the other hand, there is a fact that the liver is a major organ for metabolism of foreign substances and also functionally interposed between the site of absorption and the systemic circulation. These conditions render the liver not only the most important organ for detoxification of foreign substances but also a major target of their toxicity [11]. Therefore, the controlled experimental trial was required to be conducted in Ethiopia to investigate the effects of crude extract of khat fresh leaves and soft twigs on hepatic functions in rats since the liver toxicity effect of khat has not yet been investigated in the country.

MATERIAL &METHODS:

Plant Material collection and extraction:

Fresh khat was purchased from Merkato market, Addis Ababa. The plant sample was identified by a taxonomist. Fresh leaves and soft twigs, the chewable part of the plant, were collected from the purchased plant sample and weighed to give 2.60 kg. The plant material was repeatedly washed with distilled water, and then dried for a week in dark place. 776 g of dry khat sample was powdered and put into labeled extraction flasks. After soaking the powder with sufficient amount (i.e. a total of 1 L was utilized to soak a 100 g of the plant's powder; because the soaking step was repeated three times to exhaustively extract the components) of 70 % (V/V) hydro-ethanolic solvent (70 % ethanol and 30 % distilled water), it was put on shaker and shaken at the speed of 150 rpm for 72 hours and then placed on table until it was settled. The supernatant was decanted and filtered with Whatman number 1 filter paper.

The filtrate was then concentrated by using the rotary evaporator. The resulted extracted solution was poured to labeled plastic container and was put on water bath at 40 ^{0} until it was dried. Finally, 45 g ethanol free extract was obtained. Afterward the yield was calculated and found to be 1.70 %. The plant extract was put in desiccator until utilized.

Experimental animals:

Ethical clearance was granted by the Department Ethics and Research committee (DERC) of the Department of Biochemistry. Adult male Wistar albino rats (N = 35) aged between 14 to 16 weeks were purchased from the animal breeding centre, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, and were acclimatized for two weeks at room temperature with 12:12 h Light: Dark cycle. The rats were kept in standard plastic cages, fed with standard rat pellets and tap water was provided *ad libitum*.

Grouping and Dosing of Rats:

Adult male Wistar albino rats (N = 35) aged between 14 to 16 weeks were randomly allocated into five groups of 7 animals each. Rats in group I were given 1mL of distilled water, and served as control. Rats in group II, III and VI were treated with crude extract of 150, 250, 350 and 450 mg/kg doses of khat respectively. The khat extract was orally administrated by using oral gavage, daily for a period four weeks. The doses for the khat extract were selected based on the average amount of khat fresh leaves and soft twigs chewed daily by humans

in Ethiopia [4] and yield value was then converted into rats based on the body surface area.

Blood collection and preparation of serum samples:

After the end of the experimental period, all animals were made to fast for 12 hours and blood samples were collected, immediately after cervical dislocation, via cardiac puncture. The collected whole blood samples were carefully transferred to serum separator test tube and were left on working table for 15 minutes until it clot at room temperature. After centrifugation at 3000 rpm for 15 minutes, the sera were aspirated with clean and dry pipettes and stored at -20 ° until analysis.

Biochemical assays:

The Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) were determined with an automated analyzer (Hitachi 902, Germany). While the total bilirubin and direct bilirubin were analyzed by the semi-automated machine (Humanlyzer-3000, Germany). Total protein was measured by the refractometric method. The assays were conducted using standard assay kits (Human Gesellchaft für Biochemica und Diagnostica mbH, Germany) according to the manufacturer's guidelines.

Statistical analysis:

The results were computed statistically with IBM SPSS software package version 20 using one-way analysis of variance (ANOVA) for mean difference between groups, and then post hoc Dunnett test was followed for comparing the tested groups to the single normal control.

A linear regression was performed to analyze the correlation between the change in dose of crude extract of khat and the change in biochemical indices of liver functions including AST, ALT, and ALP, total protein, total and the direct bilirubin. Data were expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ were considered as statistically significant.

RESULTS: In the current study, serum level of ALT, AST, and ALP were significantly increased ($P < 0.05$) in all groups of rats treated with different doses of crude extract of *Catha edulis* fresh leaves and soft twigs as compared with those of control group rats (**Table 1**). In **group** III, IV and V rats treated with 250, 350 and 450 mg/kg of khat, the serum level of the total and direct bilirubin were significantly increased where as the serum level of total protein was significantly reduced ($P < 0.05$) as compared to that of control group rats.

However, the serum level of total and direct bilirubin of group II rats (treated with 150 mg/kg crude extract of khat) and group I (control group rats) was not significantly ($P < 0.05$) different (**Table 1**).

TABLE 1: LEVEL OF LIVER ENZYMES IN THE SERUM OF CONTROL AND CRUDE EXTRACT OF *CATHA EDULIS* TREATED RATS

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	T P (g/dl)	TB (mg/dl)	DB (mg/dl)
I	**138.00 ± 3.51**	**57.17 ± 2.59**	**182.83 ± 10.00**	**6.33 ± 0.08**	**0.28 ± 0.06**	**0.05 ± 0.01**
II	167.83 ± 1.19*	76.17 ± 1.35*	238.33 ± 5.42*	5.88 ± 0.05*	0.52 ± 0.05	0.11 ± 0.02
III	198.83 ± 1.74*	90.33 ± 2.17*	303.17 ± 14.43*	5.47 ± 0.03*	0.89 ± 0.03*	0.16 ± 0.02*
IV	254.17 ± 10.90*	105.33 ± 1.80*	368.50 ± 6.16*	5.10 ± 0.04*	1.24 ± 0.07*	0.16 ± 0.02*
V	399.83 ± 11.04*	135.00 ± 4.87*	465.17 ± 20.64*	4.17 ± 0.20*	1.51 ± 0.09*	0.21 ± 0.02*

Tabular values represent mean ± standard error of mean (n = 7 rats per each group). The symbol (*) denoted a significant difference at $P < 0.05$. TP (Total protein), TB (total bilirubin) and DB (direct bilirubin).

Increment in the dose of crude extract of khat was a significantly correlated ($P < 0.05$) with the change in serum level of liver biomarkers (**Table 2**).

DISCUSSIONS: The current study conducted in rats has demonstrated that the oral administration of crude extract of khat (*Catha endulis)* fresh leaves and soft twigs resulted in significant increase ($P < 0.05$) in serum level of AST, ALP and ALT. This indicates the leakage of these enzymes into extracellular fluid as a result of toxic damage of liver tissue by the extract may be by damaging membrane of liver cells. The result of the present study is in line with those of Al-Hashem et al., [7]

who reported that the oral administration of hydro-ethanolic crude extract of khat shrubs in rats, for a period of one month, increased liver enzyme levels and concluded that *Catha edulis* extract had toxic effects on the liver of treated rats as evidenced by alterations in biomarkers of oxidative stress. Accordingly, Al-Mehdar et al., [8] reported that the oral administration of *Catha edulis* crude extract in rats significantly increased level of liver enzymes in serum. They suggested different mechanisms mediating the effect, including sympathomimetic effect and induction of oxidative stress.

TABLE 2: CORRELATION BETWEEN CHANGE IN DOSE OF CRUDE EXTRACT OF KHAT AND SERUM LEVEL OF LIVER BIOMARKERS

Independent variable	Dependent variable	Pearson's correlation coefficient (r)	P value
Change in dose of crude extract of khat	AST (U/L)	0.89	0.00
	ALT (U/L)	0.96	0.00
	ALP (U/L)	0.95	0.00
	T P (g/dl)	− 0.93	0.00
	TB (mg/dl)	0.94	0.00
	DB (mg/dl)	0.78	0.00

AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), TP (total protein), DB (direct bilirubin) and TB (total bilirubin).

In fact, it is well documented that the alkaloids of khat including cathinone, cathine and norephedrine have peripheral sympathomimetic effect which cause increasing the release of norepinephrine from adrenergic nerve terminals, and also cause inhibition of its reuptake as well as inhibition of its metabolic inactivation by monoamine oxidase enzyme [12]. Consequently, the increased norepinephrine concentration in postsynaptic space could exert hepatic vasoconstriction via stimulation of postsynaptic α1-receptors hence ischemic hepatitis may develop as a result of decrease hepatic blood flow [8].

During the drug metabolism in the liver hepatocytes, the oxidative phase-I and the conjugative phase-II of drug metabolism mechanism can result in hepatotoxic metabolites, although some parent xenobiotics are also known to induce oxidative stress in several mechanisms [14]. Accordingly, some chemical components of the khat extract might have been converted to prooxidant metabolites or the extract might have induced decreased synthesis/activity of the antioxidant system in treated rats that may resulted in damage in membrane integrity of liver cells by inducing oxidative stress hence those mitochondrial and cytosolic enzymes were leaked to the extracellular fluid [8]. In contrast, Al-Zubairi et al., [10] reported that the sub-chronic administration of Catha edulis (khat) crude extract in rats has no significant effect on liver enzymes concluding that the sub-chronic administration of Catha edulis crude extract has no hepatotoxicity. This inconsistency was resulted may be due to the difference in geographical origin of the khat plant utilized that can affect the chemical components of khat that are responsible for the effect.

The present study revealed that oral administration of crude extract of khat in rats produced significant ($P < 0.05$) elevation in serum ALP level, in rats treated with khat crude extract as compared to the control group rats, which may indicate damage of biliary epithelium as a result of khat induced cellular damage. On the other hand, the current study revealed that serum level of total and direct bilirubin was significantly increased ($P < 0.05$). This might be due to obstruction of intrahepatic biliary ducts that may be the cause for the increase in serum levels of ALP as reported in earlier publication [8].

Current study had demonstrated that the oral administration of crude extract of khat induced significant decrease ($P < 0.05$) in serum total protein in rats treated with khat crude extract as compared to the control group rats. This is in line with Al-Hashem et al., [7] who reported that crude extract of khat produced a significant decrease in serum total protein in rats, and suggested that it might be due to decreased protein synthesis resulted from liver cell damage. On the other hand,

tannins of khat inhibit the digestive enzymes [1,13] resulting in impaired digestion and hence the absorption of nutrients that, in this case, might caused decrease in synthesis of protein by the liver by reducing digestion and hence the absorption of amino acids.

Increased serum total and direct bilirubin have been linked to liver disease [14]. In this study, rats treated with 250, 350 and 450 mg/Kg crude extract of khat showed significant increase ($P < 0.05$) in serum level of total and direct bilirubin, as compared to the control group rats, suggesting a direct toxic effect of the extract on liver cells leading to decreased uptake and conjugation of bilirubin and reduced secretion into bile ducts [7]. As revealed by the current study, 150 mg/Kg crude extract khat did not induce statistically significant increase ($P < 0.05$) in serum level of total and direct bilirubin, unlike the other doses, as compared to the control group rats. This may indicate that chewers using high dose of khat are more vulnerable to khat induced liver damage [15].

Furthermore, the current study demonstrated that there is a positive relationship between the increases in dose of crude extract of khat and the change in serum level of AST, ALT, ALP, total bilirubin and direct bilirubin in rats treated with crude extract of khat. Pearson's correlation coefficient (r) is close to 1, indicating that there is a strong relationship between the two variables. This means that increase in dose of crude extract of khat is strongly correlated with increase in the serum level of AST, ALT, ALP, total bilirubin and direct bilirubin.

However, the result of the current study illustrated that there is a negative correlation between the increases in dose of crude extract of khat and the serum level of total protein in rats treated with crude extract of khat. The Pearson's correlation coefficient (r) is close to 1, indicating that there is a strong relationship between the two variables. This means that the increase in dose of crude extract of khat is strongly correlated with decrease in the serum level total protein.

CONCLUSIONS: It could be concluded from the present study that oral administration of crude extract of khat fresh leaves and soft twigs had toxic effects on the liver in rats as evidenced by alterations in serum level of the liver enzymes, total protein and bilirubin. In current study, the increase in dose of crude extract of khat was strongly correlated, linearly, to the change in serum level of liver biomarkers assayed.

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