Protective Effect of Ethanolic Extract of Saussurea Lappa on Paracetamol Induced Toxicity in Female Rabbits

Mariam A Kadhem¹ and Shatha A Kadhum²

¹ Department of Physiology, pharmacology and Chemical, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.
² Department of Mathematics, College of Sciences, University of Basrah, Basrah, Iraq.

E-mail: maremhussain60@gmail.com

Abstract. The current study was carried out to investigate the protective effects of ethanolic extract of Saussurea lappa on paracetamol induced toxicity in female rabbits. Eighteen female rabbit adult were divided into three groups each (group/6 rabbits). First group (negative control) was administered distilled water for 14 day. Second group (positive control) was administered paracetamol at dose 300 mg/kg B.W orally for 14 day. Third group was administered paracetamol at dose 300 mg/ kg B.W orally and treated with ethanolic extract of Saussurea lappa at dose 300 mg/kg B.W orally for 14 day. The animals were sacrificed and blood samples were collected from heart by cardiac puncture. These samples were used for the measurement of biochemical parameter like ALT, AST, TP and Albumin as well as hormonal assay in addition to histological examination for liver, ovary and uterus. The obtained results showed a significant increase (P≤ 0.05) in ALT, AST and significant decrease (P≤ 0.05) in TP, Albumin, FSH and LH level in control positive group compared with control negative group while the results showed a significant decrease (P≤ 0.05) in ALT, AST and increase TP, Albumin, FSH and LH level in rabbits treated with ethanolic extract of Saussurea lappa. Histological studies showed many pathological changes in liver, ovary and uterus in control positive group but in the Saussurea lappa group the histopathological changes near to the normal status. The protective effects of Saussurea lappa extract on paracetamol induce toxicity resulting in improvement effect on liver and reproductive system could be attributed to their antioxidant actions.

Keywords: Saussurea lappa, Paracetamol, Female rabbits, Pharmacological effect, Histological effect

1. Introduction.

Paracetamol (Acetaminophen) is a non-prescription drug commonly used for antipyresis and analgesia.(1) Although generally considered a safe drug, it continues to be a cause of death either through overdose, idiopathic reaction,. Death from acetaminophen overdose is thought to be secondary to liver failure, which is caused by massive hepatic necrosis, the hallmark pathological feature of acetaminophen toxicity. In addition to liver, however, many organ systems may fail under acute overdose such as renal, cardiac, and central nervous systems (2). It is thought that the liver is the target organ for acetaminophen toxicity because this is primarily where the drug is detoxified. Under normal
conditions, acetaminophen is mainly metabolized by undergoing sulfation and glucuronidation (3). It has been proposed that a small amount of drug goes through the cytochrome P450 mixed function oxidase system and is metabolized into the reactive intermediate N-acetyl-P-benzoquinoneimine (NAPQI),1 which is in turn detoxified by reaction with glutathione (4, 5). When large quantities of acetaminophen are consumed, the detoxification pathways become Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments (6). Costus roots (Saussurea Lappa) are one of these herbal plants. It belongs to the family Asteraceae which is commonly known as sunflower family. Costus is popularly known as Kuth root or Costus and used in various traditional medicines as anti-ulcer, anticonvulsant, anticancer, hepatoprotective, anti-arithmetic, antiviral, and antioxidant(7,8,9,10) because it contains many active compounds such as flavonoids, steroids, terpenes, alkaloids sesquiterpenes, costunolide, dehydrocostus lactone, cynaropicrin, and chlorogenic acid(11). To the best of our knowledge, studies concerning the toxic effects of paracetamol on the liver and female reproductive function and the counter effects of Saussurea lappa are quite limited. Therefore, this study was designed to evaluate the toxic effects of paracetamol on the reproductive organ and functions in female rabbits and the possible preventive and curative effects ethanolic extract of Saussurea lappa

2. Material and methods:

2.1. Plant Material

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

2.2. Preparation Ethanolic extract from Saussurea lappa

50 gm of powder were put in the round bottle flask, 200 ml of ethanol (70 %) were added to flask and extracted for 12 hrs at 70 °C. The extract was filtered by using whatmann filter paper, then the extract were put in the petridish and left at room temperature under the shade, the collection extracts were kept in tight closed container and stored until using (12).

2.3. Experimental animals:

Eighteen adult female rabbit weighting between (1500-2000) gm, the animals kept in suitable cages in the animal house of Veterinary Medicine College and were feeder standard diet and water ad libitum.

2.4. Experimental design:

The animals were divided in to three groups each group containing 6 animal as follow:-
Group 1: (Negative control) normal rabbits received distilled water.
Group 2: served as positive control group, rabbits received paracetamol at dose 300 mg/kg B.W for 14 day.
Group 3: rabbits received paracetamol at dose 300 mg/kg B.W then treated with ethanolic extract of Saussurea lappa at dose 300 mg/kg B.W for 14 day.

2.5. Collection of Blood Samples.

Blood samples (10ml) were collected from each animals at end of experiment by the heart (cardiac puncture). blood was deposited into tube without anticoagulant and then the blood samples
were centrifuged at (3000 rpm) for 15 minutes and serum samples stored in polyethylene eppendorff tubes at (-20ºC), which then used to study biochemical parameters (ALT, AST, TP, Albumin, and hormonal assay such as FSH, and LH).

2.6. Biochemical analysis:

AST and ALT activity were enzymatically determined by using standard assay (SYRBIO chemical-kit based on the method of Reitman and Frankel in 1957 (13). Determination of Serum total proteins carried out by using Biuret method, Proteins form a violet color complex in present copper ions in alkaline solution(14). Albumin was measured in serum base on method performed by Doumasin 1971 (15). Albumin reacts with bromocresol green to yield green color

2.7. Histological techniques

The animals were sacrificed at the end of the experiment and the organ samples were taken as liver, ovary and uterus. 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 Haematoxyline and Eosin (H & E stain) (16).

2.8. Statistical analysis:

The mean and standard deviation (mean ±SD) were calculated for all values. Comparison between the control and the treated group, were done using the student's t-test. Difference were considered statistically significant at p≤0.05 (17).

3. Results:

3.1. Effect of of Ethanolic extract of Saussurea lappa on liver enzymes, total protein and albumin in female rabbits treated with paracetamol.

The results of ALT and AST revealed a significant increase (p≤0.05) in control positive group compared with control negative group and group treated with ethanolic extract of Saussurea lappa but the treatment with ethanolic extract of Saussurea lappa decreased the ALT and AST compared with control positive group. The result of TP and Albumin revealed a significant decrease (p≤0.05) in control positive group compared with control negative group and group treated with ethanolic extract of Saussurea lappa but the treatment with ethanolic extract of Saussurea lappa group increased the TP compared with control positive group while the results showed non-significant (p≤0.05) in Albumin of rabbits treated with Ethanolic extract of Saussurea lappa group compared with negative control group (table 1).

Table (1): effect of Ethanollic extract of Saussurea lappa on ALT, AST, TP, Albumin in female rabbits treated with paracetamol.

| Parameters    | Group 1     | Group 2     | Group 3     |
|---------------|-------------|-------------|-------------|
| ALT(U/I)      | 15.000±2.966 | 39.583±3.023 | 19.800±1.524 |
|               | C           | A           | B           |
| AST(UI)       | 22.250±3.416 | 43.416±1.908 | 26.100±0.894 |
|               | C           | A           | B           |
| TP(g/dl)      | 7.150±0.868  | 3.300±0.644  | 5.880±0.228  |
|               | A           | C           | B           |
| Albumin (g/dl)| 3.833±0.206  | 2.166±0.417  | 3.500±0.402  |
|               | A           | B           | A           |
Value are expressed as mean ISD of 6 guinea pigs in each group. Capitals letters denote significantly different (p≤0.05) compared to control group.

3.2. Effect of Ethanolic extract of Saussurea lappa on FSH and LH in rabbits treated with paracetamol.

The results of FSH and LH revealed a significant decrease (p≤0.05) in control positive group compared with control negative group and group treated with ethanolic extract of Saussurea lappa, but the results showed non-significant (p≤0.05) in FSH and LH of female rabbits treated with Ethanolic extract of Saussurea lappa group compared with negative control group (table 2).

Table (2): effect of Ethanolic extract of Saussurea lappa on FSH and LH in female rabbits treated with paracetamol.

| Parameters  | Group 1 | Group 2 | Group 3 |
|------------|---------|---------|---------|
| FSH (mIU/ml) | 6.28±0.541 | 3.31±0.397 | 6.68±0.432 |
| LH (mIU/ml) | 11.60±0.821 | 3.40±1.077 | 11.73±1.687 |

Value are expressed as mean ISD of 6 guinea pigs in each group. Capitals letters denote significantly different (p≤0.05) compared to control group.

Histological examination.

Liver:
The liver of female rabbits (control negative) appeared normal architecture of liver, hepatocyte in addition to normal central vein and sinusoid as shown in figure (1), while the liver in control positive group revealed histopathological changes including minimal diffuse vacuolation of hepatocytes, septal fibrosis and inflammatory cells, mostly mononuclear cell as shown in figure (2), but the liver of female rabbits treated with Saussurea lappa exhibited normal hepatocyte normal central vein, sinusoid as shown in figures (3).

Ovary:
The ovary in control negative rabbits appeared normal ovarian cellular tissue with normal Graafine as shown in figure(4) while the ovary of control positive rabbits treated with paracetamol revealed histopathological changes included very clear disturbance in ovarian tissue or parenchyma, primary
follicles, follicles enclosed by fibrosis, tertiary follicles appeared with degeneration and enclosed by fibrosis matrix as shown in figure (5) but ovary of female rabbits treated with *Saussurea lappa* appeared normal ovarian cellular tissue with normal graafine follicles, normal primary follicles and secondary follicles as shown in figures (6).

![Figure (4): Section of ovary of control rabbit. Showing normal ovarian cellular tissue with normal Graafine follicles (G). stain (H&E) 400X.](image4)

![Figure (5): Section of ovary of control positive rabbit. Showing primary follicles, follicles enclosed by fibrosis(F), tertiary follicles appeared with degeneration and enclosed by fibrosis matrix(E). stain (H&E) 400X.](image5)

![Figure (6): Section of ovary of female rabbit treated with *Saussurea lappa* showing normal ovarian cellular tissue with normal graafine follicles (G) and secondary follicles(S). stain (H&E) 400X.](image6)

**Uterus:**
The uterus of control negative rabbits appeared normal endometrium, normal uterine gland, normal uterine lumen as shown in figure (7). While the uterus of control positive rabbits treated with paracetamol revealed histopathological changes, this changes included thickened uterine wall with prominent myofibrosis stroma, also part of the uterus lined by papillary vacuolated proliferating epithelium as shown in figure (8) but the uterus of rabbits treated with *Saussurea lappa* exhibited amelioration of uterine tissue normal endometrium (proliferative phase), normal uterine glands, dilated lumen and papillary epithelium as shown in figures (9).

![Figure (7): Section of uterus of control negative rabbit. Showing normal endometrium, normal uterine gland, normal uterine lumen, stain(H&E) 400X.](image7)

![Figure (8): Section of uterus of control positive rabbit induced by paracetamol. Showing thickened uterine wall(T) with prominent myofibrosis stroma(f), stain (H&E) 400X.](image8)

![Figure (9): Section of uterus of rabbit treated with *Saussurea lappa* showing normal uterine glands (U), dilated lumen and papillary epithelium (P), stain (H&E) 400X.](image9)

4. **Discussion:**

Paracetamol, a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses (18, 19, 20). In the present study, the serum level of hepatic enzymes ALT, AST levels in control positive group were increased and reflected the
hepatocellular damage in the paracetamol-induced hepatotoxicity animal model. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver (21). However the total protein and albumin levels were decreased, in our study, decreased total serum protein level in paracetamol treated rabbits may be attributed to impaired protein synthesis by damaged liver tissue. Moreover, hepatotoxicin impaired the capacity of liver to synthesize albumin (22). The reversal of increased serum enzymes in paracetamol induced liver damage by the ethanolic extract of Saussurea lappa, in addition elevation total protein and albumin may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity, due to phytochemical compound of Saussurea lappa extract like flavonoids and chlorogenic acid which act as antioxidant substance serve a inhibition free radicals-induced lipid peroxidation and suppression paracetamol toxicity (23).

Our study showed that administration of over dose of paracetamol to adult female rabbits resulted in pituitary and gonadal dysfunctions manifested by marked decrease in the plasma levels of FSH, LH, thus paracetamol induced gonadal impairment might due to the direct effect of paracetamol on gonads or central effect on the pituitary gland but the results show the positive effects of Saussurea lappa extract on increasing the plasma levels of FSH, LH compared to animals that were treated with paracetamol in control positive group was due to the ability of Saussurea lappa extract on removing toxic affectivity of paracetamol. Saussurea lappa mainly contains flavonoids , steroids, and chlorogenic acid known characteristic antioxidant and detoxification resulting from paracetamol, therefore the extract exhibit gonadotrophin activity in female rabbits, which caused an increase of FSH, LH(11,24)

5. References.

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