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Pharmacogn. Mag.
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ORIGINAL ARTICLE

Catha edulis-induced Skeletal Muscle Toxicity in Experimental Rats via Regulation of Rhabdomyolysis Biomarkers

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Submitted: 01-04-2019 Revised: 16-05-2019 Published: 26-08-2019

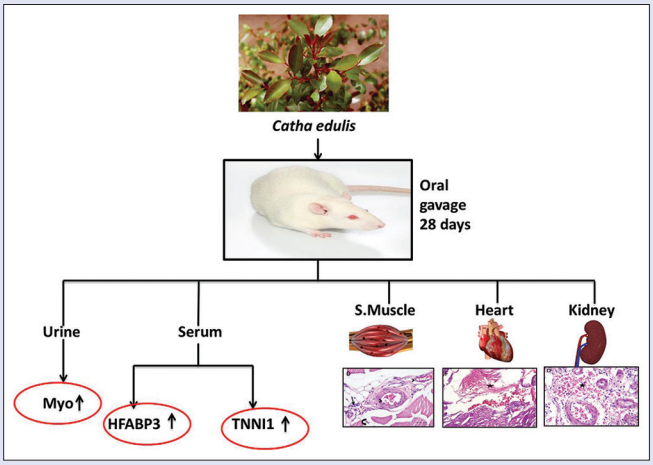
ABSTRACT

Background: Recently, there are clinical reports on the potential of *Catha edulis* (khat) to induce muscular toxicity. *C. edulis* (khat) is an evergreen shrub and a well-known controversial plant due to the content of natural stimulant, cathine and cathinone. **Objective:** The main objective of the study is to evaluate the possible effect of *C. edulis* leaves extract to induce rhabdomyolysis *in vivo*. **Materials and Methods:** Sprague Dawley rats were grouped and treated with khat extract at two different doses (250 and 500 mg/kg), while atorvastatin was used as positive control for 28 days. Body weight was measured throughout the study period. Overnight urine was collected from each rat at the 28th day for myoglobin (Myo) analysis. Terminal blood samples were collected from sacrificed animals for the measurement of serum biomarkers and clinical chemistry. The standard clinical pathology assays aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum creatinine (CR) were monitored. Skeletal muscle, cardiac muscle, and kidney were collected for histopathological examination. **Results:** Animals received 250 mg/kg khat extract had shown mild-to-no gait disorders, while at higher dose extract (500 mg/kg) had reduced the body weight of rats with marked increase of gait disorders compared to control. CR, AST, and ALT were elevated in high-dose administration and in rats received ethanol. The tested biomarkers such as heart-type fatty acid-binding protein (HFABP) 3, Troponin I Type 1 slow skeletal (TNNI1), and Myo were significantly increased in khat high dose and statin treatment, but not in low-dose extract and alcohol. The increase in HFABP and TNN1 results were well reflected in histopathological findings of skeletal myofiber degeneration and in the hemorrhages and pyknosis of nucleus observed in the cardiac muscle. **Conclusion:** These results provide evidence that khat chewing contributes to the development of muscle toxicity and probable rhabdomyolysis. The current subject thus warrants detailed studies which could emphasize on the cardiac complications and muscular toxicity mechanisms.

Key words: *Catha edulis,* khat, muscle weakness, myoglobin, rhabdomyolysis

SUMMARY

- *Catha edulis* (khat) is an evergreen shrub and a well-known controversial plant due to the content of natural stimulant, cathine and cathinone
- There are clinical reports on the potential of *C. edulis* (khat) to induce muscular toxicity
- Higher dose extract (500 mg/kg) had reduced body weight of rats with marked increase of gait disorders compared to control
- The tested biomarkers such as heart-fatty acid-binding protein 3, Troponin I Type 1 slow skeletal, and myoglobin were significantly increased in khat high-dose dose and in statin treatment.



Abbreviations used: CR: Creatinine; fsTnI: Fast skeletal troponin I; GIT: Gastrointestinal tract; HFABP: Heart-type fatty acid-binding proteins; KA: Khat alcohol; KH: Khat high dose; KL: Khat low dose; Myo: Myoglobin; ssTnI; SWGDRUG: Scientific Working Group for the Analysis of Seized Drugs.

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DOI: 10.4103/pm.pm_142_19

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INTRODUCTION

Catha edulis (khat) is an evergreen shrub and a well-known controversial plant due to the content of natural stimulant, cathinone.[1] Chewing the fresh leaves of this plant is a traditional habit among the people of Uganda, Ethiopia, Yemen, and some part of the Arabian Peninsula.[2] The leaves are in aromatic odor, astringent, and slightly sweet in taste. Khat chewing is pleasant, pleasurable, cheerful, and euphoretic in the beginning, but later leads to mild issues such as emotional instability, lethargy, and loss of appetite. It is not limited

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Cite this article as: Mohan S, Shaheen E, El-Amir YO, Khadashia HA, Ncibi SS, Farasani A, *et al.* *Catha edulis*-induced skeletal muscle toxicity in experimental rats via regulation of rhabdomyolysis biomarkers. Phcog Mag 2019;15:S359-65.

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to the mild side effects but is well documented for moderate-to-very severe health hazards.[3]

The existence of khat leaves is found in 44 different types.[4] The so-far evaluated studies show that this plant leaves contain various types of chemicals such as terpenoids, flavonoids, glycosides, sterols, tannins, and alkaloids. Among the alkaloids, phenylalkylamines are the major one, which comprises of two important chemicals such as cathinone and cathine.[5] These compounds are structurally similar to amphetamine. Among cathinone and cathine, only cathine is a stable compound, whereas cathinone decomposes easily upon leaves drying. Hence, the chewers of the khat prefer to keep the fresh leaves in wrapped banana leaves and consume as much as fresh they can.[6]

Various pharmacological and toxicological studies have been done with khat in both animal models and in clinical samples. Since it is a psychotropic plant, majority of the studies have been focused but not limited to its effects in and issues related to behavioral and psychotropic aspects. Central effects of khat exhibit psychosis and manic illness.[7] In very high dose of khat (KH) consumption, it may lead to hallucinations and suicidal depression.[8] Bogale and Engidawork[9] have found schizophrenic-like symptoms in khat consumed rat models. Odenwald *et al.*[10] also stated that in some cases, khat consumption is the primary agent, causing the onset of psychosis. Apart from these, those patients with family traits of psychosis and schizotypal peoples also showed an increased risk of khat-induced psychosis. Besides psychotic disorders, the available literature show that regular use of khat in individuals causes much cognitive impairment such as problems associated with learning and memory, behavioral flexibility, and extinction.[11]

In the peripheral level, khat affects many systems. It has exhibited various gastrointestinal tract (GIT) disorders such as dry mouth, polydipsia, delayed intestinal absorption, dental caries, periodontal disease, chronic gastritis, constipation, hemorrhoids, paralytic ileus, weight loss, duodenal ulcer, upper GI malignancy, and oral keratotic white lesions.[8] The effect of khat on GIT is believed due to the astringent effect of tannins and sympathomimetic activity of cathinone. Khat effect in the liver and kidneys such as fibrosis, cirrhosis, acute kidney damage, and enzyme inhibition are found to be due to cathinone accumulation, autoimmune reactions, acting as a substrate, degenerative changes in the kidney, and oxidative stress.[12] Khat has the capacity to induce metabolic and endocrine effects such as hyperthermia, perspiration, and hyperglycemia. Effects such as tachypnea and bronchitis also have been noted in the respiratory system.[13]

It is significant to note that khat chewing also affects adversely in the musculoskeletal system. Chewing khat for a long time is closely associated with muscular weakness.[14] It is of high significance when it comes to skeletal muscle damage, cardiac complications, and renal issues. Cathinone has been proved to induce a severe negative inotropic effect on the cardiac muscle earlier. Structurally similar compound amphetamine and methamphetamine had earlier showed significant rhabdomyolysis with myoglobinuria.[15] Regardless of these extensive studies on khat with animal and human experimental models, no effort has been made to expedite the other cofactors associated with muscular toxicity in khat chewers. Recently, there are selected studies had reported some clinical findings shed light on the probability of rhabdomyolysis associated with khat. One among that is a clinical case found in a hospital in the US, which suggest severe rhabdomyolysis associated with khat consumption.[16] Earlier reports from hospitals suggest that high number of khat chewing patients with sympathomimetic toxicity has been found to be significantly linked with severe rhabdomyolysis.[17]

Since there are no preclinical studies on the probability of developing skeletal muscle toxicity and cardiac muscle toxicity associated with khat consumption, we determined to evaluate these factors in detail in an animal model by keeping in mind the probabilities of rhabdomyolysis reported earlier in clinical finding.

MATERIALS AND METHODS

Animal husbandry

Male Sprague Dawley rats approximately 7 weeks old (180–210 g) were obtained from the animal house, Jazan University. Following physical examination by a veterinarian, the rats were allowed to acclimate for 7 days before the dosing. All animals were individually housed in polypropylene cages with dust-free softwood bedding. The animals were maintained at standard conditions of temperature, humidity, and light on standard pellet diet and water *ad libitum*. The rats were moved to clean cages once per week. All rats were observed at least once daily for general condition. Body weights were measured upon arrival. The study was carried out with the approval of the Institutional Scientific Research Ethics Committee (REC39/3-269).

Test materials

C. edulis leaves were provided for this research by the Ministry of Interior, Saudi Arabia. The fresh bundles were transported to the laboratory and kept at −80°C immediately. The leaves were tested and confirmed by the Faculty of Science, Botany Department, Jazan University. The

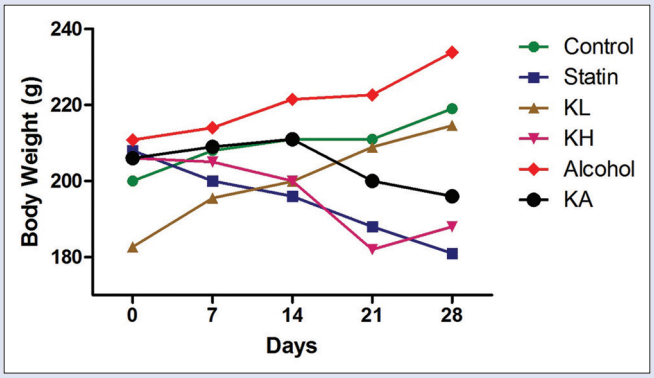

Figure 1: Body weight of animals from day 0 to 28

Table 1: Clinical chemistry parameter and biomarker in rat serum

Treatment	ALT (U/L)	AST (U/L)	SC (μM/L)	HFABP (ng/mL)	TNNI1 (ng/mL)
Control	57±7.22	105±12.84	45±7.76	2.08±0.05	1.21±0.01
Statin	360±47.81*	630±56.07*	89±7.01**	2.28±0.01*	4.09±0.05*
KL	60±4.72*	165±23.11*	46±4.82	2.10±0.03	1.28±0.07
KH	210±30.10**	530±31.23**	67±5.22*	2.31±0.05*	3.04±0.05*
Alcohol	96±7.02*	211±19.15*	39±6.10	2.08±0.07	1.88±0.01
KA	221±29.00*	418±59.00*	72±2.00*	2.27±0.01*	2.94±0.04*

*The parenthesis, **Statistical significance at 0.05 and 0.01 level, respectively. All values were average of value obtained±SD. ALT: Alanine transaminase; AST: Aspartate transaminase; SC: Serum creatinine; HFABP: Heart-type fatty acid-binding proteins; TNNI1: Troponin I1 slow skeletal type; KA: Khat alcohol; KH: Khat high dose; KL: Khat low dose; SD: Standard deviation

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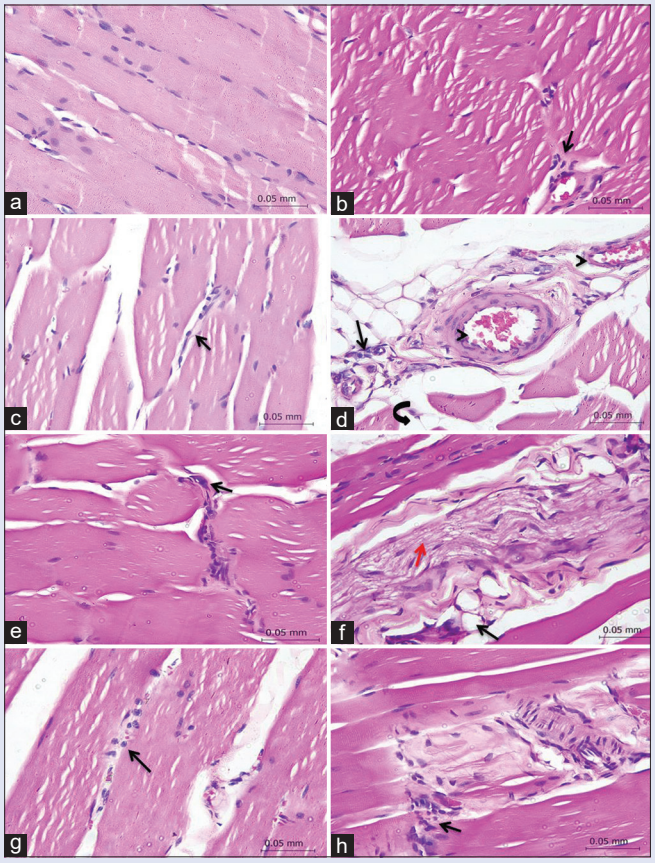

Figure 2: Photomicrograph of the skeletal muscle. (a) Control group had shown normal architecture of skeletal muscle; (b) Statin group. Splitting within the myofiber and mild perivascular inflammatory infiltrates as shown in arrow; (c and d) KL group, whereas (c) interstitial cellular infiltrates and (d) congestion (arrowhead), edema (curved arrow) and perivascular inflammatory infiltrates (arrow); (e and f) KH group, whereas (e) hyalinization and increase eosinophilia of the myofiber. Infiltration of inflammatory cells and satellite cells (arrow) in the interstitium. (f) Area of necrosis replaced by fibrous connective tissue (red arrow) and adipose tissue (black arrow); (g) Ethanol group, whereas infiltration of inflammatory cells in the interstitium (arrow); (h) KE group, whereas area of necrosis infiltrated with inflammatory reaction (arrow) (hematoxylin and eosin stain)

Table 2: Urinary myoglobin

Treatment	Myo (ng/mL)
Control	0.76±0.01
Statin	5.61±0.84**
KL	0.78±0.02
KH	3.29±0.50**
Alcohol	0.77±0.01
KA	3.71±0.074**

The parenthesis **Statistical significance at 0.01. All values were average of value obtained±SD. Myo: Myoglobin; KA: Khat Alcohol; KH: Khat high dose; KL: Khat low dose; SD: Standard deviation

extraction of the plant materials has been done according to the protocol of Scientific Working Group for the Analysis of Seized Drugs organization with slight modification. The detailed extraction procedure and liquid chromatography-mass spectrometry were reported in our earlier publication.[18] The standard drug atorvastatin was purchased from The Jordanian Pharmaceutical Manufacturing Company, Jordan.

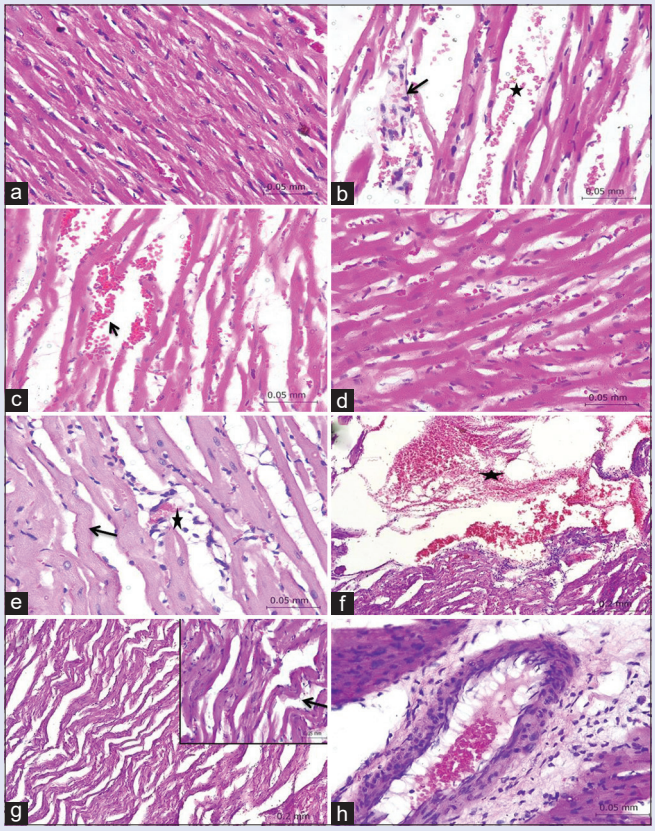

Figure 3: Photomicrograph of the heart. (a) Control group, whereas branching, striated, and uninucleate myofibers as evident. (b) Statin group, whereas severe hemorrhage (star) and edemaare shown together with infiltration of inflammatory cells (arrow). (c) Khat low dose group showed whereas hemorrhage (arrow) and necrosis. It also showed hyalinization of the myofiber with pyknotic nuclei. (d) Khat high dose group which showed necrosis of the myocardium and inflammatory cellular infiltrates in the interstitium. (e) Ethanol group had shown mild necrosis of the myofiber with interstitial inflammatory reaction (star). (f and h) KE group had shown severe hemorrhage (star), (g) thin wavy fibers (arrow), Loss of striation. (h) Perivascular inflammatory reaction (hematoxylin and eosin stain)

Other chemicals and kits were from the commercial manufactures as mentioned in the text.

Treatment

The rats were randomly allocated to six groups with ten animals in each group and were treated over a period of 28 days via oral gavage. Animals were randomly allocated in six groups: (Group: 1/Control) control group received normal saline; (Group: 2/Statin) atorvastatin 10 mg/kg; (Group: 3/khat low dose [KL]) khat 1000 mg/kg; (Group: 4/KH) khat 2000 mg/kg; (Group: 5) ethyl alcohol 4 g/kg; and (Group: 6/khat alcohol [KA]) 2000 mg/kg khat + ethyl alcohol 4 g/kg. The doses were selected according to the literature and our pilot acute toxicities studies earlier. Khat was found to be safe up to 2000 mg/kg. Hence, the maximum dose used in the study was 2000 mg/kg. The treatment was done every day for 28 days. Body weights of all the rats were measured every week till scarifies.

Blood collection

From all rats, samples of blood were collected through intracardiac puncture for clinical chemistry and biomarker analysis.

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Urine collection

Urine was collected from all the rats once the study reaches the 28th day. Each rat was maintained in a metabolic cage without food but with access for water overnight. The urine samples were collected overnight and stored at 2°C–8°C and subsequently deeply frozen until analysis.

Clinical pathology and biomarkers

Analysis was performed on the same day of blood collection. Hematology parameters were evaluated with laboratory enzyme-linked immunosorbent assay (ELISA) kit (Human diagnostic, Germany) according to the manufacture's procedure. For biomarker analysis, 100 ml serum was stored at −80°C. Biomarker analysis was performed using commercial kit from MyBioSource, Inc., CA, USA. The assay has been performed according to the manufacture's protocol mentioned in rat heart-type fatty acid-binding protein (HFABP) ELISA kit and Rat Troponin I Type 1 slow skeletal (TNNI1) ELISA kit.

Urinary biomarker

Urinary myoglobin (Myo) (rat Myo ELISA kit competitive, My BioSource Inc., CA, USA) was evaluated according to the manufacturer protocol using an ELISA reader.

Histopathology

Skeletal muscles, heart, and kidney were collected for histopathologic evaluation. All tissues were fixed in 10% neutral-buffered formalin and processed for routine hematoxylin and eosin staining. Tissues were evaluated without knowledge of treatment group by histopathologist.

Statistical evaluation

All values were reported as mean ± standard deviation. The statistical significance of differences between groups was assessed using one-way ANOVA followed by *Post hoc* Tukey's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical observations

Animals received 1000 mg/kg khat extract had shown mild-to-no gait disorders from day 7 to day 28. Their body weight was observed and found to be increasing normally regardless of the extract consumption. However, the 2000 mg/kg dose received rats had exhibited significantly reduced body weight with marked increase of gait disorders compared to control. Ethanol alone received groups has shown no reduction in body weight, in contrary, KA group had mimic similar kind of observations as shown in KH dose group. The positive control used in the study had reduced the weight and showed sever gait disorders as expected earlier, when compared to healthy control rats [Figure 1].

Clinical pathology and biomarker in serum and urine

Since increase in blood creatinine (CR) level is a late marker of muscle and kidney damage, we observed the level of CR in serum. It has been observed that the positive control statin increased the level of CR significantly at $P < 0.01$. KH and KA also showed marked increase in CR, whereas low dose of extract KL and alcohol had shown no role in this mechanism. In contrary, KL had shown a mild significance in the elevation of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT), while KH had shown four-folds escalations. It is worth to note that both AST and ALT increased while alcohol administration to the rats [Table 1].

Among the serum biomarkers investigated, HFABP3 and TNNI1 were increased in statin received group and in high dose of extract (KH). There was a slight increase in low dose of extract, but was not significant. In vehicle-treated control rats, neither serum HFABP3 nor TNNI1 was as routinely detected [Table 1]. The Myo detected in urine sample was statistically significantly increased in KH dose and in statin treatment but not in low-dose extract and alcohol [Table 2].

Histopathological analysis

Histopathological lesions in the skeletal muscle, heart, and kidneys of different groups were summarized in Table 3. Examination of skeletal muscle of control group revealed normal architecture which

Table 3: Score of histopathological lesions in the skeletal muscle, heart, and kidneys in different groups

Lesions	Groups					
	Control	Statin	KL	KH	Ethanol	KE
Skeletal muscle						
Splitting within the myofiber	−	+++	+++	+++	+++	+++
Perivascular cellular infiltrates	−	+	+	+++	++	+++
Interstitial cellular infiltrates	−	−	+	+++	++	+++
Focal area of necrosis	−	−	−	+++	−	+++
Heart						
Hemorrhage	−	++	++	++	+	+
Hyalinization	−	+++	+++	+++	+++	+++
Loss of striation	−	+++	+++	+++	+++	+++
Pyknosis of nucleus	−	+++	++	+++	+++	+++
Wavy myofibers	−	+	+	++	++	++
Interstitial cellular infiltrates	−	++	+	+++	++	++
Perivascular cellular infiltrates	−	++	+	++	++	++
Kidneys						
Hemorrhage	−	+++	+++	+++	+++	+++
Congestion	−	+++	+++	+++	+++	+++
Atrophy of the glomeruli	−	+++	−	+++	+++	+++
Vacuolation of renal epithelium	−	−	−	+++	−	+++
Focal necrosis of the renal tubularepithelium	−	++	+	++	++	+++

Symbols: −: No, +: Mild, ++: Moderate, +++: Severe. KH: Khat high dose; KL: Khat low dose; KE: Khat ethanol

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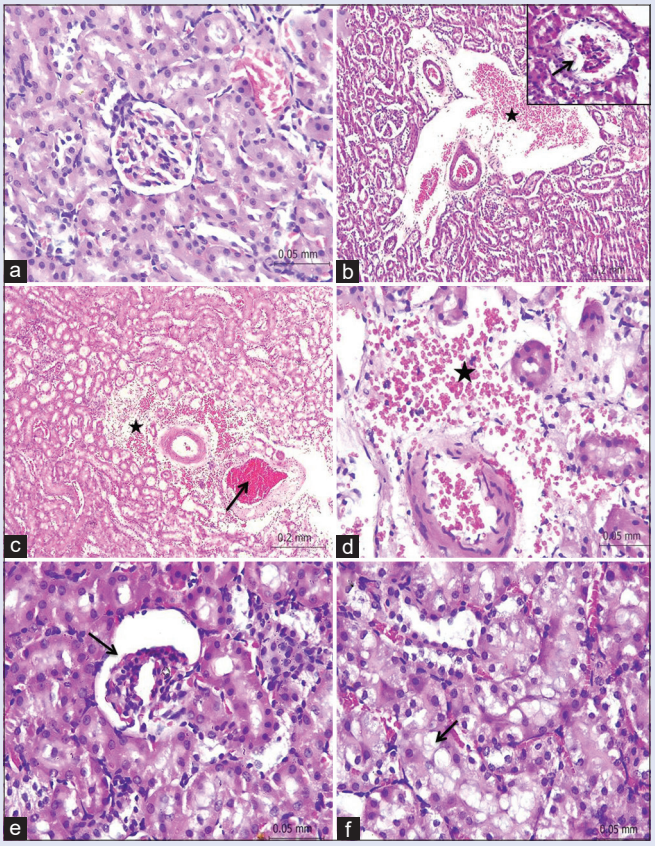

Figure 4: Photomicrograph of the kidneys. (a) Control group had shown normal architecture of the kidney with normal renal tubules and renal corpuscles. (b) Statin group, whereas hemorrhage (star) and congestion are visible together with atrophy of the renal glomerulus (arrow). (c) Khat low dose group had shown mild hemorrhage (star) and congestion (arrow). (d and f) Khat high dose group showed severe hemorrhage (star), (e) atrophy of renal glomeruli (arrow) and (f) vacuolation of renal tubules (arrow) (hematoxylin and eosin stain)

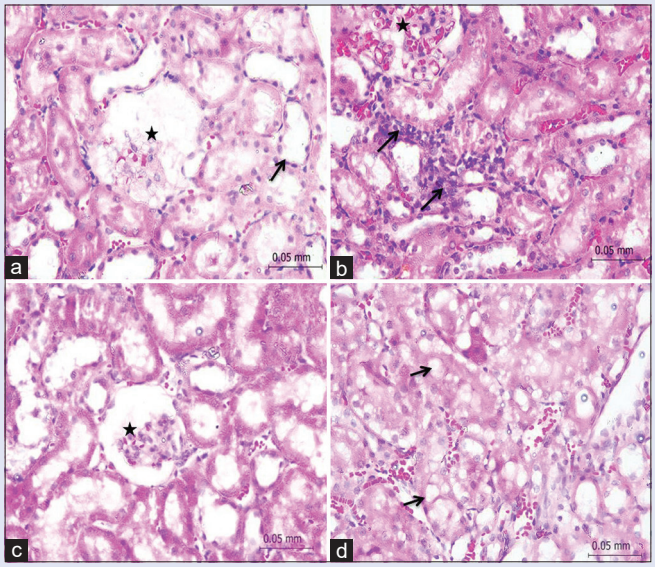

Figure 5: Photomicrograph of the kidneys. (a and b) Ethanol received group, whereas (a) atrophy of the glomerulus (star) and atrophic tubules with flat epithelium (arrow) and (b) severe inflammatory reaction in the interstitial tissue (arrows) and glomerular congestion (star).(c and d) represents KE group. (c) Atrophy of the glomerulus (star). Necrosis of renal tubular epithelium manifested by increase eosinophilia and loss of nuclei. (d) Vacuolation of the renal tubule (arrows) and congestion (hematoxylin and eosin stain)

characterized by long, cylindrical, multinucleated, and striated myofiber [Figure 2a]. Administration of statin-induced degenerative changes in the skeletal muscle manifested by splitting within the myofiber and mild perivascular inflammatory reaction [Figure 2b]. KL produced splitting of the myofiber with mild perivascular and interstitial inflammatory cellular infiltrates. Congestion of blood vessels and edema can be also detected [Figure 2c and d]. KH induced severe degeneration and necrosis of the skeletal muscle which characterized by hyalinization, increased eosinophilia, and infiltration of inflammatory cells and satellite cells in the interstitium [Figure 2e]. In some areas, the myofiber is replaced by fibrous connective tissue, adipose tissue, and inflammatory reaction [Figure 2f]. Administration of ethanol induced degenerative changes of the myofiber and infiltration of inflammatory cells in the interstitium[Figure 2g]. Coadministration of khat at high dose with ethanol induced severe degeneration and necrosis of skeletal muscle with infiltration of macrophages[Figure 2h].

Microscopic examination of cardiac muscle of control rats showed normal myocardium which characterized by branching, striated, and uninucleate myofibers [Figure 3a]. Statin induced severe hemorrhage associated with degenerative and necrotic changes of the myocardium which manifested by hyalinization, increased eosinophilia, loss of striation, interstitial inflammatory reaction, and pyknosis of nuclei [Figure 3b]. The same lesions could be seen in KL and KH groups and ethanol group with increasing severity in both high dose and ethanol group [Figure 3c, d and e]. Coadministration of ethanol and khat induced severe necrotic changes of the myocardium with severe perivascular infiltration of inflammatory cells. Most of the myocardial fibers were wavy and thinner with pyknotic nuclei [Figure 3f, g and h].

Kidney of control group revealed normal renal tubules and renal corpuscle [Figure 4a]. Statin induced hemorrhage, congestion, and atrophy of the glomeruli [Figure 4b]. The vascular changes could also be seen in the kidneys of KL group animals with degenerative changes in the renal tubular epithelium [Figure 4c]. Administration of KH induced severe hemorrhage and necrotic changes of the kidneys which characterized by atrophy of the glomeruli and vacuolation of the renal tubular epithelium [Figure 4d, e and f]. Ethanol induced atrophy of some glomeruli and severe congestion of other glomerular capillaries [Figure 5a and b]. There is also infiltration of inflammatory cells in the interstitium. Coadministration of ethanol and KH induced atrophy of the glomeruli, congestion and necrosis of the renal tubular epithelium [Figure 5c and d].

DISCUSSION

The recreational plant *C. edulis* has been in the limelight of research for many decades. It is mainly due to its capacity to produce a pleasurable euphoretic effect with mild-to-moderate addiction. In addition to the addiction capacity, it also plays a significant role in other psychological factors, including controlling cognitive skills. The presence of several phytochemicals has been proved to be the reason for these activities and noticeable side effects too. So far, many researchers have been established its toxic effects in the various organ system. However, no focus has been given to its ability to induce muscle toxicity, starting from mild myalgia to severe rhabdomyolysis. Very recently, there are some clinical reports which shed light on the findings of possible rhabdomyolysis associated with khat chewing. Hence, the current research designed to

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investigate this, and we found that *C. edulis* can produce mild-to-severe rhabdomyolysis with noticeable changes in clinical and pathological features in an *in vivo* model.

We designed our experiment to collect the skeletal muscle from the quadriceps and then the heart and kidney. These are the main organ of target in case of rhabdomyolysis. In addition, clinical chemistry also has been performed. We observed marked elevation of AST and ALT in KH received groups and in statin group. Elevations of aminotransferases are very common in clinical practice associated with rhabdomyolysis.[19,20] Even though the liver is the main organ for AST production, it also found in other organs such as the heart, skeletal muscle, kidney, and brain in declining order of concentration. Moreover, it has been found that extrahepatic reasons such as skeletal muscle should be the significant source of AST elevation in rhabdomyolysis.[19] The increases in AST correlated with histopathological findings of skeletal myofiber degeneration and focal areas of necrosis.

In contrast, the ALT is of primarily from the liver, but not limited to. The existence of ALT is also found in the skeletal muscle, heart, and kidney. In our study, it has been noted that the elevation of ALT is less than of AST elevation by khat administration. A study by Nathwani *et al.*[21] had shown that elevated aminotransferases associated with muscle injury and AST elevations will be greater than ALT elevations. Even though we found aminotransferase elevation, particularly AST, it is too early to conclude that the observed AST or ALT is solely due to the rhabdomyolysis. In rhabdomyolysis, the damage of skeletal muscle will lead to breakdown of muscle products into the circulation, and it has the potential to induce kidney injury. The severity of kidney damage and the direct measurement of rhabdomyolysis can be measured by estimating the serum CR. Our results showed that serum CR has been showed marked elevation in both statin and KH group.

HFABP, TNNI1, and Myo were useful to detect minimal pathologic alterations in skeletal myofibers. FABP3 modulates the fatty acid uptake in the muscle cells, especially in the skeletal muscle and heart. In combination with other skeletal muscle biomarkers, HFABP is considered as a predictive biomarker for skeletal muscle necrosis.[22] We have found that statin and KH significantly increased the level of FAB protein in serum. It was mildly increased in KL but reached to significant level only in high doses compared to control mean. The increase in HFABP correlated with the magnitude of histopathological findings in skeletal myofiber of degeneration and in the hemorrhages and pyknosis of nucleus observed in the cardiac muscle.

Detection of skeletal muscle injury is measured using serum concentrations of skeletal troponin I (sTnI). sTnI exists in 2 isoforms, slow sTnI (ssTnI) and fast sTnI, representing slow- and fast-twitch muscles, respectively. Our results revealed a highly significant elevation of ssTnl with KH administration, which reveals the probability of severe complications and reported muscular weakness with khat chewing.[23] The expression of HFABP and sTnI in our research shall be considered as an endorsement to studies earlier which suspect rhabdomyolysis with khat chewers[24] and by users of synthetic cathinones.[25] Even though the elevation of HFABP was found in our study, there is a probability that it could be happens due to either cardiac or skeletal muscle injury or kidney damage. Then, we estimated the amount of Myo in the urine. Myo is an oxygen carrier which is abundantly present in both cardiac and skeletal muscle. It is expected to leak into serum in the case of skeletal muscle fiber damage. As expected, we found a significant elevation of Myo in KH treatment. Myo is present mainly in the skeletal muscle compared to the heart muscle, where it is only present in negligible amount. Therefore, the results obtained may reflect the overall total mass of the skeletal muscle than in the heart. But in general, the estimated Myo is considered to increase to a greater extent with skeletal muscle toxicity than cardiac damage. Meantime, the Myo is found to be in normal range in healthy control rats. The magnitude of biomarker elevations in serum correlated with the magnitude of histopathological alterations [Table 3].

Rhabdomyolysis has been previously described in chronic alcoholics.[26] In a retrospective review of dialysis-dependent acute renal failure from rhabdomyolysis and drug misuse, alcohol was the most commonly abused substance, being implicated in 54% of cases.[27] Recent reports suggest that khat chewing is now associated with consumption of alcohol.[28,29] This is mainly seen in African khat users and those migrated to Europe from Africa. In contrary, this trend is not reported in Ethiopian and Yemen consumers. In Uganda, this composition is well known as "mixers."[30,31] Hence, we included one group of animals with mixer of khat and ethanol and another group only with ethanol. Both AST and ALT were found to be elevated in ethanol received group. This may be due to alcoholic liver issues, particularly in the setting of an elevated gamma-glutamyl transferase. Histopathological finding was scored, where Khat ethanol (KE) group showed similar kind of severity in lesions in the muscle, heart, and kidney. Ethanol alone group could not show similar severity as KE group, except in splitting within the myofiber. HFABP, TNNI1, and Myo elevations were not significant in ethanol alone groups. But in KE group, it showed significance as of KH dose treatment. Hence, it shall be seen that the dose of ethanol alone received in the current study and KE combinations could not produce any superior or augmented muscle toxicity than khat alone group.

CONCLUSION

Our research at the dose selected showed noticeable elevation of rhabdomyolysis-related biomarkers together with marked elevation in the lesions as observed in the histopathological analysis. The current subject thus warrants detailed studies which could emphasize on the cardiac complications, muscular toxicity mechanism, and the phytochemicals related to it. The clinicians should be aware of such rare khat-induced rhabdomyolysis to recognize and design the treatment protocol.

Acknowledgements

The authors would like to express their utmost gratitude and appreciation to the Deanship of Scientific Research, Jazan University (JUP8//000269).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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