

Khat (*Catha edulis*) Extract Increases Oxidative Stress Parameters and Impairs Renal and Hepatic Functions in Rats

Fahaid H Al-Hashem, MBBS, PhD* Ismaeel Bin-Jaliah, MBBS, PhD** Mohammad A Dallak, MBBS, PhD** Luke O Nwoye, PhD, PD (Physiol)* Mahmoud Al-Khateeb, MSc*** Hussein F Sakr, MBBS, PhD** Refaat A Eid, MBBS, PhD**** Khalid S Al-Gelban, MBBS, SSCFM***** Hasan S Al-Amri, MBBS, SSC-Psych***** Mohamed A Adly, BSc, PhD*****

Background: The habit of khat chewing represents a major socio-economic problem in many countries but research into its hepato-renal toxic effects has produced contradictory results.

Objective: To evaluate the subacute effects of Khat (*Catha edulis*) extract on hepatic and renal functions in white albino rats.

Design: Randomized experimental animal study.

Setting: Physiology laboratory, medical school of King Khalid University.

Method: Twenty white albino rats aged between 14 and 16 weeks were included in the study. The rats were assigned randomly into two groups, ten each. Treated rats received orally administered hydro-ethanol extract of *Catha edulis* for four weeks. Control rats received corresponding amounts of normal saline.

Result: There was statistically significant increase in the activities of hepatic enzymes in treated rats compared to the control group. In addition, serum urea, bilirubin and phosphorous concentrations were significantly increased compared to a decreased serum total protein and albumin concentrations. Oral administration of the extract induced lipid peroxidation and oxidative stress in hepatic and renal tissues as shown by significant increases in lipid peroxidation biomarkers thiobarbituric acid reactive substances (TBARS) and significant decreases in levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Histological examination of *Catha edulis* treated rats revealed marked hepato-renal pathological changes compared to the control group.

Conclusion: These results indicate that orally administered *Catha edulis* extract exerts severe hepato-nephro toxicity and the mechanism of this damage may be related to oxidation, increased lipid peroxidation, and generation of free radicals inside these tissues.

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* Associate Professor, Department of Physiology
 ** Assistant Professor, Department of Physiology
 *** Lecturer, Department of Physiology
 **** Assistant Professor, Department of Pathology
 ***** Associate Professor, Department of Family and Community Medicine
 ***** Assistant Professor, Department of Internal Medicine, College of Medicine

*****Assistant Professor, Department of Biology
College of Science
King Khalid University
Saudi Arabia
Email: fahaid999@yahoo.com

Khat (*Catha edulis*) is an evergreen shrub which grows in some high altitude areas of East Africa and the Arabian Peninsula. People chew fresh young khat leaves for their stimulant and pleasurable effects which are attributed mainly to cathinone^{1,2}. The habit of khat chewing represents a major socio-economic problem in the countries of Southern Arabia and the Horn of Africa². Although the use of khat has spread worldwide, it has remained most deeply rooted in the source countries because only the fresh leaves have the potency to produce the desired effects. It is estimated that there are five to ten million regular khat users, worldwide³.

The stimulating and euphoric effects of khat provide a strong inducement for the user to obtain daily supplies and to engage in regular khat chewing sessions, especially as tolerance develops with regular use. This strongly suggests development of psychic or physical dependence or both in the user⁴. The medical and socio-economic impact of khat use on society has generated a debate as to whether khat should be considered an illegal drug and banned or tolerated as an innocuous stimulant, similar to caffeine. The World Health Organization takes the former view and classifies khat as a substance of abuse while the source countries maintain an ambiguous legal position on khat⁵.

Khat use has been reported to affect the cardiovascular, digestive, respiratory, endocrine, hepatobiliary and genito-urinary systems³. Recent studies demonstrated toxic effects of khat leaves, both short and long-term, on rabbit liver, as evidenced by significant increases in plasma levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)^{1,4}.

In other studies on rats, administration of khat was reported to cause a reduction in the liver enzyme, alkaline phosphatase (ALP), and increased activities of acid phosphatase, lactate dehydrogenase (LDH) and increased total bilirubin⁶. In yet another study, on humans, it was shown that regular khat chewing may cause kidney damage, as total serum protein levels were reduced in khat consumers while the levels of urea and creatinine were greatly increased⁷. Aside from these biochemical changes, other studies have reported histopathological changes in both livers and kidneys of treated rats^{1,8}.

The mechanism of khat toxicity on liver and kidney is uncertain. However, the generation of free radicals and oxidants is now seriously implicated in khat toxicity as oral administration of khat in rats was associated with decreased serum free radicals metabolizing enzymes such as superoxide dismutase (SOD) and catalase (CAT)^{8,9}. However, these studies have measured these components in serum and no study has measured them in liver or kidney tissues.

The first aim of this study is to revisit hepato-nephro toxicity and to evaluate the mechanism of khat cellular damage by measuring antioxidant components and lipid peroxidation in renal and hepatic tissues.

METHOD

Preparation of *Catha edulis* Shrub Extract and Dose Selection

This study was performed from 10th October to 10th December, 2009. Fresh shrubs of *Catha edulis* (stem tips and leaves) were used. The plant material was washed, dried and extracted with 500 ml of water ethanol-mixture (70/30%, V/V) at room temperature and then filtered. The filtrate was evaporated in a vacuum at 40°C to remove all traces of ethanol. The resulting ethanol-free extract (20 gm), which constituted about 10.7% of the original dry material, was dissolved in freshly prepared normal saline to a final concentration of 200 mg/ml; 0.5 ml of the extract was administered orally to the animal. The dose of the extract used was about 100 mg/rat (equivalent to 500 mg/kg of dry plant). Dose selection was based on the average amount of khat leaves chewed daily by khat chewers^{9,10}.

Experimental Animals

Twenty white albino rats aged between 14 and 16 weeks and weighing between 180-200 gm were used for the study, fed standard rat pellets and allowed free access to water. They were housed in plastic cages (5 rats/cage) at a controlled ambient temperature of 22 ± 2°C and 50 ± 10% relative humidity, with 12 hour light/12 hour dark cycles. The study was approved by the National Institute of Health Guide for the Care and Use of Laboratory Animals¹¹.

Experimental Design

The rats were assigned randomly to two groups, ten each. The animals were identified with differently colored tail marks and housed in different segments of the cage, according to their group. The experimental group received single doses (500 mg/kg body weight) of *Catha edulis* extract daily while the control group received equivalent amounts of normal saline daily. At the end of day 28, the rats were subjected to overnight fasting and then blood samples were collected directly from tail veins.

Preparation of Liver and Kidney Homogenates

Rats were sacrificed; the livers and kidneys were quickly removed, washed in ice-cold, isotonic saline and blotted individually on ash-free filter paper. Some liver tissues were then homogenized separately in 0.1 M Tris-HCl buffer, pH 7.4 using a Potter-Elvehjem homogenizer at 4°C with a diluting factor of 4. The crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 minutes in cold centrifuge; the supernatant was kept at -20°C for estimation of TBARS, GSH, SOD and CAT activities.

Histological Studies

Routine histological examination of liver and kidney was performed. Tissue fixation was carried out with 10% neutral buffered formaldehyde solution (pH 7.0).

Biochemical Assays

Uric acid, creatinine, urea, total protein content and albumin in the serum were estimated using a Refletron automated analyzer. Liver enzymes AST, ALT, ALP and glutamyl transpeptidase (γ -GT) were assayed. All spectrophotometric measurements were carried out using a Jenway UV visible spectrophotometer.

Estimation of Lipid Peroxidation and Oxidative Stress Parameters in Tissue Homogenates

The concentration of TBARS in liver and kidney homogenates was determined by the method of Okhawa¹².

The GSH content of liver and kidney homogenates was measured at 412 nm using commercially available kits (Randox Laboratories, UK).

CAT activity in liver and kidney homogenates was assayed using commercially available catalase activity assay kits (Biovision, K773-100).

The activity of SOD in liver and kidney homogenates was assayed using commercially available SOD activity assay kits (Biovision, K335-100).

Results were expressed as the mean value \pm SEM. Statistical differences between groups were assessed by Student's t test. Values of $p < 0.05$ were considered significantly different.

RESULT

Table 1 shows that administration of *Catha edulis* extract to rats for a period of 28 days resulted in statistically significant increases in the activities of ALT, AST, γ -GT and ALP in treated rats compared to the control group. In addition, the extract caused significant increases in the levels of serum urea, bilirubin and phosphorus ions, accompanied by significant decreases in serum total protein and albumin levels.

Table 1: Levels of Hepatic Enzymes, Urea, Total Protein, Albumin, Bilirubin and Phosphorus in the Serum of Control and *Catha edulis* (CE) Treated Rats

Parameter	Control group	CE-treated group
ALT (U/L)	31.8 + 1.30	65.2 + 3.19*
AST (U/L)	73.6 + 1.34	123 + 3.51*
ALP (U/L)	75.3 + 2.17	117 + 2.55*
GTT (U/L)	12.6 + 1.14	21.0 + 1.87*
Urea (mg/dl)	30.2 + 1.92	53.2 + 3.96*
Protein (g/dl)	6.06 + 0.207	4.24 + 0.321*
Albumin (g/dl)	3.66 + 0.219	1.16 + 0.114*
Bilirubin (mg/dl)	0.38 + 0.030	0.76 + 0.08*
Phosphorus (mg/dl)	6.7 + 0.877	8.7 + 0.965*

Values are given as mean \pm SEM for groups of 10 animals each. Values are statistically significant at $*P < 0.05$. *Catha edulis* treated rats were compared with control rats. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase and GTT: γ -glutamyl transpeptidase

Table 2 shows a significant increase in the serum level of TBARS, and significant decrease in the levels and activities of GSH, SOD and CAT in liver homogenates of *Catha edulis* treated rats compared to rats treated with normal saline. Similar results were obtained with kidney homogenates, as shown in table 3. Treatment with *Catha edulis* extract resulted in a significant increase in TBARS level and significant decreases in the levels of GSH, SOD and CAT.

Table 2: Thiobarbituric Acid Reactive Substances (TBARS), Reduced Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) Levels in Liver Homogenate of Control and *Catha edulis* (CE) Treated Rats

Parameter	Control group	CE-treated group
TBARS (mM/100g of tissue)	1.04 ± 0.07	2.28 ± 0.30*
Glutathione (mg/100g of tissue)	57.6 ± 3.75	31.23 ± 2.56*
SOD (U/mg of protein)	10.05 ± 0.870	5.76 ± 0.67*
CAT (U/mg of protein)	65.34 ± 2.01	39.9 ± 2.1*

Values are given as mean ± SEM for groups of 10 animals each. Values are statistically significant at * $P < 0.05$. *Catha edulis* treated rats were compared with control rats. TBARS: Thiobarbituric acid reactive substances, SOD: Superoxide dismutase and CAT: Catalase.

Table 3: TBARS, GSH, SOD and CAT Levels in Kidney Homogenate of Control and *Catha edulis* Treated Rats

Parameter	Control group	CE-treated group
TBARS(mM/100g of tissue)	0.85 ± 0.003	1.68 ± 0.004*
Glutathione(mg/100g of tissue)	37.6 ± 1.67	21.23 ± 1.54*
SOD(U/mg of protein)	21.03 ± 1.89	10.56 ± 1.01*
CAT(U/mg of protein)	42.14 ± 3.1	31.0 ± 2.9*

Values are given as mean ± SEM for groups of 10 animals each. Values are statistically significant at * $P < 0.05$. *Catha edulis* treated rats were compared with control rats. TBARS: Thiobarbituric acid reactive substances, SOD: Superoxide dismutase and CAT: Catalase.

Histopathological examination of the livers of *Catha edulis* treated rats revealed marked degenerative changes compared to the control animals, see figure 1, C-F. Observed degenerative alterations include disorganization of hepatic cords, cytoplasmic vacuolization of hepatocytes and invasion of infiltrative inflammatory cells. Furthermore, necrotic changes were seen.

Microscopic examination of the kidneys of treated rats also showed major changes, see figure 2. Atypical tubules and amorphous Malpighian corpuscles as well as invasion of infiltrative inflammatory cells were observed. As seen in figure 2 (B), Malpighian corpuscles showed hypertrophied glomerular capillaries and dilated Bowman's capsules. Some tubular cells exhibited cytoplasmic vacuolar degeneration, see figure 2, E. Major changes were detected in some kidneys of *Catha edulis* treated rats. These included amorphous glomerular capillaries and infiltrative inflammatory cells as well as complete cytoplasmic vacuolization of tubular cells, see figure 2, D-F. In a few instances, complete destruction of glomerular capillaries was observed, see figure 2, D.

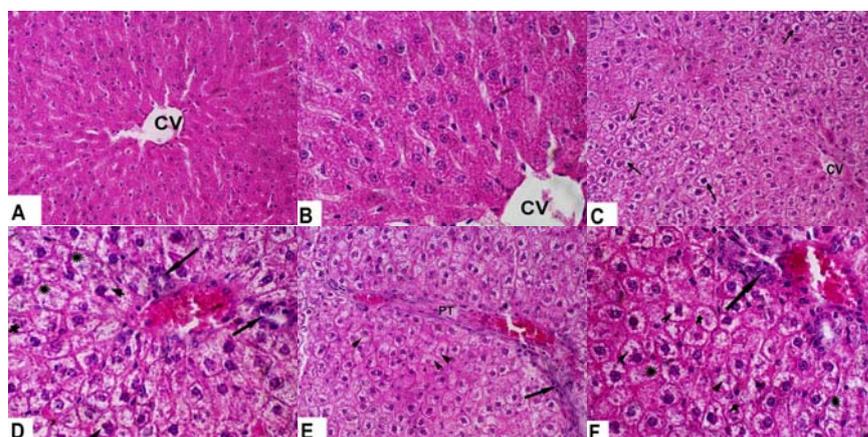


Figure 1 (A-F): Liver of Normal and Khat-Treated Rats

- A, B:** Liver of normal control rat, 20x and 40x
C: Liver of Khat-treated rat, showing two central veins and disorganized hepatic cords with most hepatocytes having cytoplasmic vacuolization (arrows), 20x
D: Large magnification of the liver of Khat-treated rat showing infiltrative inflammatory cells (arrow) and hepatocytes with apparent cytoplasmic vacuolar degeneration (arrowheads) as well as necrotic changes. Note karyorrhexis (stars) and karyolysis (asterisks), 40x
E: Liver of Khat-treated rat, showing a portal tract, inflammatory infiltration and vacuolized hepatocytes (arrowheads), 20x
F: Large magnification of the liver of Khat-treated rat showing more infiltrative inflammatory cells (arrow) and more hepatocytes with cytoplasmic vacuolar degeneration (arrowheads) as well as necrotic alterations. Note karyorrhexis (stars) and karyolysis (asterisks), 40x H & E stain

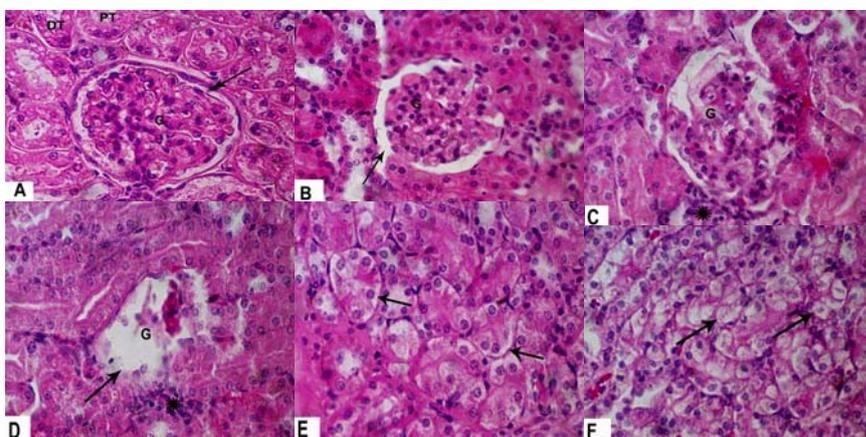


Figure 2: Kidney of Normal and Khat-Treated Rats

- A:** Normal kidney with intact Malpighian corpuscle and convoluted tubules, 40x
B: Kidney of Khat-treated rat showing hypertrophied glomerular capillaries and injured dilated Bowman's capsule (arrow)
C: Kidney of Khat-treated rat showing amorphous glomerular capillaries and infiltrative inflammatory cells (asterisk), 40x
D: Kidney of Khat-treated rat, showing complete destruction of glomerular capillaries (arrow) and infiltrative inflammatory cells (asterisk), 40x
E: Kidney of Khat-treated rat, showing partial epithelial cell vacuolar degeneration of the tubules (arrows), 40x
F: Kidney of Khat-treated rat, showing major epithelial cell vacuolar degeneration of the tubules (arrow), 40x H & E stain

DISCUSSION

Levels of hepatic enzymes AST, ALT, ALP and GGT are elevated in liver damage due to tissue necrosis or membrane damage¹³. In the current study, the activities of these enzymes were elevated in the serum of *Catha edulis* extract treated rats, indicating their leakage into extracellular fluid as a result of toxic damage of hepatic tissue by the extract. Our findings are consistent with those of Al-Habori et al who reported that long term feeding of khat leaves to New Zealand white rabbits increased liver enzyme levels and concluded that

prolonged exposure to *Catha edulis* leaves may lead to toxic hepatocellular jaundice⁴. Hyperbilirubinemia is often the first and sometimes the only manifestation of liver disease¹⁴. Oral administration of *Catha edulis* hydro-ethanol extract in our study significantly increased serum bilirubin, 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.

In this study, there was a significant decrease in serum total protein and albumin of *Catha edulis* extract treated rats as compared to control rats. This indicates impaired liver function, decreased protein synthesis, either primary as in liver cell damage or secondary to diminished protein intake and reduced absorption of amino acids¹⁵.

Increased serum urea and creatinine have been linked to kidney disease¹⁵. In this study, rats treated with *Catha edulis* extract had significantly increased serum creatinine suggesting impaired renal function due to a reduced ability to excrete these products. These effects could originate from changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate^{6,7,16}. *Catha edulis* extract also increased serum phosphate concentration, another evidence of impaired renal excretory function. These results are in broad agreement with those of Al-Motarreb et al, Al-Meshal et al and Dimba et al, all of whom reported khat induced cytotoxicity in livers and kidneys after oral administration of khat to animals^{1,17,18}.

Many studies have focused on the direct histopathological features of *Catha edulis* on oral tissues, few have addressed its systemic effects and little is known about the effect of *Catha edulis* extract on liver and kidney histology^{19,20}. The degenerative changes in liver and kidney seen in this study, including cytoplasmic vacuolization of hepatic and tubular cells, invasion of infiltrative inflammatory cells, etc, support earlier findings of liver function abnormalities and urinary disorders (poor micturition and reduced urine flows) associated with khat chewing^{21,22}.

Lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury²³. In our study, TBARS (a marker of redox balance and lipid peroxidation in cells) level increased two folds in the liver and kidney homogenates of *Catha edulis* extract treated rats. Some constituents of the extract might have been converted to prooxidant metabolites or the extract might have induced decreased synthesis/activity of the antioxidant system in treated rats. In addition, the extract could have increased fatty acyl coenzyme A oxidase activity leading to increased lipid peroxidation.

In this study, the activities of SOD and CAT were decreased in rats treated with *Catha edulis* suggesting that the extract generated free radicals or directly inhibited synthesis of antioxidant enzymes. This finding is similar to a recent study in which administration of *Catha edulis* extract or its alkaloid fraction was shown to alter the activities of the free-radical metabolizing/scavenging enzyme system⁹. GSH is a thiol which plays a central role in coordinating the body's antioxidant defense processes. The role of glutathione as a protective agent against oxidative organ damage has been the subject of extensive studies²⁴. The exposed sulfhydryl groups in glutathione bind to a variety of electrophilic radicals and metabolites that may cause cell damage¹⁵.

As expected, *Catha edulis* treatment markedly depleted hepatic GSH stores in rats. It could be hypothesized that administration of the extract led to saturation of detoxification pathways in the liver so that intermediate metabolites accumulated and caused liver damage by covalent binding to tissue molecules and proteins such as GSH. Another hypothesis could be

that *Catha edulis* extract contains an oxidizing agent or causes suppression of GSH synthesis leading to increased production of reactive oxygen species and induction of oxidative stress¹⁷.

The results presented in this paper have confirmed the toxic effects of khat extract on hepatic and renal functions in white albino rats. They also show, for the first time, that this toxicity may be related to lipid peroxidation and oxidative stress in hepatic and renal tissues as indicated by a significant increase in lipid peroxidation biomarkers (TBARS), and a significant decrease in levels of the antioxidant components SOD, CAT and GSH.

CONCLUSION

Our data reveal that oral administration of *Catha edulis* extract had diverse toxic effects on the liver and the kidney of treated rats as evidenced by alterations in biomarkers of oxidative stress and biochemical indices of liver and kidney function with corresponding histological changes.

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