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Modulatory effect of *Saussurea costus* ethanolic extract on kidney male rats expose oxidative stress

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Abstract

Tissue destruction is the primary pathological abnormality associated with diseases or as an adverse drug reaction, blocking of which has been of great importance for investigators using different modalities as pharmacological agents or herbal remedies. The present study aimed to identify the tissue-protective effects of ethanolic extract of *Saussurea costus* using hydrogen peroxide-induced tissue damage in a rat model. To do so, 25 male rats were used and assigned into four groups (5 rats control received distilled water plus 15 rats exposed to H_2O_2 for 14 days; subdivided into five rats per group based on blocking concentration of *S. costus* extract G1=0.1mg/kgW.T, G2=0.2mg/kg, and G3=0.3mg/kg) given oral gavage needle. Blood samples were withdrawn from all rats at day 0 and after 14 days of exposure to 1% H_2O_2 in the control or treated group. The testis and kidney at day 14 were excised and fixed for histological studies. Lipid profile, renal function tests, testosterone level, and histological parameters were considered for all subjects. The results indicated that the ethanolic extract of *Saussurea costus* blocked the tissue-destructive effects of rat testis and kidneys, reducing the lipid derangement effects induced by H_2O_2. In conclusion, *S. costus* has provided cytoprotective results against H_2O_2-induced tissue destruction, especially at a relatively modest dose.

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Introduction

The commonly used drugs in clinical settings might induce genital, renal, and metabolic derangements, such as proton pump inhibitors (1,2), antipsychotic drugs (3,4), hypouricemic drugs (5), and hypoglycemic drugs (6). This harmful impact has been attributed to oxidative stress (3,4,6), proinflammatory effects (7,8), electrolyte dysregulation (9), or direct tissue damage (3,4,10). Several herbal treatment modalities have been suggested to protect these metros from inducing these defects and maintain the quasi-equilibrium status of the surrounding milieu (11-14). Hydrogen peroxide (H_2O_2) is a reactive oxygen species that regulates several oxidative stress-related states (15). The perennial plant *Saussurea costus* (also known as *Saussurea lappa*) is used for the treatment of many different diseases, including gastrointestinal dysfunction (indigestion, diarrhea, vomiting, and dyspepsia) (16,17). It's also used for cough, inflammation, and diuretics (18). These effects may be the result of the presence of therapeutic molecules such as costunolide, sesquiterpenoids, dehydrocostus lactone, monoterpenes, chloropicrin, flavonoids, lignans, triterpenes, steroids, and glycosides (19). Typically, Saussurea, while consumed properly orally, costus root could be harmless. However, Saussurea aristolochic acid, a pollutant that may be present in costus and could have nephrotoxic and carcinogenic consequences, could be present. Security of *Saussurea costus* has still not been identified in pregnant or nursing women. In those that are allergic to Saussurea species, especially their sesquiterpene lactone contents

(STLs), *Saussurea costus* may trigger an allergic reaction. Contact dermatitis has been seen in those who have been exposed to STLs. Concerns have been expressed concerning these chemicals' potential to be genotoxic and embryotoxic. Assays conducted in vitro and in vivo have revealed that STLs are mutagenic (20,21).

So, the aim of the study is to study of effects of H_2O_2 on the kidney, and determination of the tissue protective effect of the ethanolic extract of *Sasurea costus* plant.

Materials and methods

Ethical approve

The scientific committee has approved this study of the college of veterinary medicine- University of Baghdad at the seventh congress dated 11/1/2022, that the concurrent conducting experiment did not violent the laws of animal rights and the euthanasia is applied in accordance of this guidelines, and approval issue number and date is UM.VET.2022.014.

Preparation of ethanolic extract of *S. costus*

The dried roots are ground into a relatively coarse powder and then kept in air-tight vessels for use in the separation process. This is how *Sassurea costus* ethanolic extract (SCEE) is made. To obtain a crude *S. costus* extract, 200g of the raw herb root was ground into a powder (22), extracted using a simple maceration at room temperature with 70% ethanol, macerated for 72 hours, filtered three times, and dried using a rotary evaporator. Before giving the test animals the root extract's semi-solid slurry, it was dissolved in bi-distilled water for the experimental animals.

Experimental design

A total of 25 rats (4 weeks old; 200-250g weight; white male Albino rats) were kindly provided by the animal house of the University of Mosul, fed with a standard diet, free access to tap water, and kept in light/dark daily cycle with 12:12 hours light: opaque process at approximately 25ºC. These 25 rats were divided into five equal groups. C group (Control group): given distilled water only for 14 days. G1 group given 1% H_2O_2 provided in drinking water orally (23) for 14 days. G2 group given 1% H_2O_2 provided in drinking water alongside 0.1mg/kg/day of *S. costus* ethanolic extract orally by gavage needle. G3 group given 1% H_2O_2 provided in drinking water alongside 0.2mg/kg/day of *S. costus* ethanolic extract (24) orally by gavage needle. G4 group given 1% H_2O_2 in drinking water alongside 0.3mg/kg/day of *S. costus* ethanolic extract orally by gavage needle.

Collection of blood samples

The blood samples were withdrawn from the rats initially and following 14 days post-therapy. The serum was separated by centrifugation, collected, and stored at -20ºC to be ready for further analysis.

Biochemical analysis

According to manufacturer instructions provided in the data sheet of the commercial kits, the biochemical parameters were measured. The lipid parameters (Serum lipids profile), Serum total cholesterol, and HDL-c were determined according to the colorimetric method. Serum triglycerides were determined by the enzymatic method using a standard enzymatic assay (Fortress/UK kit). LDL and VLDL-c were calculated. The testosterone concentration was determined using the Testosterone Enzyme Immunoassay kit (Beckman couiter, USA).

Serum total cholesterol (TC), triacylglycerols (TG), and high-density lipoproteins (HDL) were determined by enzymatic colorimetric test using kits supplied by SPINREACT, Spain. LDLc was calculated according to the formula. VLDL-c (mg/dl) = Triglycerides/5. While LDL-c was calculated in mg/dl by theis formula LDL-c=Total cholesterol - (HDL-c + VLDL-c). Measurement of serum albumin was done using a modified bromocresol green colorimetric method. The concentration was determined by measuring the absorbance at 630 nm and comparing it with the absorbance of the standard solution. Urea was determined by the enzymatic colorimetric method. I was using urease to hydrolyze urea into ammonia and carbon dioxide. The concentration was determined by measuring the absorbance at 572 nm. Creatinine was determined spectrophotometrically by the kinetic method. The absorbance was read at 30 seconds and 2 minutes later. Creatinine concentration was calculated using a standard concentration of 2 mg/dl (cobas111) of Japanese origin.

Histopathology study

Rats were sacrificed by cervical dislocation for histological examinations after completing a study period. Immediately after sacrifice, the kidney and testis were fixed in a born solution for 1 hour and transferred 48 hours in 10% neutral buffered formalin saline. Tissues were preserved in paraffin and sectioned at five µm thickness using a rotary microtome. Kidneys were removed, washed with ice-cold saline, and immersed in 10% neutral buffered formalin tissue pieces for five days. Sections were stained with hematoxylin-eosin (H&E) to study histopathological changes in the testes for light microscopy examination (23).

Statistical analysis

The results of the present study have been saved in Excel-sheet (2016) for statistical analysis using the software GraphPad Prism (version 9.4.1, USA). The data is presented as mean and standard deviation. One-way analysis of Variance (ANOVA) was conducted among groups to identify differences, followed by the Duncan test. T A series of t-tests were conducted between groups to identify the significant difference at a p-value of less than 0.05.

Results

Weight changes

The rats enrolled in the present study were weighed at day 0 before commencing exposure to H_2O_2 and the costus extract. The weight in different groups showed a non-significant (P>0.05) difference between before the exposure to interventional substances or after whether in treated or in control groups, and the values were close to the weight (g) in the control group (Figure 1).

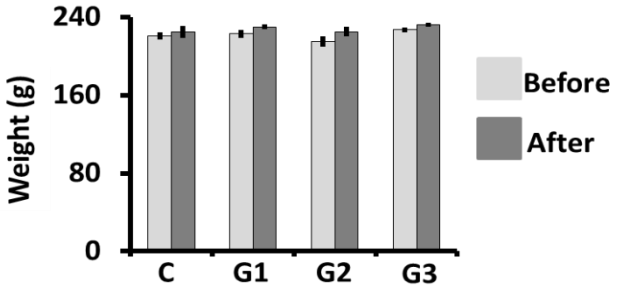

Figure 1: *Saussurea costus* ethanolic extract maintained rat weight in a 1% H_2O_2-exposed rat model. (C) the control group received distilled water, (G1) received H_2O_2 and 0.1 mg/kg/day of *Saussurea costus* extract, (G2) received H_2O_2 and 0.2 mg/kg/day of *S. costus* extract, (G3) received H_2O_2 and 0.3 mg/kg/day of *S. costus* extract-data expressed as mean±SD.

Biochemical changes

The measurement of the lipid profile in the intervention group versus the control group was plotted in figure 2. The extract of the costs has significantly (P<0.05) elevated the total cholesterol (TC) concentration (mg/dl) in the G1 group (127±7.9) and G3 group (113±4.5) compared to the control group (92.2±2.9). The extract of the costs has significantly (P<0.05) elevated the triglyceride (TG) concentration (mg/dl) in the G1 group (115±10) and G3 group (99±7.5) compared to the control group (30.9±2.2). The extract of the costs has significantly (P<0.05) elevated the VLDL concentration (mg/dl) in the G1 group (23±2) and G3 group (19.8±1.5) compared to the control group (16.2±0.5). Non-significant changes (P>0.05) have been quantified in the G2 group compared to the control group regarding TC, TG, and VLDL. HDL and LDL have shown a non-significant difference between the studied groups or compared to the control group.

The measurement of renal function tests in the intervention group versus the control group was plotted in figure 3. The extract of the costs has significantly (P<0.05) elevated the urea concentration (mg/dl) in the G1 group (64±3.7) compared to the control group (43±1.6). Non-significant changes (P>0.05) in urea have been quantified in the G2 and G3 groups compared to the control group.

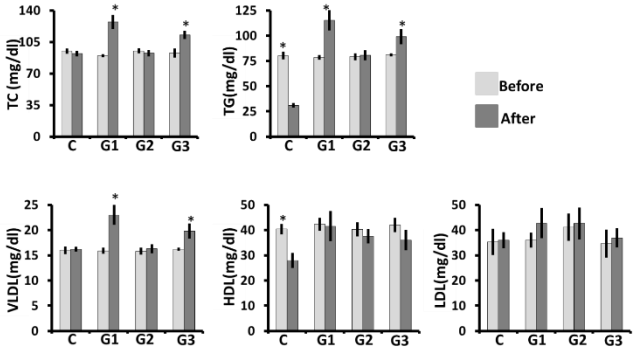

Figure 2: In a rat model, *Saussurea costus* ethanolic extract blocked lipid dysmetabolism induced by 1% H_2O_2. (C) the control group received distilled water, (G1) received H_2O_2 and 0.1 mg/kg/day of *S. costus* extract, (G2) received H_2O_2 and 0.2 mg/kg/day of *S. costus* extract, (G3) received H_2O_2 and 0.3 mg/kg/day of *S. costus* extract-TC=total cholesterol, TG=triglycerides, VLDL=very low-density lipoprotein, HDL=high density lipoprotein, and LDL=low density lipoprotein. Data expressed as mean±SD, *P<0.05 compared to before or after.

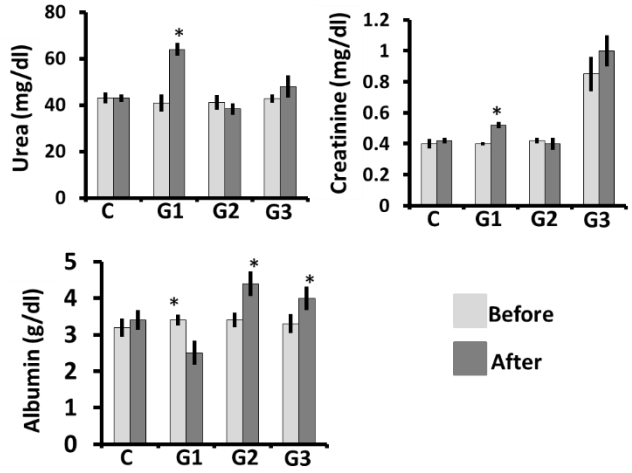

Figure 3: In a rat model, *Saussurea costus* ethanolic extract maintained normal renal function induced by 1% H_2O_2. (C) the control group received distilled water, (G1) received H_2O_2 and 0.1 mg/kg/day of *S. costus* extract, (G2) received H_2O_2 and 0.2 mg/kg/day of *S. costus* extract, (G3) received H_2O_2 and 0.3 mg/kg/day of *S. costus* extract. Data expressed as mean±SD, *P<0.05 compared to before or after.

The extract of the costs has also significantly (P<0.05) elevated the creatinine concentration (mg/dl) in the G1 group (0.52±0.01) and has no effect in G2 (0.4±0.02) or G3 group (1±0.1) compared to the control group (0.42±0.02). The extract of the costs has significantly (P<0.05) elevated the albumin concentration (mg/dl) in the G2 group (4.4±0.34)

and G3 group (4±0.32) compared to the control group (3.4±0.27). The extract of the costs has significantly ($P<0.05$) reduced the testosterone concentration (nmol/l) in the G1 group (3±0.23) compared to before the intervention (4.1±0.29). Non-significant changes ($P>0.05$) have been quantified in G2, G3, or control groups compared to before intervention (Figure 4).

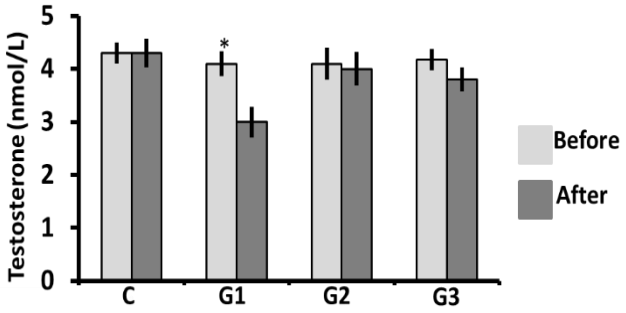

Figure 4: In a rat model, *Saussurea costus* ethanolic extract maintained average testosterone levels altered by 1% H_2O_2. (C) the control group received distilled water, (G1) received H_2O_2 and 0.1 mg/kg/day of *S. costus* extract, (G2) received H_2O_2 and 0.2 mg/kg/day of *S. costus* extract, (G3) received H_2O_2 and 0.3 mg/kg/day of *S. costus* extract. Data expressed as mean±SD, *$P<0.05$ as compared to after exposure to H_2O_2.

Histological changes

The results obtained by Hematoxylin and Eosin staining of the treated animals with H_2O_2 showed various histological lesions in kidneys and testes using light microscopic observation compared with the control and plant-treated groups, indicating damaging tissue. The microscopic examination of kidney specimens of the control group showed standard architecture of renal tissue as proximal and distal convoluted tubules, glomeruli, glomerular tuft, and interstitial tissue (Figure 5). While the histological changes of treated groups with H_2O_2 show changes in glomeruli, cortex, and medulla, showed swelling and coagulative necrosis of tubular epithelium of proximal and distal convoluted tubules, leading to stenosis of the lumen of the renal tubules, chronic inflammatory cells infiltration in the interstitial tissue also there is bleeding in the interstitial tissue, decrease in cellularity of glomeruli led to the expansion of Bowman's space, and generalized congestion of blood capillaries also observed (Figures 6-8). This, due to the harmful effects of H_2O_2 co-administration with the plant, showed a slight improvement in the histological lesions of kidneys, revealing degeneration and swelling of the epithelium of proximal and distal convoluted renal tubules; these pathological lesions were lighter than those of H_2O_2 group (Figure 9).

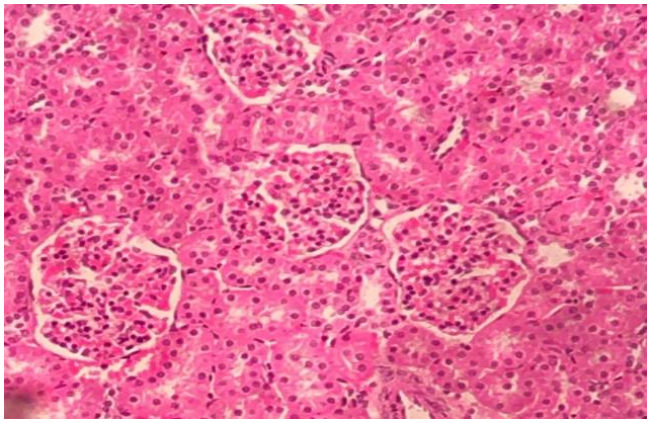

Figure 5: The histological section of a control group of rats' kidneys showed normal renal tissue architecture. H&E. 100X.

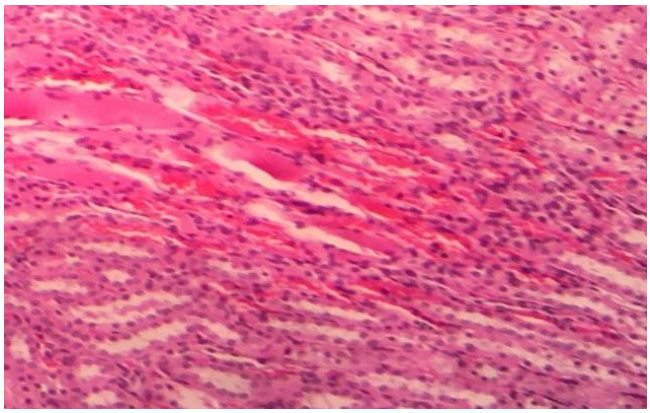

Figure 6: The histological section of the kidney of a rat treated with H_2O_2 showed swelling and coagulative necrosis or renal epithelial cells. H&E. 100X.

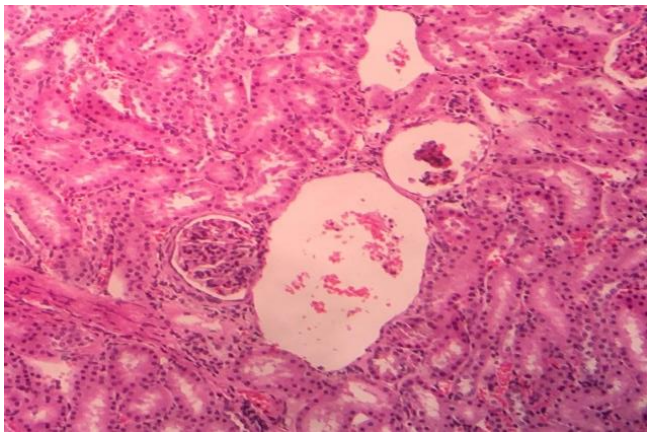

Figure 7: The histological section of the kidney of a rat treated with H_2O_2 showed decreased cellularity of glomeruli with expansion of bowman space. H&E. 100X.

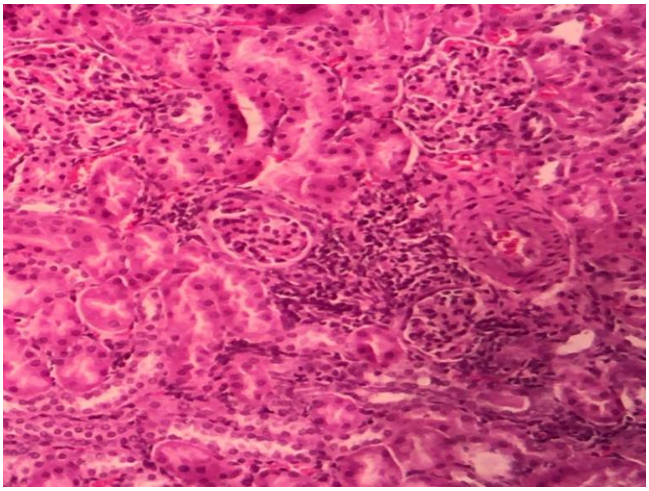

Figure 8: The histological section of the rat kidney treated with H_2O_2 showed infiltration of chronic inflammatory cells within the interstitial. H&E. 100X.

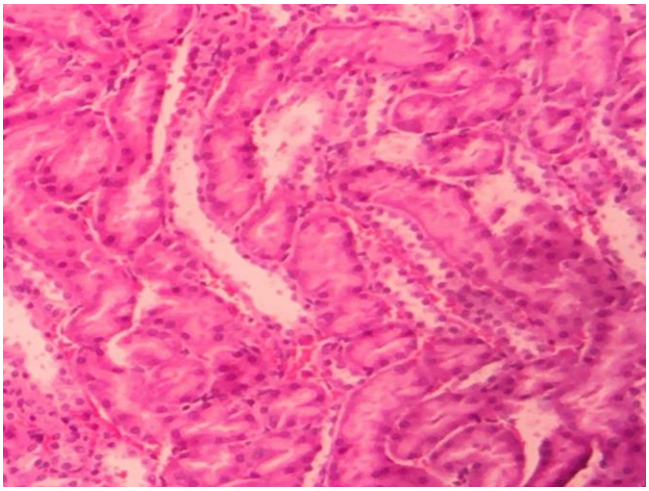

Figure 9: Histological section of the rat kidney treated with H_2O_2 co-administrated with the plant showed degeneration and swelling of renal epithelial cells. H&E. 100X.

The histological examination of the testes of the control and plant-treated groups showed the normal histological appearance of the testis composed of tunica albuginea surrounding several seminiferous tubules separated by interstitial tissue containing connective tissue and Leydig cells. The seminiferous tubules are lined by germinal epithelial cells, composed of layers of spermatogenesis cells (Figure 10). The light microscopic examination of the testes specimens of the H_2O_2 treated group for 14 days showed histological lesions characterized by congestion of the blood vessels in the interstitial tissue degeneration and necrosis of spermatogenic and Sertoli cell distortion in the spermatogenesis, obstruction the lumen of some seminiferous tubules by the necrotic debris, detachment and splitting of germ cells layer from the basement membrane, there is reduction in spermatogenesis (Figures 11 and 12). In comparison with those of the H_2O_2 and the plant-treated group, they revealed a noticeable improvement in the histological appearance, which was characterized by standard histological architecture of seminiferous tissues, which means that the plant caused good recovery as indicated by the typical arrangement of the seminiferous tubules (Figure 13).

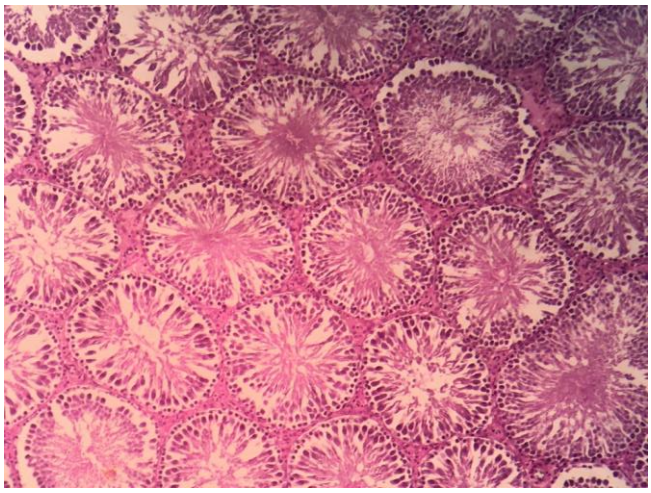

Figure 10: The histological section of the testis of the rat of the control group showed normal architecture of the testis and normal seminiferous tubules. H&E. 100X.

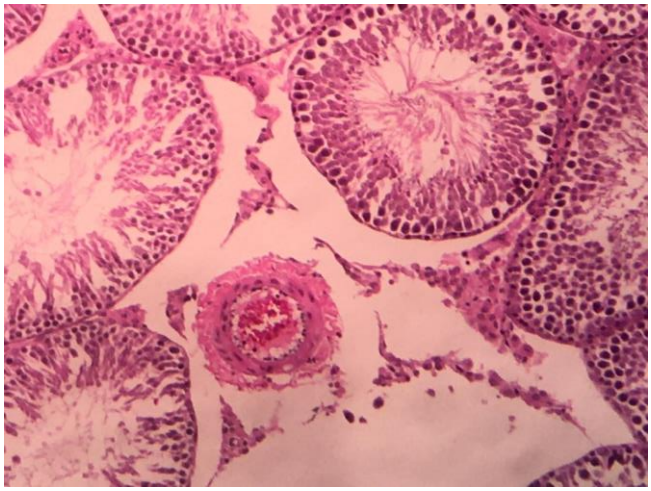

Figure 11: Histological section of the testis of rats treated with H_2O_2 showed degeneration and necrosis of spermatogenic and Sertoli cells and congestion of blood vessels. H&E. 100X.

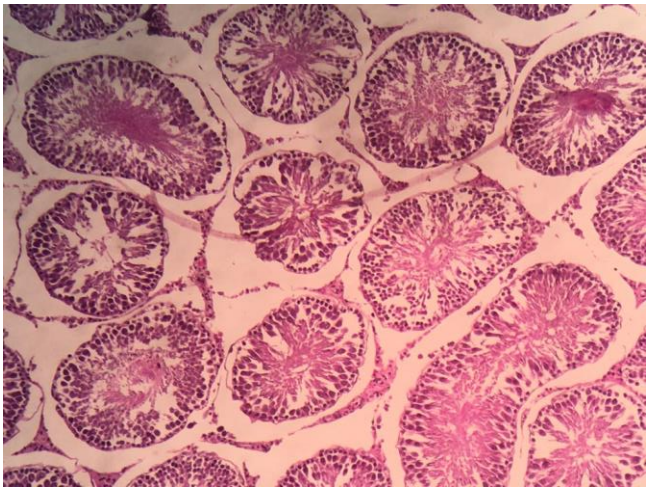

Figure 12: The histological section of the testis of rats treated with H_2O_2 showed distortion of spermatogenesis obstruction of the lumen of the seminiferous tubules. H&E. 100X.

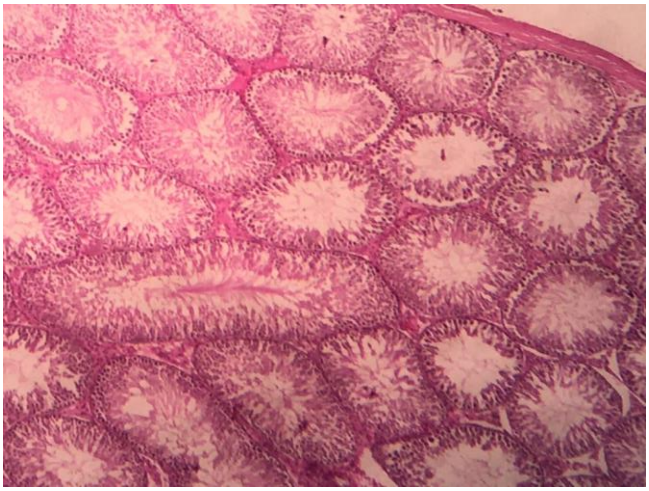

Figure 13: Histological section of testis of rat treated with H_2O_2 and plant showed improvement in the testicular arrangement of seminiferous epithelium with spermatogenesis and Sertoli cells and sperms and spermatozoa in the lumen. H&E. 100X.

Discussion

The ethanolic extract of *Saussurea costus* has had no impact on the weight of rats, whether exposed to hydrogen peroxide in the presence or absence of *Saussurea costus* and even at different doses of the plant extract. Total cholesterol, triglycerides, high-density lipoprotein, very low-density lipoprotein, and low-density lipoprotein were affected by the plant extract; the extract at low dose (0.1) and high dose (0.3) increases TC, TG, and VLDL. However, moderate amounts do not impact the lipids LDL or HDL. Low doses of *S. costus* increased urea and creatinine, while high doses have no impact. Albumin at low doses of plant extract has shown reduction, while at high doses, it has shown elevation. An insufficient amount of the plant has shown reduced testosterone, while high doses have no impact on testosterone. The histological changes confirmed modulation of histology at protection against H_2O_2 at all amounts of costus.

The present study has confirmed no weight changes in all groups regardless of the used dose of *S. coatus*. These findings were similar to those of Anyanwu *et al.* (21), who reported that Costus had protected rat body weight in rats exposed to heavy metal poisonous compared to the control non-treated group. Correspondingly, Nakade *et al.* (22) reported that rats exposed to lead have reduced body weight, an impact reserved in the presence of costus extract.

Lipid profile has shown significant changes in cholesterol, triglycerides, and HDL levels. The costs have increased the cholesterol and triglyceride levels at low doses of costus extract (0.1 and 0.2 mg/kg/day) while maintaining and improving the levels at high concentrations (0.3 mg/kg/day). Different studies have reported these results. Anyanwu *et al.* (21) have reported that heavy metal alteration of the lipoid profile is blocked by costus extract. Moreover, Nakade *et al.* (22) said lead had disturbed the lipid profile, leading to elevated total cholesterol and triglycerides and decreased HDL levels. Nonetheless, these actions are blocked by costus extract.

In a study conducted by Ashry *et al.* (23), who reported that costus had reduced the damaging effects of oxaliplatin-induced renal damage and reduced creatinine and urea, these findings agreed with our results. Moreover, a study conducted by Yamada *et al.* (24) reported that *S. costus* protected against damaging effects on the kidney induced by a nephrotoxic drug (Oxaliptan anticancer drug), reducing the creatinine and urea levels with the improvement of histological sections in a rat model. Similarly, Biswas *et al.* (25) reported that *S. costus* protected against kidney tissue damage induced by oxaliplatin nephrotoxic drugs; these two studies have said that the protective action of costs was related to their antioxidant and metabolic effects alongside the protective effects of renal tissues. Our results confirmed that only a low dose of Costus produced no products. In contrast, higher doses (0.2mg/kg/day and 0.3 mg/kg/day) provided protection and prevented the damaging effects of H_2O_2 compared to a control group exposed to distilled water. Therefore, *S. costus* has improved kidney function, indicated by reduced tissue-adverse impact on the kidney and improved creatinine, urea, and albumin levels. These studies have related these beneficial effects to the antioxidant activity of the present phenolic compounds (25-32). The testosterone level has been reduced by low concentrations, but no impact could be detected at high concentrations. Therefore, higher concentrations could be better considered for protection against H_2O_2.

Conclusion

A high concentration of *S. costus* has improved the biochemical parameters in rats exposed to hydrogen peroxide and protected the tissues from the damaging effects of H_2O_2, especially at high concentrations. The lipid profile and renal function tests have been negligibly affected by a low concentration of costus, whereas the higher concentration has modulated the lipid and renal function tests; H_2O_2 has a severe toxic effect on the histology of the kidney and testis and on the biochemical testes, the treatment with costus has a benefit protective effect against the histological alterations of testis and kidney induced by H_2O_2.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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التأثير التعديلي لمستخلص الإيثانول للقسط الهندي على الكلى لذكور الجرذان المعرضة للجهد التأكسد

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الخلاصة

تدمير الأنسجة هو التشوهات المرضية الرئيسية المرتبطة بالأمراض أو كرد فعل دوائي ضار، وبذلك فان منعه لاقى اهتماما كبيرا لدى الباحثين الذين يستخدمون طرائق مختلفة كعوامل دوائية أو علاجات عشبية. كان الهدف من هذه الدراسة هو تحديد التأثيرات الوقائية للأنسجة للمستخلص الإيثانولي لنبات القسط باستخدام تلف الأنسجة الناجم عن بيروكسيد الهيدروجين في نموذج الفئران. للقيام بذلك، تم استخدام عشرون جرذيا موزعين على أربعة مجاميع، تلقت خمسة جرذان ماء مقطر بالإضافة إلى خمسة عشر جرذيا تم تعريضهم لبيروكسيد الهيدروجين ولمدة أربعة عشر يوما؛ مقسمة إلى خمسة جرذان لكل مجموعة بناء على تركيز مستخلص القسط المستعمل المجموعة الأولى ٠,١ والمجموعة الثانية ٠,٢ والمجموعة الثالثة ٠,٣. تم سحب عينات الدم من جميع الجرذان في اليوم الأول قبل إعطاء مستخلص النبات أو الهيدروجين بيروكسيد وبعد أربعة عشرة يوما من التعرض لبيروكسيد الهيدروجين سواء في المجموعة الضابطة أو المعالجة. تم استئصال الخصية والكلى في اليوم ١٤ وتحضيرها مختبريا للدراسات النسيجية. تم قياس الدهون واختبارات وظائف الكلى ومستوى هرمون التستوستيرون والمعلمات النسيجية لجميع الجرذان. أشارت النتائج إلى أن المستخلص الإيثانولي لنبات القسط منع الآثار المدمرة للأنسجة في خصية الجرذان والكلى إلى جانب تقليل تأثيرات تغييرات الدهون التي يسببها بيروكسيد الهيدروجين. في الختام، اعطى نبات القسط تأثيرات واقية للخلايا ضد تدمير الأنسجة الناجم عن بيروكسيد الهيدروجين خاصة بتركيز معتدل نسبيا.