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# A preliminary study on the effects of Khat (*Catha edulis*) on liver of mice

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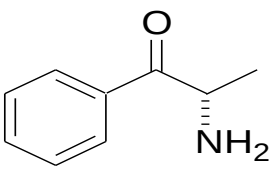
## Abstract

Khat is a psychostimulant and its use is gradually expanding worldwide. Khat is associated with health problems affecting the cardiovascular, respirator, reproductive and gastrointestinal systems. Effects of Khat on liver are not well documented. The study aim was to evaluate Khat extract effects on liver of mice. Khat alkaloids were extracted from fresh Khat shoots and leaves. The Khat extract were administered orally through intubation to seven groups of male albino mice, six weeks old with an average weight of 20-25g. Khat extract was administered in combination of 2ml tea, 2ml water, 2ml milk, 2ml coffee, 2ml coke, 2g patico sweet and 2g groundnut. To each group Khat extract was administered at a dosage of 2000mg/Kg/day. Two other groups were used as a negative and positive control. The negative control group consumed only rodent pellet and water. The positive control group was administered with indomethacin at a dosage of 2mg/Kg/day (as a twice dose per day). Before, during and at the end of the study, animal were monitored for morbidity and mortality. After thirty days of Khat extract oral administration, blood samples were collected from all animals for hematological and biochemical analysis. The biochemical analysis included the liver function tests such as serum aspartate aminotransferase, alkaline phosphatase and serum total bilirubin. The histological and cytological studies were done using H & E staining technique. There was an increased level of hepatorenal biochemical markers in all albino mice serum into which Khat extract was administered. The hepatorenal biochemical enzyme markers were more elevated in the positive control groups. The levels of hepatorenal biomarkers were within the normal range in the negative control group. There was a significant different between levels of hepatorenal biochemical enzymes biomarkers at $p<0.05$. There were signs of adverse effects of Khat on liver of mice.

**Keywords:** Khat, Cathine, Cathinone, Albino mice, Hepatotoxicity

## Introduction

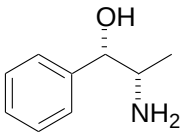
Khat scientifically known as *Catha edulis* (Vahl.) Endal., Celastraceae is a slow growing shrub inhabiting the tropical regions of Arabian Peninsula and East Africa. Countries that Khat mainly grows are: - Ethiopia, Kenya, Madagascar, Somalia, South Africa, Sudan, Afghanistan, Pakistan and Yemen [1]. Khat leaves are consumed while fresh to avoid loss of stimulatory potency. Khat leaves losses stimulatory potency after 3-4 days of harvesting [2]. It causes mild euphoria with excitement similar to those associated with strong coffee after consumption. Khat consumption also leads to talkativeness, mydriasis, constipation, and increased rate of the heart, raised blood pressure, ulcers, low libido and darkening of the teeth [3]. It is consumed to increase work performance [4] and also has medicinal value to those suffering from pain, arthritis, fever and depression [5]. Khat is also used for social functions such as marriages in some communities and also to enhance contacts with gods in some communities and religions [3]. It contains alkaloids called cathinone (Figure 1) and cathine (Figure 2) which is are amphetamine like [6]. Cathinone is the most central nervous system (CNS) stimulant active ingredient in the Khat[3].




**Cathinone**

**Figure 1:** Chemical structure of cathinone

Young fresh leaves contains 0.1-0.3% (-) cathinone[3]. Cathinone is difficult to isolate or synthesize hence not suitable for marketing in form of a pure substance [6]. Cathinone is relatively unstable, decomposing to (+) norpseudoephedrine (cathine) and norphedrine after harvesting or as the leaves are dried which are related to dexamphetamine structurally [7]. Cathinone leads to release of serotonin and dopamine in central nervous system (CNS). Dopamine release in the dopamine terminals offers dopaminergic pathway increased activities [8]. Cathinone is metabolised rapidly. Metabolism mainly occurs at the first passage in the liver [8]. Cathine has minimal function in the stimulatory effect of Khat and is associated with unwanted effects on the systemic [6].




**Cathine**

**Figure 2:** *Chemical structure of cathine*

**Drug induced liver injury**

Hepatotoxicity of drugs can be principally metabolism-dependent, parent-drug-dependent, or a combination of both. Metabolism takes place largely in the liver, which accounts for its susceptibility to drug induced injury [9]. The metabolites may be electrophilic chemicals or free radicals that deplete glutathione stimulating hormone (GSH), covalently bound to proteins, lipids, or nucleic acids or induced lipid peroxidation [10]. The liver can be damaged when even exposed to drugs even for only 15 minutes [11]. Liver metabolises majority of exogenous substances within the body before they are taken to systemic circulation [12].

Indomethacin, a methylated indole belongs to an acetic acid derivative class of non-steroidal anti-inflammatory drug and is associated with cases of drug induced liver disease. It inhibits the intracellular cyclooxygenases isoenzymes I and II leading to decreased synthesis of prostaglandins the potential mediators of pain and inflammation. Indomethacin causes a decrease of hepatic microsomal cytochrome p – 450 dependent monooxygenase system and prostaglandin depletion [13, 14, 15]. Although the mechanisms of indomethacin liver injury are not clearly established, mitochondrial dysfunction, altered calcium homeostasis and apoptosis related protein are considered for its potential hepatotoxicity. An overdose of indomethacin damages liver by activating multiple stress pathways that causes liver disorders [16, 17].

**Materials and Methods**

**Plant collection and processing**

Freshly picked *Catha edulis* young shoots consisting young leaves from a farm were harvested in early morning hours and presented as a small bundles of twigs. The plant samples were collected from 30 different Khat plants from Meru County, Kenya in the month of September 2013. The plants were sampled from 5 different farms. Approximately 20 Kilograms were collected. The bundles were wrapped with fresh banana leaves before and during transportation in order to preserve their freshness. Neither pesticides nor fertilizer had been applied for more than six months on the plants from which the sample materials were collected in order to avoid chemical residues. In Kenyatta University Biochemistry laboratories, the plant sample were divided into smaller sample portions and were stored at temperature of approximately $4^0$C before extraction of cathinone and cathine.

**Plant extraction**

Protocol by Adugna and Dagne (2009)[18] was followed when extracting alkaloids.

**Reconstitution of plant extract for animal experimentation**

Khat extract weighing 1g was dissolved in 5ml DMSO; this was used to prepare a working concentration of 200mg/ml by, the Miller and Tainter protocol (1970) [19]; was used to determine the dosage for Khat extract to be administered. To determine the volume of the extract to be administered for example to a mouse weighing 26.5g at 2000mg/Kg body weight, the weight of the mouse was multiplied by 2000mg/Kg then divided by 1000g and 200mg/ml. Therefore, a mouse weighing 26.5g was administered with 265µl of Khat extract. The accurate measurements of the extract were made possible by use of micropipettes. The dose of the extract was adjusted to the final volume of 0.5ml using normal saline and administered orally.

**Experimental animals**

Male albino mice with an average age of 6 weeks weighing 20-25g were used. The mice were bred in the Department of Zoological Sciences, Kenyatta University. The study was approved by Kenyatta University animal care and use committee (KU – ACUC) number 14402/09/2014. The albino mice were housed in group of five in metal wire meshed cages approximately 30 × 30 × 30cm and then placed on a 0.75m raised surface in the animal house. Nine groups of the study animal were housed in separate cages. Each cage was labeled with cage number (one to nine). Wood shavings beddings were replaced daily. The albino mice had access to standard rodent pellets. The temperature in the animal room was approximately $25^0$C. Before further studies commenced, all albino mice were clinically observed daily for mortality, morbidity and any physical abnormality.

**Toxicity testing**

The experimental animals were treated as indicated in the Table 1 for *Catha edulis* toxicity [19].

**Table 1:** Treatment of experimental animals with *Catha edulis* extract and different accompaniments as used by *Catha edulis* users in duration of 30 days.

| Group number (n=5) | *Catha edulis* extract dosage per day (mg/Kg) | Type of accompaniment | Amount/dosage of accompaniment in a day |
|---|---|---|---|
| 1 | - | water | 2ml |
| 2 | - | Indomethacin | 0.3mg/Kg |
| 3 | 2000 | water | 2ml |
| 4 | 2000 | coke | 2ml |
| 5 | 2000 | coffee | 2ml |
| 6 | 2000 | milk | 2ml |
| 7 | 2000 | tea | 2ml |
| 8 | 2000 | Patico sweet | 2g |
| 9 | 2000 | groundnut | 2g |

**Hematological tests**

The hematological testing which was carried out was only the blood clotting time test. The blood samples for hematological test were collected in a container without an anticoagulant. The blood samples were collected from the tail [20].

**Histocytological tests**

The experimental animals were sacrificed on the 31st day to obtain liver for physical, morphological, histological and cytological processing and examination to determine liver damage. Histological and cytological examinations were carried out using haematoxylin and

eosin staining technique and observed under light microscope for any histopathological and cytopathological changes [20].

**Specimen collection for biochemical tests**

Blood for liver biochemical analysis tests like:- Alkaline phosphatase, serum aspartate aminotransferase and total bilirubin were collected from the veins in the tail. The blood samples were collected in red tipped vacuatainers without an anticoagulant.

**Specimen transportation, processing and storage for biochemical tests**

Specimens after collection were transported from the laboratory animal house dissection centre to the processing laboratory in ice packed cool boxes within one hour. Once clotted, the blood specimens were centrifuged at 3000 revolutions per minute for two minutes and serum separated immediately into well labeled cryovials. Serum specimens were then stored in laboratory refrigerator at $4^0$C awaiting laboratory analysis at clinical laboratory at Kenyatta National Hospital.

**Biochemical laboratory analysis**

The liver function tests determined were alkaline phosphatase, aspartate aminotransferase and total bilirubin. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Kenyatta National Hospital Laboratory using Cobas Integra® 400 plus automatic Biochemistry Analyzer (Roche Diagnostics, Mannheim, Germany).

**Quality assurance (QA)/ Quality control (QC)**

All the pre-analytical and post analytical precautions were put into consideration to ensure accuracy and precision of the test results. Internal QC materials from Roche diagnostics were run daily while the external QC material for the process was from American Proficiency Institute (API). This was used at Kenyatta National Hospital to monitor the performance of the institution laboratories. The American proficiency institute external QC were run twice daily according to manufacturers' instruction and QC protocol. The quality control was automatically performed by the system as per the specification of the test definition [21].

**Data analysis**

For statistical analysis SPSS software (version 17 SPSS Inc. Chicago. Illinois) was employed for arithmetic means and standard deviation of biochemical, histocytological, physical and hematological tests were calculated. The p-values were examined by paired students t-tests. The results were considered significant by a p-value <0.05. All end points were analyzed using 2-tailed test. The average values of biochemical, histocytological, physical and hematological tests were presented using tables and bar graphs.

**Results and Discussion**

The serum levels of the alkaline phosphatase (ALP) were higher in mice which were administered with Khat extract. According to the study, the average level of alkaline phosphatase (U/L) was increased in the mice administered with indomethacin (187.82±6.06) as compared to group taking rodent pellet and water alone and the groups to which Khat extract was administered with combination of milk, water, coke, tea, coffee, groundnut and patico sweets (Table 2). There was a significant different in the levels alkaline phosphatase between the negative control group (not administered with Khat extract) and the positive control group (administered with Khat extract) (Table 3).

**Table 2:** Biochemical assayed levels of serum alkaline phosphatase units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Group | Treatment | Alkaline phosphatase (U/L) Mean±SD | P < 0.05 |
|---|---|---|---|
| 1 | No Khat extract (- ve control) | 146.12±18.65 | - |
| 2 | Indomethacin (+ ve control) | 187.82±6.06 | 0.017* |
| 3 | Extract + water | 178.38±10.76 | 0.035* |
| 4 | Extract + coffee | 178.10±11.04 | 0.042* |
| 5 | Extract + milk | 182.86±3.96 | 0.009* |
| 6 | Extract + tea | 176.24±6.16 | 0.011* |
| 7 | Extract + patico sweet | 182.92±2.85 | 0.011* |
| 8 | Extract + groundnut | 181.24±11.55 | 0.019* |
| 9 | Extract + coke | 179.24±5.78 | 0.009* |

*Significance difference using paired t-test as compared with normal control at p < 0.05, SD =standard deviation, n= number.

**Table 3:** Serum alkaline phosphatase significance difference at p < 0.05 using paired t- test in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Treatment | NC | PC | EW | EC | EM | ET | EP | EG | ECk |
|---|---|---|---|---|---|---|---|---|---|
| NC | - | 0.017* | 0.035* | 0.042* | 0.009* | 0.011* | 0.011* | 0.019* | 0.009* |
| PC | 0.017* | - | 0.200 | 0.175 | 0.570 | 0.082 | 0.225 | 0.313 | 0.158 |
| EW | 0.035* | 0.200 | - | 0.935 | 0.395 | 0.750 | 0.452 | 0.781 | 0.878 |
| EC | 0.042* | 0.175 | 0.935 | - | 0.337 | 0.802 | 0.424 | 0.745 | 0.867 |
| EM | 0.009* | 0.257 | 0.395 | 0.337 | - | 0.143 | 0.982 | 0.804 | 0.353 |
| ET | 0.011* | 0.082 | 0.750 | 0.802 | 0.143 | - | 0.033 | 0.327 | 0.079 |
| EP | 0.011* | 0.225 | 0.452 | 0.424 | 0.982 | 0.033 | - | 0.710 | 0.198 |
| EG | 0.019* | 0.313 | 0.781 | 0.745 | 0.804 | 0.327 | 0.710 | - | 0.740 |
| ECk | 0.009* | 0.158 | 0.878 | 0.867 | 0.353 | 0.079 | 0.198 | 0.740 | - |

*indicates a significant difference at p < 0.05**.** ALP =Alkaline phosphatase, NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

According to the study, the average level of the aspartate aminotransferase (U/L) was increased in the positive control group (administered with Indomethacin) (213.86±7.08) as compared to negative control group (not administered with Khat extract) (153.10±7.07) (Table 4). The concentration of aspartate aminotransferase was significantly different between the negative control (not administered with Khat extract) and the test groups (administered with Khat extract) (Table 5).

**Table 4:** Biochemical assayed levels of serum aspartate aminotransferase in units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Treatment | Group | Aspartate aminotransferase (U/L )Mean±SD | P<0.05 |
|---|---|---|---|
| No Khat extract (-ve control) | 1 | 153.10±7.07 | - |
| Indomethacin(+ve control) | 2 | 213.86±7.08 | 0.006* |
| Extract + water | 3 | 210.38±5.76 | 0.007* |
| Extract + coffee | 4 | 206.80±7.43 | 0.008* |
| Extract + milk | 5 | 208.86±9.46 | 0.004* |
| Extract + tea | 6 | 210.72±5.80 | 0.008* |
| Extract + patico sweet | 7 | 208.74±5.95 | 0.011* |
| Extract + groundnut | 8 | 209.80±8.28 | 0.006* |
| Extract + coke | 9 | 205.38±9.53 | 0.005* |

*Significance difference as compared with normal control at p<0.05 using paired t test.SD = standard deviation, n =number

**Table 5:** Serum aspartate aminotransferase significance difference at p < 0.05 in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Treatment | NC | PC | EW | EC | EM | ET | EP | EG | ECk |
|---|---|---|---|---|---|---|---|---|---|
| NC | - | 0.006* | 0.007* | 0.008* | 0.004* | 0.008* | 0.011* | 0.006* | 0.005* |
| PC | 0.006* | - | 0.370 | 0.140 | 0.488 | 0.141 | 0.202 | 0.529 | 0.277 |
| EW | 0.007* | 0.370 | - | 0.280 | 0.827 | 0.874 | 0.238 | 0.912 | 0.388 |
| EC | 0.008* | 0.140 | 0.571 | - | 0.739 | 0.340 | 0.604 | 0.424 | 0.746 |
| EM | 0.004* | 0.488 | 0.827 | 0.739 | - | 0.795 | 0.987 | 0.787 | 0.391 |
| ET | 0.008* | 0.141 | 0.874 | 0.340 | 0.795 | - | 0.344 | 0.881 | 0.446 |
| EP | 0.011* | 0.202 | 0.238 | 0.604 | 0.987 | 0.344 | - | 0.851 | 0.594 |
| EG | 0.006* | 0.529 | 0.912 | 0.424 | 0.787 | 0.881 | 0.851 | - | 0.076 |
| ECk | 0.005* | 0.277 | 0.388 | 0.746 | 0.391 | 0.446 | 0.594 | 0.076 | - |

*indicates a significant difference at p <0.05**.** NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

The average level of the total bilirubin (mM/L) was increased in the positive control group (administered with Indomethacin) (24.24±3.63) as compared to negative control group (not administered with Khat) (12.44±1.18) (Table 6). There was no significance difference at p < 0.05 in levels of total bilirubin between the positive control group and the group to which Khat extract was administered with combination of milk, water, coke, tea, coffee, groundnut and patico sweets. There was a significant different in the levels of total bilirubin between the negative control group with the positive control and the groups unto which Khat extract were being administered (p<0.05). Khat extracts altered the levels of total bilirubin in the serum of the albino mice unto which it was administered. The alteration of the serum total bilirubin did not significantly differ irrespective of the non-alcoholic drink substance Khat was administered with.

**Table 6:** The mean serum biochemical assayed levels of total bilirubin mMol/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Treatment | Group | Total bilirubin (mMol/L) Mean±SD | P<0.05 |
|---|---|---|---|
| No Khat extract ( -ve control) | 1 | 12.44±1.18 | - |
| Indomethacin (+ve control) | 2 | 24.24±3.63 | 0.004* |
| Extract + water | 3 | 20.60±3.87 | 0.009* |
| Extract + coffee | 4 | 22.08±2.86 | 0.000* |
| Extract + milk | 5 | 17.80±2.80 | 0.011* |
| Extract + tea | 6 | 18.66±4.06 | 0.017* |
| Extract + patico sweet | 7 | 21.16±6.17 | 0.024* |
| Extract + groundnut | 8 | 19.12±5.29 | 0.035* |
| Extract + coke | 9 | 20.02±2.75 | 0.003* |

*Significance difference as compared with normal control at p<0.05,
SD = standard deviation, n =number.

**Table 7:** Serum total bilirubin significance difference at $p < 0.05$ in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Treatment | NC | PC | EW | EC | EM | ET | EP | EG | ECk |
|---|---|---|---|---|---|---|---|---|---|
| NC | - | 0.004* | 0.009* | 0.000* | 0.011* | 0.017* | 0.024* | 0.035* | 0.003* |
| PC | 0.004* | - | 0.165 | 0.381 | 0.073 | 0.071 | 0.504 | 0.166 | 0.061 |
| EW | 0.009* | 0.165 | - | 0.571 | 0.209 | 0.140 | 0.884 | 0.689 | 0.816 |
| EC | 0.000* | 0.381 | 0.571 | - | 0.100 | 0.137 | 0.705 | 0.212 | 0.233 |
| EM | 0.011* | 0.073 | 0.209 | 0.100 | - | 0.714 | 0.284 | 0.613 | 0.282 |
| ET | 0.017* | 0.071 | 0.140 | 0.137 | 0.714 | - | 0.471 | 0.892 | 0.585 |
| EP | 0.024* | 0.504 | 0.884 | 0.705 | 0.284 | 0.471 | - | 0.584 | 0.756 |
| EG | 0.035* | 0.166 | 0.689 | 0.212 | 0.613 | 0.892 | 0.584 | - | 0.567 |
| ECk | 0.003* | 0.061 | 0.816 | 0.233 | 0.282 | 0.585 | 0.756 | 0.567 | - |

*indicates a significant difference at $p < 0.05$

NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and cok

The elevation ALP in serum is an indicator of possibility of hepatotoxicity [22]. Aspartate transferase and alkaline transferase lack specificity because they are found in a number of tissues hence their increase in serum concentration can not only represent liver, but also bones, muscles and heart damage [23]. Alalanine transferase activity inhibition could be a good indicator of normal functioning of the liver because it's a more specific marker of hepatocytes integrity [24]. The elevation of serum Aspartate aminotransferase (AST) in the positive control and the tests animals suggests leakage into circulation due to rupturing of the cell membrane of the hepatocytes due to organ injury [25]. Aspartate transferase is distributed in various organs, but it is more concentrated in the hepatocytes [26]. Aspartate transferase (AST) can be elevated in cases of striated muscles which is associated with increased levels of creatine kinase (CK) [24]. Fragmentation of the haem from haemoglobin of the red blood cells which are aged or damaged lead to formation of the bilirubin. Bilirubin is conjugated in the liver hence liver damage leads to accumulation of bilirubin in the serum leading to jaundice [27].

**Hematological tests**

The clotting time for the positive control group had a significant difference with that of the negative control. The blood clotting time between all the test groups (treated with Khat extract) didn't show any significant difference at $p < 0.05$ (Table 4.13). The clotting time for the positive control group was higher than that of negative control group and other tests groups (Figure 4.10). The average clotting time for other groups was almost the same.

**Table 8:** The mean blood clotting time in minutes in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

| Group | Treatment | Clotting time in minutes Mean±SD | P<0.05 |
|---|---|---|---|
| 1 | No Khat extract (-ve control) | 5.22±0.37 | - |
| 2 | Indomethacin (+ve control) | 6.60±0.23 | 0.004* |
| 3 | Extract +water | 5.32±0.16 | 0.486 |
| 4 | Extract+coffee | 5.24±0.52 | 0.959 |
| 5 | Extract+milk | 5.58±0.49 | 0.149 |
| 6 | Extract+tea | 4.94±0.99 | 0.590 |
| 7 | Extract+patico sweet | 5.26±0.36 | 0.897 |
| 8 | Extract +groundnut | 5.02±0.18 | 0.249 |
| 9 | Extract+coke | 5.02±0.31 | 0.473 |

*Significance difference as compared with normal control at $p<0.05$,
SD - standard deviation, n – number, n=5.

Bleeding time indicates the time between the making of a small incision and the moment when the bleeding stops. Tissue factors leads to coagulation in arteries or in veins after tissue damage in the extrinsic coagulation pathway [28]. Apart from factor VIII, all coagulation factors are synthesized in the liver. Vitamin K is required for the synthesis of hepatic factors VII, IX, X and prothrombin [28]. The liver makes six blood clotting factors: I (fibrinogen), II (Prothrombin), IV, V, VI, and VII. Whenever there is an abnormality in any of them bleeding time will be prolonged.

Hepatic dysfunction results in the reduced production of functional coagulation factors. The coagulation disorders which are encountered in severe liver disease leads to enhanced fibrinolysis resulting to consumption of clotting factors [29]. Fibrinolysis triggers breakdown of fibrin leading to fibrin degradation which impair hemostasis by inhibiting fibrin polymerization. Liver diseases are commonly associated with blood clotting/bleeding dysfunction. Liver problems are associated with disturbed balance in the pro-coagulant and anti-coagulant factors which leads to deviation from the normal coagulation cascade [29].

**Liver histological results**

Liver sections of the negative control group (not treated with Khat extract) of the albino mice under microscopic examination showed intact cytoplasm, uncongested central vein, uniform hepatocytes and prominent nuclei of the cells. The sections also did not show any sign of necrosis, inflammation or changes in fatty acids. The sections also composed of hexagonal or pentagonal lobules whereby the central veins and the peripheral hepatic triads or tetrads were embedded in the connective. The hepatocytes were arranged in trabecules running from the central vein radiantly. There were spaces in between the cell cords which were converging towards the central veins through the Kupffer cells. The hepatocytes of the negative control group were regular and contained nucleus which were large and spherical in shape.

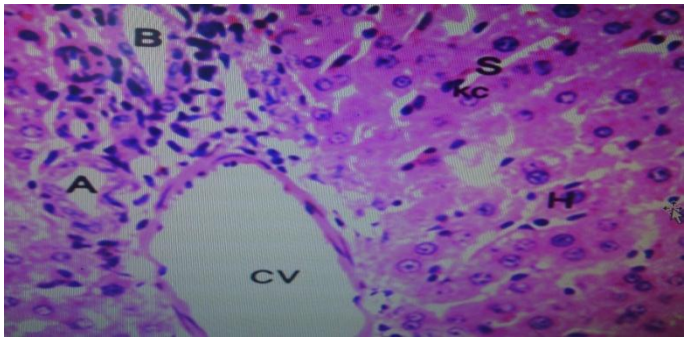



**Figure 3:** Shows a light photomicrography of a normal liver from negative control mice: (CV): central vein, (H): hepatocytes, (S): sinusoids, (A): hepatic artery, (B): bile duct, (KC): Kupffer cell. H&E. ×400

The nucleus contained nucleoli which were clearly marked with peripheral chromatin distribution. Some of the cells from the normal control group contained two nuclei (Figure 3).

In the positive control (treated with indomethacin) group and the test groups in which Khat extract was administered to male albino mice, there were signs of degeneration of hepatocytes. The hepatocytes were enlarged and had light and foamy cytoplasms filled with numerous vacuoles. The blood sinusoids walls were dilated and had large

number of Kupffer cells. There were also presence of necrosis, pycnosis and condensed chromatin in livers of all test groups. The hepatic areas of the test groups and the positive control also had presence of mononuclear cell infiltrates (Figure 4 and 5). The frequency of the abnormalities observed in the liver are demonstrated in figure 6 below.

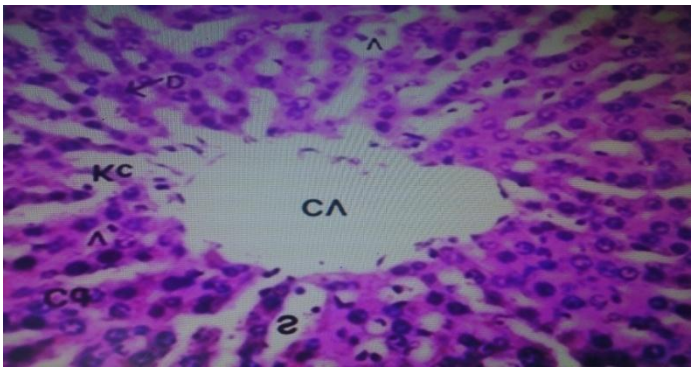



**Figure 4:** Shows liver injury of albino mice. (V): light, foamy and vacuole filled cytoplasm, (Cd): enlarged cells with condensed nuclear chromatin, (D arrow): single necrotic and pycnotic hepatocytes with contracted nuclei and condensed chromatin, (S): strongly acidophilic cytoplasm with accumulation of mononuclear cells in sinusoids, (KC): Kupffer cells around sinusoidal walls. H&E, ×400.

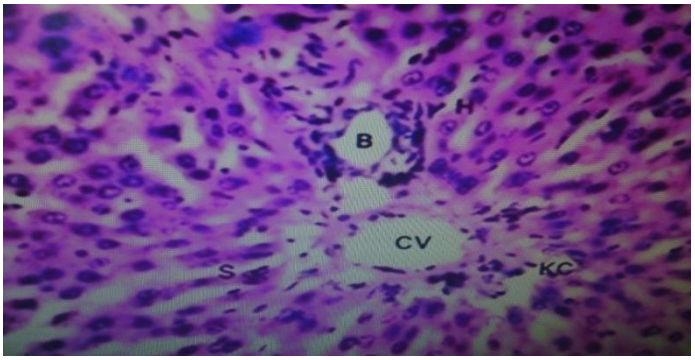



**Figure 5**: Shows liver injury of albino mice. (S): decreased widening of blood sinusoid (CV): less fragmentation, lighting of cytoplasm and infiltration of mononuclear cells around the portal system of central vein, (B): bile duct. H&E, ×400.

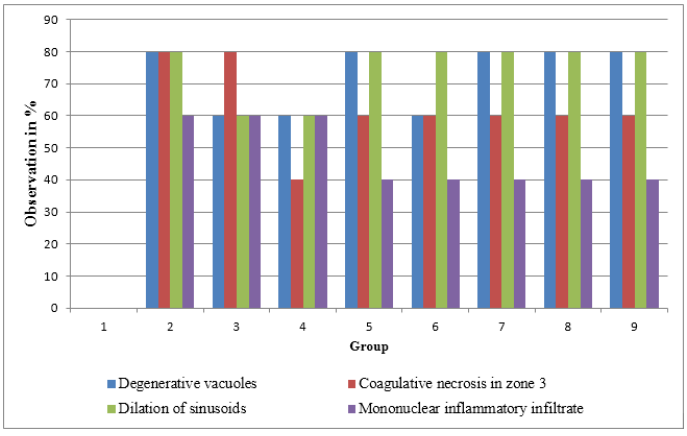



**Figure 6:** Histological observation of albino mice liver tissues.

Observed enlargement of the liver could be as a result of Khat extract exposure. Enlargement of the liver is a clear indicator of regeneration of the liver normally experienced clinically after the damage of the liver. Most xenobiotics are associated with enlargement of the liver because they have direct effect on the size of the hepatocytes [30] or on the inflammatory responses [31] this results in the histopathological and cytological changes on the hepatocytes of the mice. The mostly observed changes on the cytological and histological features of the liver were:-vacuolar and coagulative necrosis, dilatation of the sinusoids, mononuclear infiltrates, oedema, congestion and hemorrhage degeneration in parenchymal hepatocytes. The oedema may have occurred because of energy reduction which is required for the cells ion concentration regulation which leads to water retention in the hepatocytes [32]. Liver damages leads to impaired liver functioning hence interfering with plasma proteins secretion [33, 34].

Decreased secretion of plasma proteins leads to decrease in osmotic blood pressure resulting to decreased drainage of the tissue fluids hence oedema. The vacuolation of the hepatic lobule in the study predominantly started in the hepatocytes of the peripheral sites and the extended towards the center of the lobules. This can be associated with direction of blood supply in the liver lobular [35]. The vacuolation of the cytoplasm of the hepatocytes is mainly caused by disturbance of the lipid inclusions and the metabolism of the lipid due to pathological changes [36]. The vacuolar degeneration is an alteration which is produced in order to collect the injurious substances which are found in the hepatocytes [37].

## Conclusion

The study results indicated that there was a possible potential for Khat extract to cause liver damage. The elevation of alkaline phosphatase, aspartate aminotransferase and total bilirubin is an indicator of effect of Khat on liver function. The abnormal histocytological results of the liver confirm adverse effects of Khat on the liver.

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