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

MARIAM A KADHEM*
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Basra, Basra, Iraq.
Email: maremhussain60@gmail.com

Received: 21 May 2019, Revised and Accepted: 10 June 2019

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/kgB.W for 14 day.

Results: The obtained results showed a significant decrease (p≤0.05) in BW, red blood cells count, white blood cells count, neutrophil, total protein, and albumin with significant (p≤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.

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 normally 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 [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.

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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 rabbits 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 follows.

**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 centrifugation by the heart (cardiac puncture). 8 ml of blood was deposited into tubes with 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 tubes with anticoagulant which for hematological analysis (red blood cells [RBC], white blood cells [WBC], hemoglobin [Hb], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean cell Hb concentration [MCHC], packed cell volume [PCV], platelets, and 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 one set of parameters estimated in each instrument. The parameters estimated by this instrument were RBC, Hb, PCV, MCV, MCH, MCHC, platelets, WBC and, differential leucocyte.

**Biochemical analysis**

- AST and ALT activities were enzymatically determined using standard assay (SYRBIOS chemical-kit based on the method of Reitman and Frankel in 1957) [30]. Determination of serum TP's 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).
- **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≤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≤0.05) in initial B.W in all groups, but the results are observed a significant decrease (p≤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≤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≤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>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≤0.05) in differential count in rabbits treated with paracetamol compared with negative control group and treated with extract group, but it showed non-significant change (p>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° | 161.72±0.658° |
| Group 2    | 147.33±1.032° | 106.10±0.593° |
| Group 3    | 147.80±0.836° | 178.24±0.658° |

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

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

| Parameters | RBC | Group 1 | Group 2 | Group 3 |
|------------|-----|---------|---------|---------|
| percent    |     | %       | %       | %       |
| Hb (g/dl)  | 10.80±0.346° | 9.78±0.460° | 10.71±0.231° |
| PCV (%)    | 37.6±1.999° | 28.0±2.255° | 34.38±2.868° |
| MCV (fl.)  | 62.16±4.041° | 50.12±11.919° | 62.28±5.268° |
| MCH (pg)   | 19.23±1.624° | 17.72±1.513° | 19.33±1.211° |
| MCHC (%)   | 29.15±1.247° | 27.34±1.489° | 28.78±1.059° |

Values are expressed as mean±standard deviation of six rabbits in each group. Capitals letters denote significantly different (p<0.05).
of blood rabbits treated with the ethanolic extract of S. lappa group compared 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 (10³/µl)       | 5.13±0.31 | 2.45±0.40 | 5.11±0.80 |
| Lymphocyte %       | 47.3±8.16 | 53.0±32.20 | 46.0±3.47 |
| Neutrophil %       | 5.08±5.87 | 35.2±6.97 | 51.20±2.50 |
| Monocyte %         | 4.18±0.19 | 4.73±0.23 | 4.58±0.29 |

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 (UI)   | 13.58±3.27 | 39.53±3.02 | 19.83±1.36 |
| AST (UI)   | 21.16±2.90 | 43.41±1.98 | 26.75±1.78 |
| TP (g/dl)  | 6.66±0.87 | 3.61±0.36 | 5.64±0.29 |
| Albumin (g/dl) | 3.58±0.18 | 2.58±0.35 | 3.32±0.13 |

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

**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 | 0.73±0.93 | 2.44±0.39 | 0.84±0.49 |
| Urea (mg/dl)    | 27.83±2.31 | 53.33±7.52 | 32.33±2.06 |
| MDA (nmol/mg of pt) | 1.86±1.96 | 5.90±0.37 | 2.10±0.34 |

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

**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 staur 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 against hematoxity 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].
This study demonstrated significant decline (p≤0.05) in WBC of rabbits received paracetamol in positive control group comparison with negative control group indicating 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. Co-administration 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 costusindole 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 intoxicated 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 creatinine and urea in 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≤0.05) in the MDA level of the treatment group as compared with the positive control group, 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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