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Research Article

PROTECTIVE OF ETHANOLIC EXTRACT OF *SAUSSUREA LAPPA* AGAINST PARACETAMOL-INDUCED HEPATIC AND RENAL DAMAGE IN MALE RABBITS

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ABSTRACT

Objective: The objective of our study is to investigate the effect of the ethanolic extract of *Saussurea lappa* against paracetamol-induced hepatorenal toxicity in male rabbits.

Methods: Eighteen male rabbits were used for this study and were divided into three groups of six rabbits each. Group 1: Rabbits were the normal (negative control), Group 2: (Positive control) Rabbits were administered paracetamol at dose 300 mg/kg body weight (B.W) for 14 day, and Group 3: Rabbits received paracetamol at dose 300 mg/kg B.W then treated with ethanolic extract of *S. lappa* at dose 300 mg/kg B.W for 14 day.

Results: The obtained results showed a significant decrease ($p \leq 0.05$) in B.W, red blood cells count, white blood cells count, neutrophil, total protein, and albumin with significant ($p \leq 0.05$) increase in lymphocyte, alanine aminotransferase, aspartate aminotransferase, creatinine, urea, and malondialdehyde in rabbits of positive control group, histological studies showed many pathological changes in liver and kidney when compared with negative control group. The oral administration of the ethanolic extract of *S. lappa* significantly protected the hepatic and kidney cells from damage, the hematological and biochemical parameters were also almost normal in extract treated rabbits compared to the control group.

Conclusion: Our study indicates that the roots of *S. lappa* act as antioxidant substance and have hepato and renoprotective effect against toxicity induced by paracetamol.

Keywords: Paracetamol, *Saussurea lappa* extract, Liver, Kidney, Rabbit.

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INTRODUCTION

Paracetamol (acetaminophen) is one of the most popular and broadly used drugs for the treatment of pain and fever. Paracetamol is contained in several preparations, accessible as each over-the-counter or as many prescription only medications. Because of its wide availability paired with comparably high toxicity (compared with ibuprofen and aspirin), there is an a lot higher potential for overdose [1]. Below traditional conditions, acetaminophen is normally metabolized by undergoing sulfation and glucuronidation [2]. It has been proposed that a little quantity of drug goes through the cytochrome P450 mixed function oxidase system and is metabolized into the reactive intermediate *N*-acetyl-*P*-benzoquinoneimine (NAPQI), which is in turn detoxified by reaction with glutathione [3,4]. When large quantities of paracetamol are consumed, the three detoxification pathways become saturated. Overdose of acetaminophen in human is fairly common and is commonly related to hepatic [5,6] and renal damage [7]. Even though nephrotoxicity is less common than hepatotoxicity in acetaminophen overdose, renal tubular injury and acute renal failure can happen even without liver damage [8-10] and may also lead to death in humans and empirical animals [11,12]. Expanded utilization of synthetic drug therapy leads to several side effects and undesirable risks. Thus, there are worldwide trends to come back to natural herbal, which is culturally accepted and economically viable.

Studies are going for the investigation of defensive molecules that would give most extreme protection to the liver, kidney also other organs and virtually very little or no side effects would be applied during their function in the body [13,14]. Plantmedicine is an achievement of folk therapeutic diversity [15]. The world health organization in the early 1970s had an expectant government to effectively utilize the local knowledge of herbal medicines for disease anticipation and health promotion [16] WHO has shown great curiosity in documenting the use of medicinal plants utilized by tribes from various parts of the world [17]. Herbal medicines are cheaper, it can easily obtained and their way of preparation is additionally trouble-free, and above all, it suits the societal and cultural needs of individuals [18].

Among the medicinal plants, *Saussurea lappa* Clarke (synonym: *Saussurea costus* (Falc.) Lipschitz) (family Asteraceae) is a recognized medicinal plant growing in the Himalayan region between 2500 and 3000 m above sea level. It contains several active ingredients such as flavonoids, steroids, terpenes, alkaloids sesquiterpenes, costunolide, dehydrocostus lactone, cynaropicrin, chlorogenic acid, phenols, gum and mucilage, glycosides, and saponins [19] and has been reported to possess various biological activities such as antifungal [20], anthelmintic [21], antidiabetic[22],antitumor[23],antimicrobial[24],immuno-stimulant[25], antiulcer [26], anti-inflammatory [27], and antihepatotoxic [28]. The present study was designed to evaluate hepato and nephroprotective effects of *S. lappa* against acetaminophen-induced toxicity in rabbits, as this has not been previously investigated.

METHODS

Plant material

The ethanolic extract had been extracted from root of *S. lappa* that was used in our study. The root was hand-picked from the local market. It was turned to powder with the help of an electric grinder and kept in the dark container at 25°C.

Preparation ethanolic extract from *S. lappa*

50 g of powder was put in the round bottle flask, 200 ml of ethanol (70%) were added to the flask and extracted for 12 h at 70°C.

The extract was filtered by using Whatman filter paper; then the extract was put in the Petri dish and left at room temperature under the shade, the collection extracts were kept in tightly closed container and saved until using [29].

Experimental animals
Eighteen healthy rabbit weighing between 1500 and 2000 g, the animals kept in suitable cages in the animal house of Veterinary Medicine College and were feeder standard diet and water *ad libitum*.

Experimental design
The animals were divided into three groups each group containing six animal as follow.

Group 1
(Negative control) Normal rabbits received distilled water.

Group 2
Served as the positive control group, rabbits administered paracetamol at dose 300 mg/kg B.W for 14 days.

Group 3
Rabbits administered paracetamol at dose 300 mg/kg B.W then treated with ethanolic extract of *S. lappa* at dose 300 mg/kg B.W for 14 days.

Collection of blood samples
Blood samples (10 ml) were collected from each animal at the end of the experiment by the heart (cardiac puncture). 8 ml of blood was deposited into tube without anticoagulant, and then the blood samples were centrifuged at 3000 rpm for 15 min and serum samples stored in polyethylene Eppendorf tubes at −20°C), which then used to study biochemical parameters (alanine aminotransferase [ALT], aspartate aminotransferase [AST], total protein [TP], albumin, creatinine, urea, and malondialdehyde [MDA]). The remaining (2 ml) of blood was deposited into tube with anticoagulant which used for hematological analysis (red blood cell [RBC], white blood cell [WBC], hemoglobin [Hb], mean corpuscular volume [MCV], mean corpuscular Hb [MCH], mean cell Hb concentration [MCHC], packed cell volume [PCV], platelets, WBC counts, and differential WBC).

Study parameter
Measurement of the B.Ws
The weight of each animal was recorded in the 0 day and in the 14 days using electronic balance.

Hematological tests
The hematological tests were done in internal medicine department of veterinary medicine using Humacount 5, the instrument has 1 set tube sampling position, deposit in each specific tube sampling, and the reagents were put in special container beside the instrument. The parameters estimated by this instrument were RBC, Hb, PCV, MCV, MCH, MCHC, platelets, WBC, and differential leukocyte.

Biochemical analysis
- AST and ALT activities were enzymatically determined using standard assay (SYRBIO chemical-kit based on the method of Reitman and Frankel in 1957) [30]. Determination of serum TPs carried out using the Biuret method, proteins form a violet color complex in present copper ions in alkaline solution [31]. Albumin was measured in serum base on method performed by Doumas *et al*. 1971 [32]. Albumin reacts with bromocresol green to yield green color
- The creatinine levels were measured using a commercial kit (80107 Biolabs, France), while the urea level was estimated using a commercial kit (11537 Biosystems, Spain)
- Blood MDA assay: Serum lipid peroxide levels were determined by measuring thiobarbituric acid reactivity as described by Buege and Aust [33].

Histological techniques
The animals were sacrificed at the end of the experiment and the organ samples were taken as liver and kidney. These organs were fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentrations, treated with xylene and embedded in paraffin. Five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with hematoxylin and eosin stain [34].

Statistical analysis
Data obtained from experiments were expressed as mean ± standard deviation, the results were analyzed statistically using ANOVA by SPSS programming difference and were considered significant at $p \leq 0.05$ [35].

RESULTS

Effect of the ethanolic extract of *S. lappa* on B.W in rabbits treated with paracetamol
The results are presented in Table 1 that are non-significant change ($p \leq 0.05$) in initial B.W, in all groups, but the results are observed a significant decline ($p \leq 0.05$) of final B.W in positive control group, comparison with negative control group and treated with extract group, while results were showed a significant increase ($p \leq 0.05$) in final B.W in rabbits treated with the ethanolic extract of *S. lappa* compared with negative control and positive control groups.

Effect of the ethanolic extract of *S. lappa* on RBC count and RBC index in rabbits treated with paracetamol
The obtained results in Table 2 revealed a significant decrease ($p \leq 0.05$) in RBC count, Hb, PCV, MCV, MCH, and MCHC in blood rabbits treated with paracetamol (positive control group) compared with negative control group and treated with ethanolic extract of *S. lappa* group, while the results showed non-significant ($p \leq 0.05$) in RBC count, Hb, PCV, MCV, MCH, and MCHC of blood rabbits treated with ethanolic extract of *S. lappa* group compared with negative control group.

Effect of the ethanolic extract of *S. lappa* on WBC count and differential count in rabbits treated with paracetamol
The result of WBC count and neutrophil revealed decline ($p \leq 0.05$) in rabbit treated with paracetamol (positive control group) compared with negative control group and treated with extract of group. But it showed non-significant change ($p \leq 0.05$) in WBC count and neutrophil

Table 1: Effect of the ethanolic extract of *Saussurea lappa* on body weight in rabbits treated with paracetamol

Parameters	Body weight (g)	
	Initial	Final
Group 1	148.42 ± 1.685^{A}	161.72 ± 0.658^{B}
Group 2	147.33 ± 1.032^{A}	106.10 ± 0.593^{C}
Group 3	147.80 ± 0.836^{A}	178.24 ± 0.251^{A}

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different ($p \leq 0.05$)

Table 2: Effect of the ethanolic extract of *Saussurea lappa* on RBC count and RBC index in rabbits treated with paracetamol

Parameters	Group 1	Group 2	Group 3
RBC (10^6/mm^3)	5.875 ± 0.163^{A}	4.260 ± 0.230^{B}	5.455 ± 0.386^{A}
Hb (g/dl)	10.800 ± 0.346^{A}	7.780 ± 0.460^{B}	10.710 ± 0.231^{A}
PCV (%)	37.616 ± 1.999^{A}	28.060 ± 2.255^{B}	34.383 ± 0.868^{A}
MCV (ft.)	62.716 ± 4.041^{A}	50.120 ± 11.919^{B}	62.283 ± 3.268^{A}
MCH (pg.)	19.233 ± 1.626^{A}	17.720 ± 1.513^{B}	19.333 ± 1.211^{A}
MCHC (%)	29.150 ± 1.247^{A}	27.340 ± 1.489^{B}	28.783 ± 1.059^{A}

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different ($p \leq 0.05$). RBC: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean cell hemoglobin concentration

of blood rabbits treated with the ethanolic extract of *S. lappa* group comparison with the negative control group. The results of lymphocyte showed an increase (p≤0.05) in positive control group compared with another group, but the results showed the non-significant change (p≤0.05) in lymphocyte of blood rabbits treated with the ethanolic extract of *S. lappa* group compared with negative control group. The results of monocyte showed non-significant (p≤0.05) of blood rabbits treated with paracetamol (positive control group) compared with another group Table 3.

Effect of the ethanolic extract of *S. lappa* on liver enzymes, TP, and albumin in rabbits treated with paracetamol

The results of ALT and AST revealed a significant increase (p≤0.05) in positive control group compared with negative control group and group treated with extract but the treatment with the ethanolic extract of *S. lappa* significant low (p≤0.05) in ALT and AST, compared with positive control group. The result of TP and albumin revealed a significant decrease (p≤0.05) in positive control group compared with negative control group and group treated with extract but the treatment with the ethanolic extract of *S. lappa* group increased the TP compared with positive control group while the results showed non-significant (p≤0.05) in albumin of rabbits treated with extract group compared with negative control group (Table 4).

Effect of the ethanolic extract of *S. lappa* on creatinine, urea, and MDA in rabbits treated with paracetamol

The results of creatinine, urea, and MDA revealed a significant rise (p≤0.05) in positive control group compared with negative control group and group treated with the ethanolic extract of *S. lappa*, but the results showed non-significant (p≤0.05) in creatinine, urea, and MDA of rabbits treated with the ethanolic extract of *S. lappa* group compared with the negative control group (Table 5).

Table 3: Effect of the ethanolic extract of *S. lappa* on WBC count and differential count in rabbits treated with paracetamol

Parameters	Group 1	Group 2	Group 3
WBC 10^3/µl	5.133±0.314^A	2.450±0.403^B	5.116±0.806^A
Lymphocyte %	47.33±0.816^B	53.083±2.20^A	46.083±3.470^B
Neutrophil %	50.833±5.879^A	35.216±0.976^B	51.200±2.501^A
Monocyte %	4.183±0.194^A	4.783±0.231^A	4.583±0.299^A

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different (p≤0.05) compared to control group. WBC: White blood cells

Table 4: Effect of the ethanolic extract of *Saussurea lappa* on liver enzymes, TP, and albumin in rabbits treated with paracetamol

Parameters	Group 1	Group 2	Group 3
ALT (U/l)	13.583±3.277^C	39.583±3.023^A	19.833±1.366^B
AST (UI)	21.166±2.909^C	43.416±1.908^A	26.750±1.781^B
TP (g/dl)	6.666±0.875^A	3.616±0.365^C	5.6400±0.296^B
Albumin (g/dl)	3.588±0.188^A	2.583±0.354^B	3.320±0.130^A

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different (p≤0.05) compared to control group. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TP: Total protein

Histopathological examination

Liver

The liver of negative control group showed that normal hepatocytes are arranged in cords radiating from the central hepatic vein (Fig. 1a). While the liver of rabbits in positive control has shown a histological change which includes dilated and congested of central vein and artery of portal area, excessive vacuolated of hepatocyte, hemorrhage, necrotic as foci, aggregation of inflammatory cells, mild fibrosis in portal area, and hyperplasia in bile duct (Fig. 1b). However, liver of rabbits treated with ethanolic extract of *S. lappa* group showed the normal appearance of hepatocytes in the centrilobular area, normal portal area, and mild congestion (Fig. 1c).

Kidney

As shown in Fig. 2a, kidney of rabbits in negative control group observed normal glomeruli, and normal epithelial cells lining of the renal tubules, while the kidney of rabbits in positive control has shown histopathological changes, as shown in Fig. 2b, the changes included that glomerular size was significantly reduced, thin capsule (wrinkling), vacuolation of epithelial cell and star lumen of renal tubule, hemorrhage in the interstitial tissue, excessive sloughing of cell membrane, atrophy of glomeruli, and infiltration of inflammatory cell. However, kidney of rabbits treated with ethanolic extract of *S. lappa* group showed normal renal tubules with glomerulus Fig. 2c.

DISCUSSION

To the best of our knowledge, this is the first study to investigate *S. lappa* as protection against paracetamol-induced toxicity. Oral administration of paracetamol-induced significant lower (p≤0.05) in B.W compared with negative control group may be due to the loss of appetite observed in the course of study; furthermore, our results also observed that administration of the ethanolic extract of *S. lappa* led to improved B.W gaining in rabbits, through its contents which were flavonoids, proteins, and carbohydrates, which are necessary for growth, body repair, and maintenance. This study demonstrated significant decline (p≤0.05) in RBC count, Hb, PCV, MCV, MCH, and MCHC levels in positive control group which treated with paracetamol when compared with negative control group, that is, paracetamol has potential to prevent erythropoietin release from the kidneys. The low in erythrocyte value may be due to rise free radicals, reactive oxygen species, and peroxide radicals after paracetamol administration which lead to hemolysis anemia [36], as well paracetamol lead to hepatotoxicity and impairs protein synthesis and reduction in the serum TP, albumin, and globulin concentration, consequently, insufficiency of protein synthesis that specifically induces decline of essential amino acids and shortage of energy. Source of protein synthesis incorporated in Hb production and anemia [36]. This result in agrees with previous research that paracetamol caused destruction RBC and cause thrombocytopenia and hemolytic anemia [37]. These results show that these plants product may have therapeutic effect agonist hematotoxicity induce by paracetamol in the group which treated with the ethanolic extract of *S. lappa* and paracetamol when compared with positive control group which treated with paracetamol may be due *S. lappa* contain phytochemical compound include alkaloids, saponins, steroids, terpenes, polyphenol, flavonoids, sterols, tannins, and glycosides. These compounds are well known hemopoietic factors that have a direct influence on the production of blood and antioxidant substance serve on inhibition free radical, inhibit hemolytic anemia and ameliorate blood components [38,39].

Table 5: Effect of the ethanolic extract of *Saussurea lappa* on creatinine, urea, and MDA in rabbits treated with paracetamol

Parameters	Group 1	Group 2	Group 3
Creatinine mg/dl	0.736±0.930^B	2.448±0.396^A	0.841±0.491^B
Urea (mg/dl)	27.833±2.316^B	53.333±7.527^A	32.333±2.065^B
MDA (nmol/mg of pt)	1.866±0.196^B	5.900±0.379^A	2.100±0.334^B

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different (p≤0.05) compared to control group. MDA: Malondialdehyde

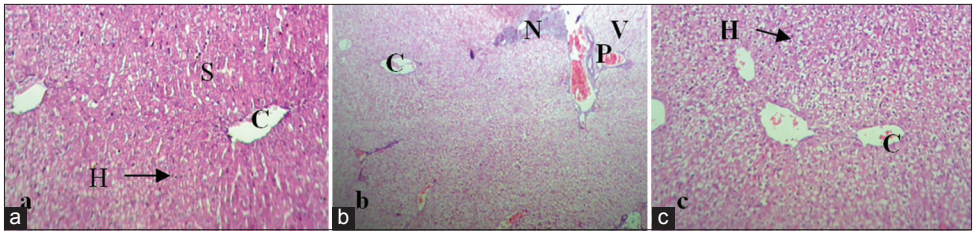

Fig. 1: Histopathological changes in liver of rabbit. (a) negative control group showing normal central vein (C), normal hepatocyte (H), sinusoid (S), (b) positive control group showing congested portal area (P), excessive vacuolated of hepatocyte, (V), hyperplasia of bile duct and necrotic area (N), (c) liver of rabbit treated with ethanolic extract of *S. lappa* showing normal central vein (C), normal hepatocyte (H)

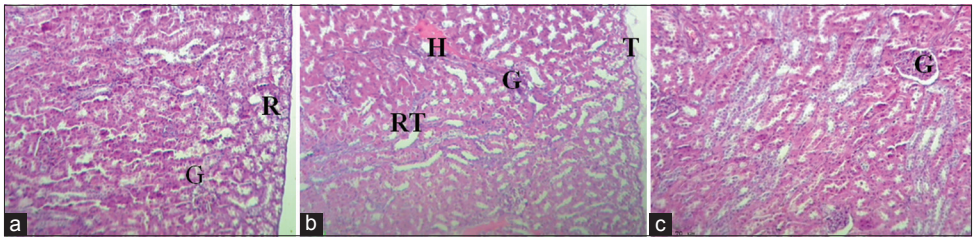

Fig. 2: Histopathological changes in kidney of rabbit, (a) negative control group, showing normal glomerul and normal renal tubules (RTs), (b) kidney of rabbit in positive control group, showing reduced in glomerular size (G) thin capsule (wrinkling) (T), dilated of RT, vacuolated of epithelial cells and hemorrhagic area (H), (c) kidney of rabbit in treated with the ethanolic extract of *S. lappa* group, showing normal RTs with glomerulus (G)

This study demonstrated significant decline ($p \leq 0.05$) in WBC of rabbits received paracetamol in positive control group comparison with negative control group indicated a suppressing of the immune system. The low in WBCs count may be due to the inability of the hematopoietic tissues to production new WBCs [40]. From the result of the differential white cells count that carried out in our study, the rise in lymphocytes count in paracetamol treated group might be due to the interaction between paracetamol and gastrointestinal macrophages, which act as a toxic material. The macrophages caused the activation of the helper T cells and the B lymphocytes, through serving as antigen presenting cell and the antigenic products [41]. They as well excrete materials called interleukin-1/-cytokines that stimulate the activation of lymphocytes and rise their count [41]. The major role of lymphocyte is the response to antigen (foreign bodies) through the expansion of cellular immunity and forming antibodies circulating in the blood [42]. Paracetamol might be a toxic effect on the neutrophils in the blood, or it has a serious impact on the bone marrow, causing the lowering of these blood cells production. Neutrophils considered as the first-line defense versus toxic materials, foreign substances, and microorganisms [43]. This might be an indicator of the disruption of immune status in the treated animals responding to the toxic effect of paracetamol. Coadministration of *S. lappa* and paracetamol showed increase WBC as a compared with control positive group and restore differential count near to control group may be due to active materials known as dehydrocostus lactone and costunolide in *S. lappa* [44,45] that refers to *S. lappa* enhanced the immunity of rabbits treated with paracetamol [46].

The larger dose of paracetamol causes hepatotoxicity, the obtain result indicated chronic paracetamol consumption induces severe liver injury and liver necrosis as observed by the rising liver enzyme AST and ALT and lowering in TP and albumin concentration in control positive group which administered paracetamol, may overdose of this caused forming reactive oxygen species and induce oxidative stress which lead to the hepatotoxicity, well rise may be attributed to the libration of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular injury of hepatocytes and necrosis. Serum AST and ALT are biomarkers in the diagnosis of hepatic injury because they are liberated into the blood circulation after cellular damage [47]. Moreover, hepatotoxin impaired the ability of the liver to synthesize albumin [48]. In our study, decline total serum protein level in paracetamol treated rabbits may be attributed to impaired protein synthesis by damaged liver tissue [49]. While administration of the ethanolic extract of *S. lappa* ameliorated effect agonist hepatotoxicity which induces by paracetamol, present study shown lowering liver enzymes AST and ALT and also height TP and albumin in Group 3 when compared with positive control group which exposure to paracetamol may be due to phytochemical compounds such as flavonoids and chlorogenic acid which acts as antioxidant substance serve a suppression free radicals induced lipid peroxidation and prevents paracetamol toxicity [50,51].

In this study, paracetamol caused nephrotoxicity was characterized by apparent elevations in serum creatinine and urea in positive control group. In nephrotoxicity and kidney diseases, the serum urea and creatinine accumulate due to the rate of production exceeds, the rate of clearance due to the deficiency in renal function [52]. Paracetamol nephrotoxicity occurs because its highly reactive metabolite NAPQI- which arylates proteins in the proximal tubule, at beginning cell death of renal tubular cells [53]. The kidneys include the excretion of various xenobiotics, pollutants, and toxins and hence they are prone to liberate rise quantities of free radicals which participate in high oxidative stress. This is included in the pathogenesis of kidney damage [54]. The present results in Group 3 toxicated with paracetamol and treated with the ethanolic extract of *S. lappa* until the end of experiment observed nearly values of creatinine and urea compared to negative control group that means nephroprotective properties of *S. lappa* on toxic effect of paracetamol, due to the high concentration of flavonoids and alkaloids they contain [55,56] as antioxidant and/or free-radical scavenging activities. The results indicated a significant excess in serum MDA level, which is a product of lipid peroxidation of the positive control group as compared with negative control group. This excess in MDA level is as a result of a rise in lipid peroxidation by the actions of the toxic metabolite NAPQI. While treatment with the ethanolic extract of *S. lappa* caused a significant low ($p \leq 0.05$) in the MDA level of the treatment group as compared with the positive control group, thus, these decreases could be as a result of the actions of the phytochemical constituents of the extract such as flavonoids in preventing the actions of the toxic metabolite NAPQI and also stabilizing the cell membranes of the intracellular proteins and other materials [57].

Histological studies on liver tissues following administration acetaminophen overdoses can cause liver damage and even failure;

cell death may occur as a result of apoptosis and necrosis [58]. Microscopic examination of histological preparations of the kidney observed decrease in glomerular size, severe tubular vacuolar, necrosis with degeneration, and with glomerular bleeding [59,60], but the histological studies on liver and kidney tissues following administration of *S. lappa* did not present any visible lesions on the tissues.

AUTHORS' CONTRIBUTIONS

The author declares that this study was done by the author named in this article.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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