Regular Khat (*Catha edulis*) Feeding Induce Toxicity in Rabbit Tissue Systems

Wafaa Ibrahim ALRajhi and Olfat Mohamed H. Yousef

***Abstract*—**The present study was evaluated to study the short-term effects of fresh leaves khat(*Catha edulis)* on hepatic and renal Functions in rabbits. . A total of 15 Male rabbit were assigned to three groups of five rats each. There was a significant increase in alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities of khat feeding rabbits. Serum total protein(TP) and albumin(ALB) concentrations were significantly decreased,while , a significant increases in blood urea (BU), creatinine (CREA] and bilirubin (Bi) concentrations . except TP and ALBU, there was still significantly decreases. Although they showed significant increases compared to *Catha edulis* consuming rabbits . The Histological investigation revealed marked changes iIn liver, disorganization of hepatic cells ,fatty degeneration, and focal necrosis in many areas of the liver.Also in kidney, it caused damage of renal corpuscles including shrunken, congested and hypertrophied of the glomeruli. However, after khat- withdrawal there was a clear improvement of the biochemical and histological structure of liver and kidney**.**

***Keywords*—** *Catha edulis*, enzymes, kidney damage, liver damage

I. Introduction

KHAT (*Catha edulis*) leaves were chewed daily as a social habit by a high proportion of the adult population of East Africa and Arabian Peninsula [1] . The main active ingredient in fresh khat leaves is Cathinone ,whose pharmacologically structure similar to amphetamine [2]. The cathinone that released after chewing khat produced feeling of euphoria. The result of regular khat chewing induced an increase in pulse rate and arterial blood pressure. In addition, cardiac complications, gastric disorders , liver damage, insomnia, anorexia and depression in long term user [3]. Also, sexual dysfunction, duodenal ulcer and hepatitis [1]. Different studies demonstrated a significant increases in plasma levels of aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of rabbit liver [4],[5]. The same results were reported by Al-Hashem et al.[6] on rats orally administered *Catha edulis* extract. Additionally, khat administration was resulted in reduction of alkaline phosphatase (ALP), and increased activities of lactate dehydrogenase (LDH) , acid phosphatase, and total bilirubin [7]. Khat extract ,in another study , causes reduction in total serum protein levels khat while the levels of creatinine and urea were significantly increased [6]. Khat chewing cause acute liver dysfunction in man and [9] chronic liver injury in animals [5,8,6]. Histopathological sings of liver were observed as hepatocytes damage and congestion of the central vien. While, there were some kidney lesions , acute tubular nephrosis, swelling in the cortical tubules, hyaline cast and fat droplets (10). This study aimed to evaluate the adverse effects of khat consuming on hepatic and renal Functions in rabbits.

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II. Material And Methods

Khat leaves were obtained regularly from a local supplier. Dose selection (2 gm/ kg of b.w.) of khat leaves consumed daily according to Hassan *et al*. [11].

Experimental Animals— Fifteen *Oryctolagus cuniculus* rabbits (1000-1200 g / w) were housed in individual cages receiving food and water *ad libitum* until the beginning of experiment.

Experimental Design — Animals were divided into three groups (5 rabbit each):-

Group 1: control group, fed standard food.

Group 2: khat group, they were given standard food containing 2 gm/ kg fresh khat leaves for 21 days .

Group 3: khat withdrawal group, the experimental animals were given standard food containing 2 gm/ kg fresh khat leaves for 14 days; followed by the standard chow for 7 days.At the end of experimental, animals were subjected to overnight fasting ,they were sacrificed by decapitation.

Biochemical Analysis— Blood was collected, sera separated at 3000rpm by centrifugation for 10 m. Serum was assayed for alkaline phosphatase (ALP)**,** alanine aminotransferase (ALT) **,** aspartate aminotransferase (AST), bilirubin (Bi), blood urea (BU), creatinin (CREA), total protein(TP) and albumin(ALBU) using enzymatic kits.

Histopathological Studies— Samples of liver and kidney were quickly removed for routine histological examination. Sections were stained in hematoxylin and eosin, microscopically examined and photomicrographs made .

Statistical Analysis— Analyzing data were made by student's t-test statistical methods. For the statistical tests a p value of less than 0.05 was taken as significant. All the results were expressed as mean ± standard error of the mean.

III. Results

Biochemical Results— Khat feeding to rabbits for 21 d. (G2) resulted in increases significantally in the activities of ALP, ALT and AST enzymes (37.40%, 40.85%, 38.00% respectively) compared to group one as showen in Table 1. In addition, there were significant increase in the levels of BU, CREA and Bi (34.25%, 25.72%, 102.2% respectively) as showen in Table II, accompanied by significant decreases

in TP and ALBU levels (14.65% , -14.84% respectively) compared to the control group as showen in Table II. In khat- withdrawal rabbits, all parameters assayed restore their normal values except TP and ALBU, there was still a significant decrease (-6.37%, -5.50%) compared to the group one. Although they showed significant increases (9.70%,10.97% respectively) compared to *Catha edulis* feeding rabbits.

TABLE I
BIOCHEMICAL ASSAYED OF HEPATIC ENZYMES, ASPARTATE AMINOTRANSFERASE [AST], ALANINE AMINOTRANSFERASE [ALT] AND ALKALINE PHOSPHATASE [ALP], IN THE SERUM OF THE DIFFERENT ANIMAL GROUPS.

Animal Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
G1(normal control)	36±0.60	79.8±0.85	59.6±0.38
G2 (khat feeding)	50±0.54*	112.4±0.61*	95.2±1.5*
G3(khat –withdrawal)	$^{-a}$35.2±0.66	$^{-a}$80.6±0.34	$^{-a}$58.6±0.69

(Mean±SE) ; n= 5 , P*>0.005 as relative to G1, P^{-a}>0.005 as relative to G2.

TABLE II
BIOCHEMICAL ASSAYED OF BLOOD UREA [BUN], CREATININE [CREA], BILIRUBIN [BI], TOTAL PROTEIN [TP] AND ALBUMIN [ALB] IN THE SERUM OF THE DIFFERENT ANIMAL GROUPS.

Animal Groups	BUN (mmol/L)	CREA (Umol/L)	Bi (Umol/L)	TP (g/L)	ALB (g/L)
G1(normal control)	6.22±0.09	97.2±0.75	4.5±0.06	62.8±0.38	18.2±0.21
G2(khat feeding)	9.46±0.06*	122.2±1.04*	9.1±0.11*	53.6±0.25*	15.5±0.07*
G3(khat-withdrawal)	$^{-a}$6.4±0.06	$^{-a}$98±0.44	$^{-a}$4.4±0.6	$^{-a}$58.8±0.68*	$^{-a}$17.2±0.06**

(Mean ±SE) ; n= 5 , P*>0.005, P**>0.05 as relative to G1, P^{-a}>0.005 as relative to G2

Histopathological Observations— Liver of control rabbit is(G1) is formed of hepatic cords radiate from the central vein and separated by narrow blood sinusoids (fig.1).

Livers of rabbit consuming khat (G2) revealed destruction of the normal hepatic architecture and severe pathological alterations. Many hepatocytes showed vacuolar degenerative changes in their cytoplasm and focal necrotic areas could be observed containing pyknotic and karyolitic nuclei (Fig.2) . Apoptotic hepatocytes were found and were characterized by condensed and fragmented nuclei noticed between the liver cells (Fig.3). The central veins were severely damaged ; they appeared dilated , congested and contained stagnant hemolyzed red blood cells with cellular infiltration (Fig.3). In addition, the hepatic sinusoids were dilated and Kupffer cells were markedly increased in size, they were activated and pushed into the sinusoidal lumens (Fig.2). Rabbit withdrawal khat (G3) revealed marked restoration of the hepatic configuration. The hepatic cords were organized and hepatocyte with little cytoplasmic vacuoles . Most nuclei exhibited normal shape, being rounded and centrally located except for a few pyknotic ones (Fig.4).

Kidney of rabbit (G1) is formed of two main portions, renal corpuscle and the uriniferous tubule (fig.1). Kidney of rabbit consuming Khat (G2) revealed variable degrees of pathological alterations of few glomeruli ; they were congested, shrunken and destructed (figs 6&7). Besides, the glomeruli became solid, hypertrophied and dilated and the urinary spaces displayed marked narrowing (fig.7). The cells of the lining epithelium of the renal tubules showed swelling, vacuolation, loss of nuclei and necrosis (figs 6&7). Also, some tubules displayed wide lumina (fig.7). The inter-tubular capillaries were congested and inflammatory cell infiltration were detected between the renal tubules (fig. 6). In khat- withdrawal rabbits (G3) the kidney revealed little pathological changes when compared with rabbit consuming khat. Bowman's capsules were intact, the glomeruli displayed normal built-up except mild congestion. The epithelial cells of the renal tubules were almost healthy except some with pyknotic nuclei and little cell debris in the lumina of few tubules (fig. 8) .

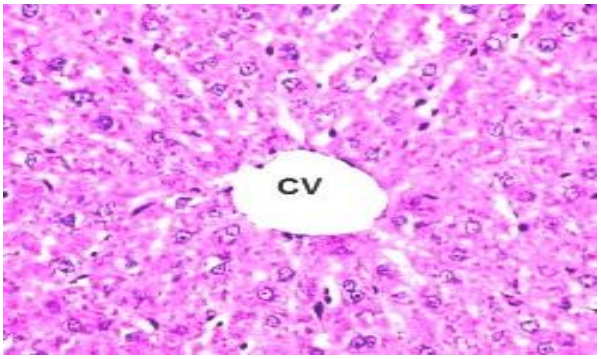

Fig.1 Section of control rabbit liver showing hepatic cords radiating from a central vein (CV) . (H&E; X40).

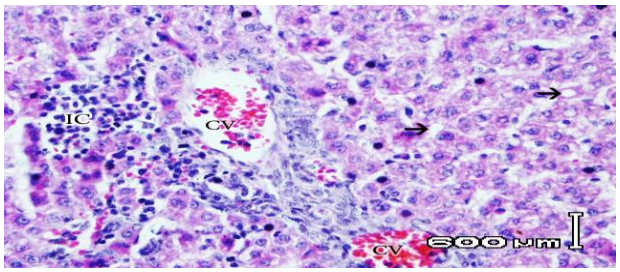

Fig.2 Khat consuming rabbit liver showing accumulation of inflammatory infiltrative cells (IC) ,dilated and congested central vein (CV) fatty degeneration(arrow) and deteriorated nuclei and loss of regular arrangement of hepatic configuration. (H&E; X40).

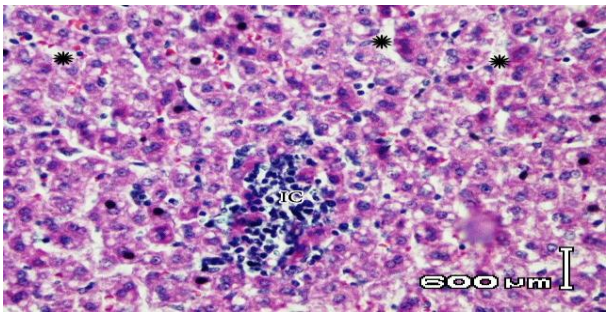

Fig.3 Liver section of khat consuming rabbit showing accumulation of inflammatory infiltrative cells (IC) ,dilated sinusoids (*) and activated Kupffer cells pushed into the lumina of the sinusoids (H&E; X40).).

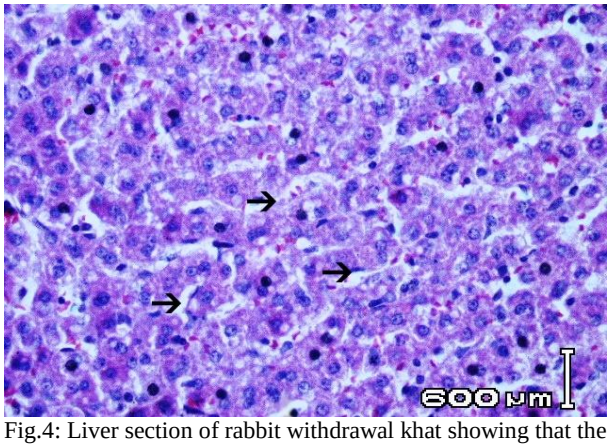

Fig.4: Liver section of rabbit withdrawal khat showing that the hepatocytes partly restored their normal configuration with slightly dilated sinusoids (arrow) (H&E; X40).

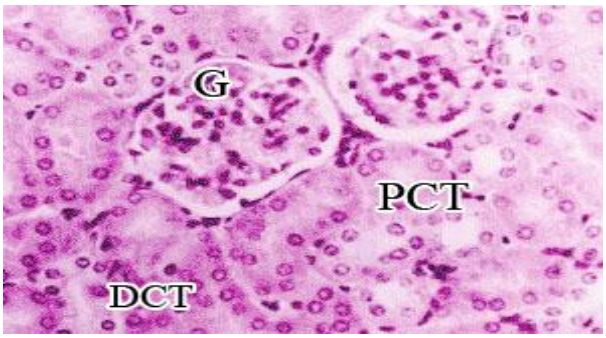

Fig.5: Control rabbit section showing , renal corpuscle (G) , proximal (PCT) and distal (DCT) convoluted tubulules . (H&E ; X40)

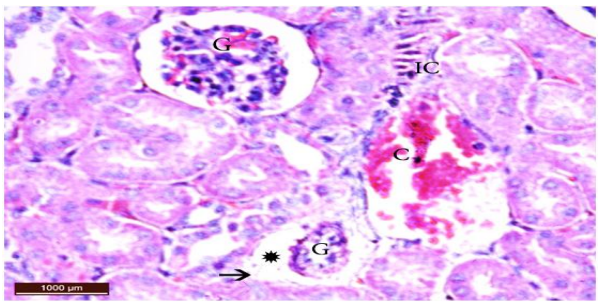

Fig6 Kidney of rabbit consuming khat showing , congested glomerulus (G) with wide urinary space (*) and distorted Bowman's capsule (arrow) congestion of the intertubular blood capillaries (C) with infiltrated inflammatory cells (IC) . (H&E ; X40)

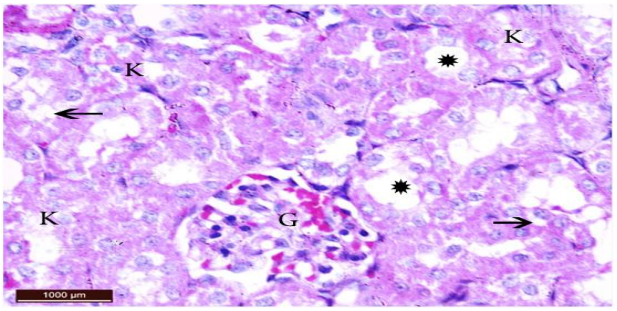

Fig7 Kidney of rabbit consuming khat revealed vacuolar degeneration of the cytoplasm of renal tubules (arrow), widening of tubular lumen (*), distruction of the brush border , necrosis and karyolysis of most renal tubular nuclei (k), hypertrophied and proliferation of the glomerulus with increase of the mesangial cells (G) . (H&E ; X40)

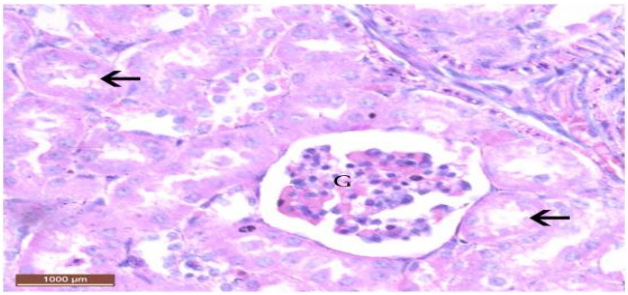

Fig.8 Kidney of rabbit withdrawn chewing khat showing congested and vacuolated glomerules (G) many tubules recovered while others still revealing swollen lining epithelium (arrow) and inter tubular inflammatory cells infiltrated . (H&E ; X40)

IV. Discussions

During tissue damage, some enzymes that did not originally originated from the serum leakages and found in the serum. Therefore, measurements of serum enzymes are available tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue [6]. In this study, both the biochemical and histopathological results demonstrate an initial signs of khat toxicity. Due to tissue necrosis or membrane damage ,the activities of hepatic enzymes AST, ALT and ALP increased in liver damage [12],[13]. In the current study, The activities of these enzymes were increased in the serum of Khat fed rabbits, indicating their leakage into extracellular fluid . The current results are consistent with Al-Habori *et al* .[4] who declared that long term feeding of Khat leaves to Neww

Zealand white rabbits elevated liver enzyme activities andlead to toxic hepatocellular jaundice. In contrast, there were other studies on rats reported that ,administration of Khat caused decrease of enzyme activities of ALS, ALT, and ALS. Hyperbilirubinemia is often the first and sometimes the manifestation of liver disease [14].The significantly increased of serum bilirubin in khat feeding rabbits, referred to, the direct toxic effect the khat on liver cells leading to conjugation of bilirubin and reduced secretion into bile ducts [6]. In this study, there was a significant decrease in serum total protein and albumin of khat feeding rabbits with compare to the control rabbits. This indicates impaired liver function ,decreased protiein synthesis due to liver cell damage or diminished of protein intak and reduced of absorption of amino acids [15].

Increased blood urea and creatinine have been linked to kidney disease [16]. In current study, khat feeding rabbits had significantly increased serum creatinine refereeing impaired renal function due to a reduced ability to excrete these product. These effects could originate from changes in the thteshold of tubular reabsorption , renal blood flow and glomerular filtration rate [17]. The present results are in agreement with the results of Al-Hashem *et al*.[6], Al-Motarreb *et al*.[5], and Dimba *et al*.[18] whoes reported that khat induced cytotoxicity to liver and kidney after . On the contrary, ,Devaki *et al* .,[19] reported no adverse effect on the functions of the liver and kidney in rats.

The changes in liver and kidney in the current study, including cytoplasmic vacuolation of hepatic cells and tubular cell invasion of infiltrative inflammatory cells, support many finding of liver and kidney disorders associated with khat chewing [5], [6], [8],[20]. The mechanism of khat toxicity on liver and kidney is uncertain. However , this toxicity may be related to lipid proxidation and oxidation stress in hepatic and renal tissues as indicated by a significant increase in lipid oeroxidation biomarkers, thiobarbituric acid reactive substances and significant decrease in level s of the antioxidant components superoxide dismutase ,catalase and glutathione[6], [8],[21].

Administration of khat extracts revealed a deranged systemic capacity to handle oxidative radicals and induces cytotoxic effects in cell of liver and kidney , as well as induction of cell death in various human leukaemia cell lines and in peripheral human blood leukocytes [22]. In addition Al-Akwa *et al*. [23] reported that khat may promote synthesis of reactive oxygen and nitrogen species in the same way that amphetamine promote free radical production. Finally, Hegazy *et al* [24] reported that the accumulative effect of khat may induce liver damages.

V. Conclusion

Alteration in the histological and biochemical indices will lead to impairment of normal functioning of the organs The results presented in this paper confirmed the toxic effects of khat chewing on hepatic and renal functions in rabbits.

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