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Evaluation of Launaea Angustifolia Extract Treatment on Rat Blood Serum Enzymes and Histology of Liver and Kidney

Aiman A. Ammari[1], Ahmad R. Alhimaidi[1], Ramzi A. Amran[1], Muath G. Al Ghadi[1], Ahmed G. Rady[1]

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ABSTRACT

Background: Launaea angustifolia (LAN) is a wild plant grown in several Arabian countries in West Asia and North Africa. Launaea is a polymorphic inter- and intraspecific genus that includes about 54 species and 10 subspecies and is classified into eight sections. This study illustrates the effects of Launaea angustifo LAN (LAn) extract on male and female body weight and blood serum enzymes alanine aminotransferase (LAT) and Alkaline phosphatase LAN, with the histology of liver and kidneys used as an animal model for humans.

Methods: A quantity of 100 mg of the extract powder was dissolved in 1 L of DMSO. Experimental rats were divided into five groups with 10 in each group (A, B, C, D, E: 100, 50 and 10 mg/kg and positive control and negative control, respectively).

Result: No significant differences were observed between the treated and control-group females. However, the mean male body weight showed some variation within the 5th group (E) given the negative control (DMSO) and all treated groups presented lower body weights than the normal control (C). Analysis of the enzymatic activities of (ALT) alanine aminotransferase showed that the females in treatment group A (100 mg) and B (50 mg) had higher levels of ALT than those of group D females (10 mg) at $P<0.05$ and females in group E at $P<0.001$). The histology section of the liver and kidney and the histological structure of the rat kidney also showed no differences in all groups. Launaea angustifolia
extract (LAn) was found to be safe, especially in the histology of the liver and kidney and for ALT and ALP blood parameters.

Key words: Launaea angustifolia extract, Rat liver, Kidney enzymes, Histology.

INTRODUCTION

Medicinal plants contain active ingredients and are free of side effects; as a result, the demand for such plants has grown, leading to a variety of applications and cheaper prices (Nikfarjam *et al*., 2016; Heidari *et al*., 2017; Farahbod and Soureshjani, 2018; Abasian *et al*., 2018).

In recent years, antioxidants, proteins, antibiotics and hormones have all been produced using therapeutic plant extracts (Alhimaidi *et al*., 2022; Abasian *et al*., 2018; Alnahdi *et al*., 2018; Divya and Anand, 2018; Ayaz *et al*., 2017; Ghafurniyan *et al*., 2015).

Launaea angustifolia (LAN) is a wild plant grown in several Arabian countries in West Asia and North Africa (Kilian, 1997). LAN is a genus of flowering plants in the family Asteraceae. It is characterized by yellowish flowers with pubescent corolla tubes. Launaea is a polymorphic genus with inter-and intraspecific inconsistencies, especially within the section Zollikoferia DC. This genus contains about 54 species and 10 subspecies and is classified into eight sections.

As reported in (Marzouk *et al*., 2021), several species of the genus Launaea are used in folk medicine to treat, *e.g*., stomach ache and skin diseases and are reported to have antitumor, insecticide and cytotoxic activities. Michel *et al*. (2020) (on coumarin constituents' antimicrobial activities reviewed the potential uses of medicinal plants from the Asteraceae and Lamiaceae families in cardiovascular diseases and found that most such plants have antioxidant effects followed by anti-hyperlipidemia, vaso-relaxant, antithrombotic and diuretic effects (from the most common to least common studies).

The chemical composition of essential oils from *Launaea resedifolia* L. was identified using the ordinary GC-MS technique, revealing the presence of 19 compounds dominated by dioctyl phthalate. Nineteen compounds in the essential oil of *L. resedifolia* were also identified, representing 86.68% of the total oil.

The compounds identified via spectral comparison were mainly esters, alcohols, ketones and terpenes. The principal constituents were dioctyl phthalate (39.84%), Decanoic acid, decyl ester (12.09%), 11-Octadecenal (11.24%) and Eucalyptol (07.31%), while others were present in relatively small amounts. Some studies also explored coumarin

[1]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia.

Corresponding Author: Aiman A. Ammari, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia. Email: aammari@ksu.edu.sa

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constituents' antimicrobial activities and neuro-pharmacological properties. The antibacterial activity of crude oil was analyzed using the disk diffusion method against seven bacterial strains. Antibacterial assays found that the oils were active against most of the tested bacterial strains. A major constituent in the visible parts was Dioctyl phthalate (39.84%) and the yield of essential oils was 0.9%.

These extracts revealed *in vitro* antibacterial activity on the studied bacteria, as confirmed by the inhibition zone diameter ranging from 11 to 37 mm and a MIC value between 0.09 and 0.69, depending on the microorganism being tested. Zellagui *et al*. 2012).

Manijeh and Maryam, (2020) carried out a cytotoxic study on the effects of Hydro-alcoholic leaf extract of Elaeagnus Angustifolia (LAn) in a hepatocellular carcinoma cell line (HepG2). Their results showed that the Lunaea Angustifolia (LAn) plant contained compounds with cytotoxicity properties, highlighting this plant as a potent candidate for liver cancer treatment. Experimental animals were used in the investigation as a human biology model. Bahadoran *et al*. (2020).

A blood serum enzyme test can reveal the body's condition and alanine aminotransferase (ALT) is an enzyme found mainly in the liver, kidneys and other organs. ALT is the most widely used clinical biomarker of hepatic health. (Ozer *et al*. 2008). As its name implies, ALT is involved in the transamination of alanine and is present in the liver at much higher concentrations than in other organs. The body uses ALT to break down food into energy and the function of ALT is to convert alanine into pyruvate for cellular energy production (Raval *et al*. 2019).

ALT can also be called serum glutamic-pyruvic transaminase (SGPT). Normally, ALT levels in the blood are low; however, if the liver or kidneys suffer from a disorder, more ALT will be released into the blood and levels will rise (Botros and Sikaris, 2013).

Thus, an ALT blood test is used to check for liver and kidney disorders in the body (Kasarala and Tillmann, 2016). Alkaline phosphatase (ALP) is an enzyme found in the blood that comes from the liver and bones.

An ALP blood test measures the level of ALP in one's blood and is one of the tests included in a comprehensive metabolic panel. High levels of ALP in the blood may indicate liver disease or certain bone disorders. Kazi *et al*. (2018). The level of alkaline phosphatase (ALP) In humans varies from 44 to 147 international units per liter (IU/L), but other lab kits recommend a range of 30 to 120 IU/L. The normal range of ALP in Wister male rats is 44 to 147 IU/L (MedlinePlus: Medical Encyclopedia 2017).

The aims of this study are to illustrate the effect of the Launaea angustifolia (LAn) extract on male and female ALT and ALP blood serum enzymes, with the histology of liver and kidney used as an animal model for humans.

MATERIALS AND METHODS

The plants were collected from February to April, 2022, from the Thomammah arid desert area northeast of the Riyadh city area. The leaves were collected and washed and then placed in a dark and dry place away from insects and dust until dry. Then, the samples were ground and extracted. The powder was mixed with 500 ml of methanol for 1 days on a shaker. Next, the solutions were left to stand and filtered via Whatman paper. The extract was poured into a Petri dish and incubated. The dried samples were collected and maintained at a temperature of -20°C. Then, 100 mg of the extract powder was dissolved in 1 liter of DMSO (Alhimaidi *et al*., 2022; Alhimaidi *et al*., 2021).

All animal treatments were performed according to the regulations and guidelines of the ethics committee and the Institutional Animal Care at King Saud University, as well as the Collaborative Institutional Training Initiative (CITI) program, Lab Animal Research ID no. Record ID 46433344.

Adult female and male rats (12-15 weeks of age) were obtained from the animal house at the Zoology Department, College of Science, King Saud University. The animals were housed in a ventilated room at 25±2°C under a 12:12 h light/dark cycle. The animals had free access to standard laboratory feed and water ad libitum. The rats were divided into five groups according to treatment concentration, with 5 females and 5 males in each group. The 1st group (GA) was treated with 100 mg/kg, the 2nd group (GB) was treated with 50 mg/kg and the 3rd group the normal control group (GC) without any treatment. The 4th group (GD) was treated with 10 mg/kg of the LAn extract via feeding tube and the 5th group (GE) was treated with DMSO and used as a negative control. Then, 100 g of the extract powder was dissolved in 1 L of DMSO for treatment. To reach the desired concentration of (LAN) extract treatments (10, 50 and 100 mg/kg), a 50 mL beaker was weighed and then filed with 5 mL of filtered fluid; this fluid was then evaporated in an isotonic incubator (at 50-60°C for approximately 5 h).

Afterward, the remaining extract was weighed and then each rat was orally fed with the desired dose according to the following formula: desired dose (10, 50, or 100 mg/kg) X rat body weight (BW, g) / (LAN) extract concentration (mg/mL) = ? ml (Alhimaidi *et al*. 1998, 2021). All animal groups were treated for two weeks and their body weights were taken at the end of the experiment. Blood samples were then collected from all rat groups for hematological and enzyme examination.

The liver and kidney enzymes from rat blood samples were analyzed to investigate how the Launaea angustifolia extract affected them. At the end of the experiment, the blood samples collected from female and male rats were placed in non-heparinized glass tubes for cell blood counts using a cell counting system and in heparinized glass tubes for serum collection. Then, the serum was separated via centrifugation at 3000 rpm for 15 min. The enzymatic activities of the alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured via diagnostic kits using the Bio-System instrument BTS-350.

Samples of the rat liver and kidney were collected for histological analysis from all treated and control rats. Each rat

was kept in a chamber containing CO_2 until dead and then the liver and kidney were extracted and kept in 10% neutral formalin for 48 h as small cube-sized samples (~0.5 cm) for fixation. Later, the rat liver and kidney samples were dehydrated using an alcohol solution (30%-50%, 70% and 100%).

The samples were next treated with xylene and then embedded in paraffin wax cubes and sectioned via microtome to a size of 3-4 mm. The sections were mounted on slides and stained with eosin and hematoxylin. Lastly, the liver and kidney sections were examined under a light microscope to analyze the hepatotoxicity and nephron structure.

The mean body weights of female and male rats were recorded and analyzed using the Mine Tab INSTAT program with an analysis of variance (ANOVA). To determine the levels of ALP and ALT enzymes, we used a simple T test.

RESULTS AND DISCUSSION

The mean body weights of the rats are shown in Table 1

The mean body weight of the females at the beginning of the experiment varied between 178 and 195 g; at the end of the experiment, the weight increased to 192-210 g. The mean male body weight at the beginning of the experiment was higher than that of females, varying from 205 to 33 g and at the end of the experiment, they varied from 162.8 to 254.6 g (Table 2).

Comparing the female treated rats with the female control showed no significant differences. However, the males showed some variation between the negative control (DMSO) and all treated groups were lower than the normal control (Table 1 and Table 2).

The alanine aminotransferase results are shown in Table 3

Female treatment groups A (100 mg) and B (50 mg) showed higher levels of ALT than the females in group D (10 mg) at $P<0.05$ and the negative control females in group E (DMSO) at $P<0.001$.

In addition, the females in treatment groups A and B showed higher levels than all male groups, especially the male groups GA, GC (normal control) and D (negative control; DMSO), at $P<0.001$ and $P<0.05$, respectively.

In addition, treated male groups A and B also showed significantly higher levels than the other male groups (Table 3), while the other male groups showed no significant differences between the normal and negative control (DMSO) and control female and male groups, as shown in Table 4. All female groups showed no significant differences between them. In addition, the female group with stronger

Table 1: Mean body weights of female and male rats, before and after treatment with Launaea Angustifolia extract for two weeks.

Rat group treatment	Means of mg	Standard Deviation+SD	Standard error of mean+SEM	P value vs. female Group
Female Group A Bwt. before (100 mg)	192.60	+10.502	+4.697	ns
Female Group A Bwt. after (100 mg)	195.00	+9.274	+4.147	ns
Female Group B Bwt. before (100 mg)	184.6 0	+9.685	+4271	P<05 vs. GC after Bwt. Before (50 mg)
Female Group B Bwt. after (100 mg)	192.00	+3.391	+1.517	ns
Female Group C Bwt. before (100 mg)	195.20	+8.815	+3.942	ns
Female Group C Bwt. after (100 mg)	210.40	+11.803	+5.278	P<0.01 vs. GD before P<0.01 vs. GE before
Female Group D Bwt. before (100 mg)	180.20	+9.550	+4.271	ns
Female Group D Bwt. after (100 mg)	202.80	+10.922	+4.8850	P<0.05 vs. GE before
Female Group E Bwt. before (100 mg)	178.00	+11.895	+5.320	ns
Female Group E Bwt. after (100 mg)	199.00	+19.481	+8.712	ns

Table 2: Mean body weights of male rats, before and after treatment with Launaea Angustifolia extract for two weeks.

Rat group treatment	Means of mg	Standard Deviation+SD	Standard error of mean+SEM	P value vs. female Group
Group A Males Bwt. before (100 mg)	233.80	+ 14.394	+ 6.437	ns
Group A Males Bwt. after (100 mg)	218.80	+23.414	+10.471	ns
Group A Males B Bwt. before (100 mg)	215.20	+215.20	+6.815	ns
Group A Males B Bwt. after (100 mg)	203.4	+20.562	+9.196	P<0.01 vs. GC after
Group A Males C Bwt. befor (100 mg)	219.20	+10663	+4.769	ns
Group A Males C Bwt. after (100 mg)	254.60	+16.727	+7.481	481 P<0.01 vs. GE before and after
Group A Males D Bwt. befor (100 mg)	229.00	+11.402	+5.099	ns
Group A Males D Bwt. after (100 mg)	232.00	+27.157	+12.145	ns
Group A Males E Bwt. befor (100 mg)	205.80	+14.856	+6.644	ns
Group A Males E Bwt. after (100 mg)	208.20	+30.194	+13.503	ns

treatment (A) showed no significant differences with any of the female or male groups. The 2nd strongest female treatment group (B) showed no significant differences with any female groups but did show a significant difference with the normal male control group (D) at P<0.05.

The normal control female group (C) presented higher levels than male group B at P<0.01 and the normal male control group (C) at P<.0.001. Additionally, the female group D presented higher ALP levels than the treated male group D at P<0.001. The negative control (DMSO) female group E showed higher ALP levels than all male groups (GA and GB at p<0.01), male GC at (P<0.05) and the male GD group at P<0.00 (Table 4).

The histological sections of the livers from female rats are illustrated in (Fig 1)

In comparison with the treated (A, B and D) and normal groups (C and E; negative control DMSO). The structure of the histological sections showed no differences between the treated group (A, B and D), the control group (C) and

Table 3: Means of blood enzymes ALT level of female and male rats group treated with Launaea Angustifolia leaves extract for two weeks.

Rat group treatment cons. mg	Means level of ALT enzymemg	Standard Deviation +SD	Standard error of mean+SEM	P value vs. female group	P value vs. male group
Female group A (100 mg ALT enzymes)	129.00	+14.983	+ 6.701	GA vs. GE females P<0.01	GA vs. G C and GD males P<0.001
Female group B (50 mg ALT enzymes)	137.60	+ 6.229	+2.786	GB vs. GC P<0.05 GB vs. GD P<0.01 GB vs. GEP<0.001	GB vs. GA male P<0.05GB vs. GD and GE male P<0.001
Female group C (control ALT enzymes)	111.60	+10.334	+ 4.622	ns	GC vs. GC male P<0.01
Female group D (10 mg ALT enzymes)	106.60	+ 13.867	+ 6.202	ns	GD vs. GC male P<0.05
Female group E (neg. control ALT enzymes)	94.20	+ 11.100	+ 4.964	ns	ns
Male GA (100 mg ALT enzymes)	108.60	+ 10.621	+ 4.750	ns	GA vs. GC male P<0.05
Male GB (50 mg ALT enzymes)	115.40	+ 7.503	+ 3.356	ns	GB vs. GC male P<.001 GB vs. GD male P<0.05
Male GC(ALT enzymes)	80.40	+ 13.667	+ 6.112	ns	ns
Male GD (10 mg ALT enzymes)	119.20	+ 13.236	+ 5.919	ns	GD vs. GC male P<0.001
Male GE (neg. control ALT enzymes)	87.20	+ 14.342	+ 6.414	ns	ns

Table 4: Means of blood enzyme ALP levels of female and male rat groups treated with Launaea Angustifolia extract for two weeks.

Rat group treatments	Means levels of ALP enzymeng	Standard Deviation +SD	Standard error of mean+SEM	P value vs. female group	P value vs. male group
GA female 100 mg ALP enzymes	220.20	+48.582	+21.726	ns	ns
GB female 50 mg ALP Enz	195.20	+53.087	+23.741	ns	ns
GC female control ALP EnZ	137.40	+25.235	+11.285	ns	ns
GD female (10 mgALP enzymes)	187.40	+34.681	+15.510	ns	GD vs. GD male P<0.001
GE female (neg. control DMSOALP enzymes)	129.40	+6.387	+2. 857	ns	ns
GA male (100 mgALP enzymes)	251.00	+108.00	48.299	ns	ns
GB male (50 mgALP enzymes)	311.20	81.922	36.637	no	GB vs. GD female P<0.05
GC male (nor. controlALP enzymes)	262.40	26.207	11.720	no	GC vs. GB female P<0.01GC vs. GD female P<0.001
GD male (10 mgALP enzymes)	345.00	+108.48	+48.512	no	GD vs. GB female P<0.05GD vs. GD female P<0.001
GE male (neg. DMSO ALP enzymes)	232.20	+21.253	+9.505	no	GE vs. GA female P<0.01Vs. GB female P<0.01 vs. GC female P<0.05GE vs. GD male P<0.001.

negative control DMSO group E (Fig 1). Therefore, treatment with the Launaea angustifolia (LAn) extract did not correspond to any significant differences in the rat liver histological structure.

The histological sections of the kidneys from female rats are illustrated in Fig 2 in comparison to the treated (A, B and D) and normal groups (C and E; negative control DMSO).

The histological structure of the rat kidney also showed no differences between the treated group (A, B and D), normal control group (C) and negative control DMSO group E (Fig 2). Therefore, treatment with the Launaea Angustifolia (LAN) extract did not correspond to any significant differences in the rat kidney histological structure.

The increase observed in the mean body weights of female and male rats in this study was attributed to continuing growth, beginning as young adults and continuing to gain weight during the experiment. The mean body weight in adult female rats is usually lower than that in males, which could be due to testosterone hormones increasing muscle mass in males, as testosterone is one of the many factors involved in the development of muscle bulk and strength.

Testosterone also increases neurotransmitters, which encourage tissue growth and interacts with nuclear receptors in DNA, thereby causing protein synthesis.

Although some males showed a decrease in body weight, this decrease could be due to certain rats becoming subordinate males within the social hierarchy of the group. Social hierarchies are ubiquitous features of virtually all animal groups. (Fulenwider *et al*. 2021).

In terms of the means of the blood enzyme ALT levels of the female and male rat groups treated with the Launaea Angustifolia extract for two weeks, the results showed no significant differences within the female groups. Thus, treatment with the Launaea Angustifolia extract did not affect liver function and according to (Lin *et al*. 2008), ALT is considered a useful test for the early detection of liver damage.

In addition, the results showed variation between female and male rat groups, which could be due to gender variation. The level of ALT in healthy males is 7–55 U/L and that in female is 7-45 U/L. The healthy thresholds for ALT are 30/19 U/L in males/females (M/F), respectively. Valenti *et al*. (2021) Although the ALT levels reported by Valenti *et al*. conflict with this study's results, the female rats' ALT levels were still higher than the males' ALT levels, which could be due to species differences. In addition, all treated male groups showed higher ALT levels than the control groups. Although elevations in ALT can be highly suggestive of liver injury, ALT is not liver-specific and extra-hepatic sources of circulating ALT include areas of skeletal or cardiac muscle damage and drugs that increase ALT gene expression (*e.g*., fenofibrate) (Yang *et al*. 2014).

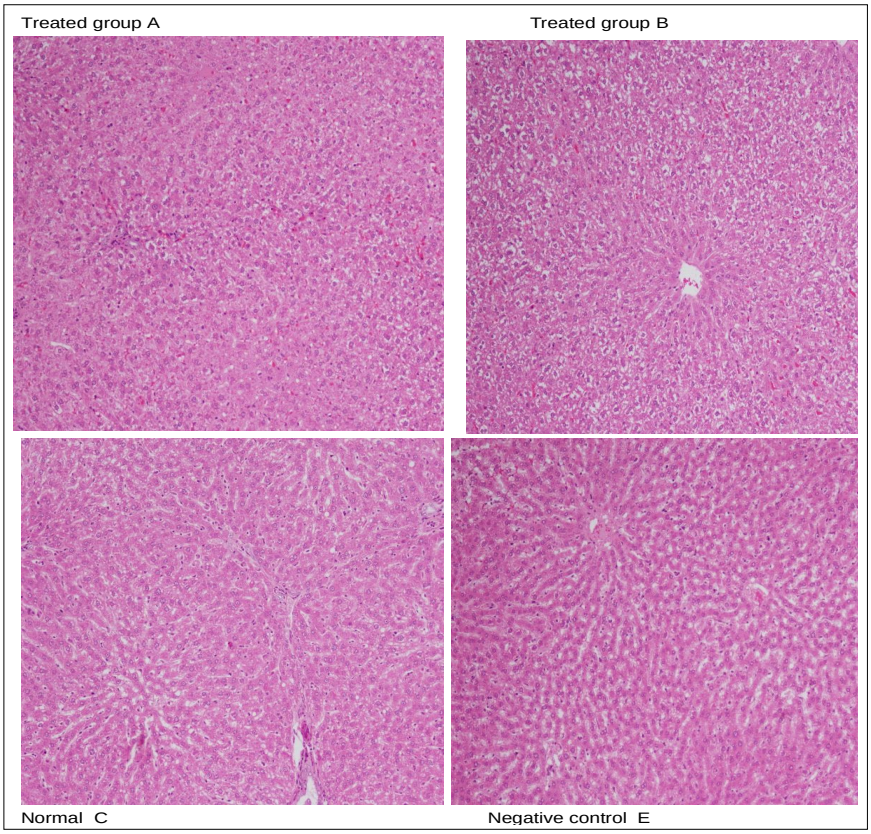

Fig 1: Histology of the livers of the rat groups treated with Laun Angustifolia extract for two weeks (A and B) compared to the normal and negative control (Groups C and E).

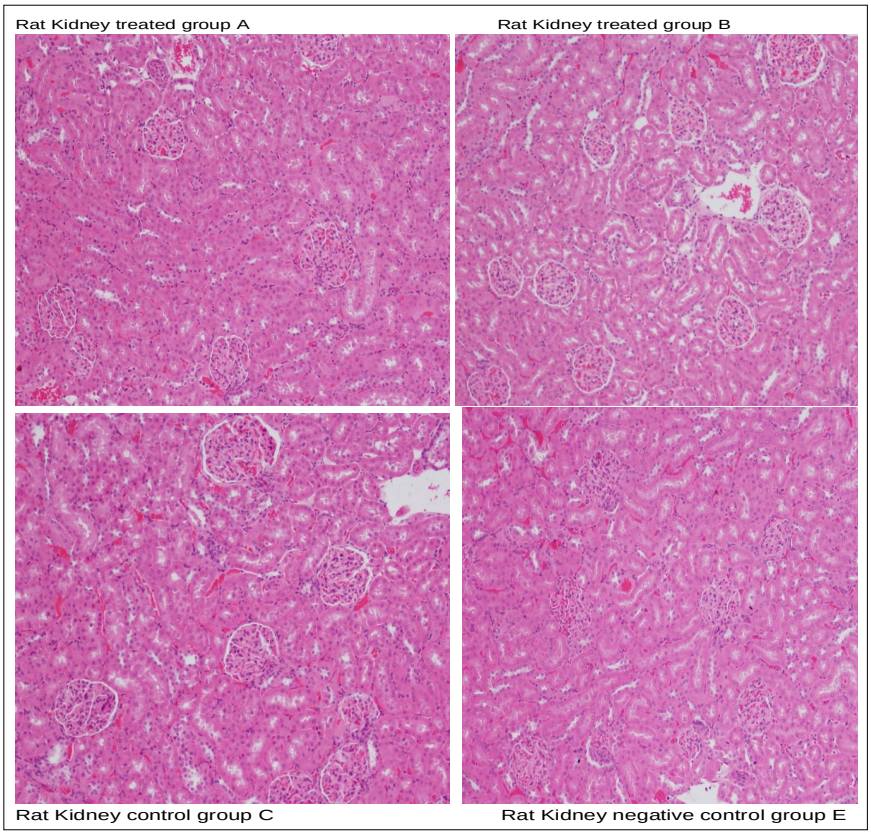

Fig 2: Histology of the kidney of female and male rat groups treated with Launaea Angustifolia extract for two weeks compared to the normal and negative control (Groups C and E).

Alanine aminotransferase sometimes presents elevated levels in serum under conditions of significant cellular necrosis and is used as a measure of liver function. Levels of ALT may be elevated in cases of hepatitis, congestive heart failure, liver or biliary duct damage, or myopathy. Diet, restraint and drug administration may also affect plasma ALT in rodents (Evans, 2009).

We also explored the means of blood enzyme ALP levels in the female and male rat groups treated with Launaea Angustifolia extract for two weeks. ALP in the blood can reflect damage to tissues or the disruption of normal body processes. The results showed no significant differences between the female or male groups, suggesting a lack of side effects on liver tissue or bile function.

Although females showed differences compared to the male groups, these differences might be due to the sexual variation between females and males. As stated, normal ALP levels vary with age and sex and in humans, ALP levels are slightly higher in males than in females. The present results showed higher levels of ALP in male Wister rats compared to the normal range.

Histological examination of the liver and kidney agreed with the blood enzyme results for ALT and ALP, which did not show any significant differences between the treated groups or control groups. Thus, use of the Launaea Angustifolia extract had no side effects.

Numerous species of the genus Launaea are used in traditional medicine to treat conditions including stomach ache and skin conditions in addition to offering anticancer, insecticide and cytotoxic properties, effectively preventing CCl4-induced oxidative injuries in the liver and revealing the extract's therapeutic role against bacterial infections as a potent antimicrobial source (Reddy and Mishra, 2012; Khan *et al*., 2012; Zellagui *et al*., 2012).

CONCLUSION

These results are consistent with those of previous experimental investigations on Launaea angustifolia (LAN) plant extract, which demonstrated its safety through its effects on liver and kidney histology and the ALT and ALP blood parameters.

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Conflict of interest: None.

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