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PHYTOCHEMICAL AND PROTECTIVE EFFECT OF SAUSSUREA COSTUS EXTRACT ON SOME PHYSIOLOGICAL AND HISTOLOGICAL PARAMETERS IN ALBINO RATS INDUCED WITH CHLORPROMAZINE IN THE TESTIS

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Abstract

This study examined the possibility of using an alcoholic extract of S. costus root as a protective agent against chlorpromazine, which causes male infertility. The study divided white male rats into five groups, each containing six rats, for 30 days. The results revealed that the rats that received doses of chlorpromazine had significant changes in the hormones LH, FSH, and testosterone, in addition to a decrease in sperm motility, distortion of some sperm, and death of others. When injecting the rats with different doses of the alcoholic extract of the S. costus plant (at concentrations of 1.25 mg/kg, 2.4 mg/kg, and 4 mg/kg), in conjunction with doses of chlorpromazine at 2 mg/kg, there was no decrease in the aforementioned attributes. The histological test of the testicles showed significant changes in the histological structure of the testis in the group that received chlorpromazine. Both the amount of Leydig and Sertoli cells were reduced, as well as the average diameter of the seminiferous tubules, as a result of taking the drug. This led to a lower number of sperm-forming cells, the presence of gaps or spaces between the seminiferous tubules, and few or no mature sperm. Compared to the control group, the interstitial tissue was similarly dense, the spacing between the seminiferous tubules was reduced, and spermatogenic cells with mature spermatogonia

appeared within the lumen of the tubules. There was a slight increase in the diameter of the seminiferous tubules and the number of Sertoli and Leydig cells compared to the control group. Seminiferous tubules and the average Leydig and Sertoli cell diameters increased significantly in the two groups at doses of 2.4 mg/kg and 4 mg/kg.

Keywords: Saussurea Costus, Rats, Chlorpromazine, Roots, Testes.

التأثير الوقائي للمستخلص الكحولي لجذور القسط الهندي Saussurea costus في بعض الجوانب النسيجية والفسلجية لخصى ذكور الجرذان المستحثة بعقار كلوبرومازين

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الخلاصة

تناولت هذه الدراسة إمكانية استخدام مستخلص كحولي من جذر القسطرة كعامل وقائي ضد chlorpromazine الذي يسبب العقم عند الذكور. قسمت هذه الدراسة ذكور الجرذان البيضاء إلى ست مجموعات، تحتوي كل مجموعة على ستة فئران لمدة 30 يومًا. أظهرت النتائج أن الفئران التي تناولت جرعات من الكلوربرومازين كان لها تغيرات معنوية في هرمونات *LH* و*FSH* وهرمون التستوستيرون، بالإضافة إلى انخفاض في حركة الحيوانات المنوية وتشويه بعضها وموت البعض الآخر. عند حقن الجرذان بجرعات مختلفة من المستخلص الكحولي لنبات القسطرة بجرعة 1.25 مجم/ كجم، 2.4 مجم/ كجم، 4 مجم/ كجم، بالتزامن مع جرعات من الكلوربرومازين بجرعة 2 مجم/ كجم؛ لم يكن هناك نقصان، الصفات المذكورة أعلاه لم تتغير، وأظهر الاختبار النسيجي للخصيتين تغيرات معنوية في التركيب النسيجي للخصية في المجموعة التي تناولت الكلوربرومازين، وانخفضت كل من اعداد خلايا *Leydig* و*Sertoli*. وكذلك متوسط قطر النبيبات المنوية نتيجة تناول الدواء. وقد أدى ذلك إلى انخفاض عدد الخلايا المكونة للحيوانات المنوية، ووجود فجوات أو فراغات بين الأنابيب المنوية، وقلة أو عدم نضج الحيوانات المنوية. المجموعة الضابطة، كان النسيج الخلالي كثيفًا بالمثل، وتم تقليل التباعد بين الأنابيب المنوية، وظهرت الخلايا المنوية ذات الحيوانات المنوية الناضجة داخل تجويف الأنابيب. كان هناك زيادة طفيفة في قطر النبيبات المنوية و *Sertoli z* و*Leydig* مقارنة بمجموعة التحكم. زادت الأنابيب المنوية ومتوسط أقطار خلايا *Leydig* و*Sertoli* بشكل ملحوظ في المجموعتين بجرعات 2.4 مجم/ كجم و4 مجم/ كجم.

كلمات مفتاحية: قسط السوسوريا، الجرذان، الكلوربرومازين، الجذور، الخصيتين.

Introduction

For thousands of years, medicinal plants have served as a source of healing in societies worldwide. For around 85% of the world's population, it is still essential as a primary healthcare delivery method. 80% of all synthetic pharmaceuticals are produced from it, making it also a resource for drug discovery (1). Furthermore, plants function as living chemical factories where various secondary metabolites (SMs) are biosynthesized. In actuality, it is these metabolites that serve as the building blocks for a large number of commercial pharmaceutical medications and herbal therapies made from medicinal plants. The pharmaceutical and food industries can benefit from the biological activity of many chemical components in medicinal plants to enhance human health. Alkaloids, terpenoids, and phenylpropanoids are several SMs considered for therapeutic development (3).

Around the world, 8-12% of marriages suffer from infertility. Its causes are varied since it might be brought on by inherited, acquired, or unknown factors that affect sperm quality. Male fertility is also impacted by several medical issues (5). About 30% of male infertility is caused by idiopathic sperm abnormalities (4). However, many medical problems, such as renal illness, liver failure, hemochromatosis, chronic obstructive pulmonary disease, cystic fibrosis, and multiple sclerosis, might impact semen characteristics (7). Iraq is a very diverse nation. Traditions, the standard of life, weather conditions, and access to healthcare are all varied. These variables strongly affect the rate of infertility. Due to the war, lifestyle, stress, smoking, occupation, nutrition, and heredity, the rate of infertility has risen in Iraq, especially in recent years (6). There are many treatment options to overcome male infertility, such as: in vitro fertilization, hormonal therapy, and assisted reproductive technology. But all of these treatments have their drawbacks. As a result, research efforts have recently focused on finding a less harmful treatment for male infertility (14). The male reproductive system's health is improved when using natural, less dangerous remedies than synthetic ones (8). owing to these natural compounds' pharmacological properties (9). Chlorpromazine is an antipsychotic medication primarily used to treat various mental disorders such as schizophrenia, bipolar disorder, and extreme agitation. As a phenothiazine medication (12), it affects the levels of certain brain chemicals, such as dopamine. These effects are due to its ability to block the action of acetylcholine, a neurotransmitter involved in various body functions, and it also affects fertility (11).

The study aimed to investigate the effect of costus extract on some physiological and histological aspects related to fertility in conjunction with taking the drug chlorpromazine, which has side effects affecting fertility.

Materials and Methods

Collection and Preparation of Plant materials: The healthy, disease-free roots of the *S. Costus* plant root were acquired from the local markets in Ramadi city, dried, and crushed using an electric grinder. The plant powder was then stored in appropriate containers until usage.

The roots of *S. Costus* were chopped and ground through a sieve of 150 microns. In a water bath for 72 hours, 1000 ml of distilled water is pulverized into an excellent powder. The mixture is stirred frequently for hours, then filtered, Bypassing it through a piece of cotton gauze. The remnant was Re-washed three times with the same amount of moisturizing vent. The filter was also re-filtered using Whatman No. 1 filter paper and was the final nomination Oven dry completely. At a temperature of 4 °C, do not store the product in the refrigerator.

Gas chromatography-mass spectrometry (GC-MS): Plant samples filtered and extracted with alcohol are placed in a GC-MS system of GC-MS-QP2010 Plus (Shimadzu, Japan). The gas is carried using helium gas of purity nine at a constant flow rate of 1 ml per minute. The column's temperature begins at 80 °C and steadily rises by 10 °C until it reaches 280 °C. In comparison, the device's heat source reaches a temperature of 350 °C. The chemical compounds in the plant samples were then compared to those in the Nist computer library connected with the GC-MS. The device was set at 80 °C and stayed there for two minutes. The detector was 280 °C in temperature (10).

Preparation of chemicals and Dose administration: According to the (13) approach, chlorpromazine was diluted in 500 ml of distilled water to obtain a concentration of 2 ml, which was used to induce sterility in laboratory animals, Clomid was obtained from one of the pharmacies in the city, and the preparation instructions were followed at a concentration of 2 mg/kg.

Experiment design: Thirty-six adult male white rats were obtained from the animal house of the College of Agriculture at Tikrit University. In the College of Education for Women /Anbar University's animal home, animals were put in plastic animal cages. The cages were covered with sawdust, with attention to the cleanliness of the cages, and the sawdust was changed three times a week. Six rats were placed in each cage when rats were distributed. The rats were kept in suitable laboratory conditions for ventilation, temperature, and lighting while receiving a constant water supply and a standard dietary requirement. The experimental rats were divided into six groups: A negative control group was given water only. A positive control group was assigned oral chlorpromazine at a concentration of 2 ml as a single dose per day. While the groups of Costus root extract were distributed according to the concentration of the extract into three groups: each one was given a concentration of 1.25 ml, 2.4. ml, 2.4. ml and group of clomid, daily for 30 days.

Biochemical analysis and histological examination: The animals were sacrificed after the end of the experiment, and the testes and epididymis were removed from the animals after anesthesia with chloroform. Then the organs were placed in plastic containers containing 10% formalin for biochemical and histological research (2).

1. Using an ELISA reader, testosterone and luteinizing hormone follicle stimulating hormone were measured using an enzyme-linked immunosorbent assay (ELISA) (14).
2. histological preparations were produced after the testicle was removed and put in plastic containers containing 10% formalin (4).

Statistical analysis: The data were analyzed statistically according to the Complete Randomized Design model (GenStat-Tenth) using the statistical program (CRD). The

significant differences of the mean, Edition Version-10.3.0.0, were also tested at least significant difference L.S.D level of $P \leq 0.05$ (15). In addition to that, Correlation Microsoft Word Excel 2016 was used.

Results and Discussion

Phytochemical analysis: The analysis and chemical detection results by GC-MS of the alcoholic extract of the roots of Indian *S. Costus* showed the presence of 14 active compounds (Table 1, Figure 1).

Table 1: Phytochemical constituents identified in *S. costus* alcoholic extract gas chromatography-mass spectrometry.

Compound	R.T. Min	Peak height	Total of %	Chemical composition
Cyclohexane,1-ethenyl-1methyl-2,4-bis(1-methyl ethenyl	10.615	1439002	0.766%	C15H24
Caryophyllene	11.020	977446	0.482%	C15H24
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	11.742	1028299	0.586%	C15H22
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methyl phenyl)	11.873	1033846	0.589%	C15H24
9,12,15-Octadecatrien	13.801	5551581	3.840%	C18H32O
9,12,15-Octadecatrien	13.890	16751181	11.669%	C18H32O
2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methyl phenyl)	15.540	1682318	1.464%	C15H22O2
2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methyl phenyl)	15.942	6962068	7.716%	C15H20O2
Acetic acid,6-(1-hydroxymethyl-vinyl)-4,8a-dimethyl-3-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	17.041	11459069	9.701%	C17H24O4
Androstane-17-one, 3ethyl-3-hydroxy	17.338	2477272	1.735%	C21H34O2
Azuleno(4,5-b)furan-2(3H)-one, 3a,4,6a,7,8,9,9a,,9b-octahydro-6-methyl-3,9-bis(methylene)	17.462	3032581	2.555%	C15H18O2
Azuleno(4,5-b)furan-2(3H)-one, 3a,4,6a,7,8,9,9a,,9b-octahydro-6-methyl-3,9-bis(methylene)	17.698	23927441	57.705%	C15H18O2
3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone	21.045	1310604	0.723%	C28H25NO7
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	23.509	585104	0.466%	C30H52O

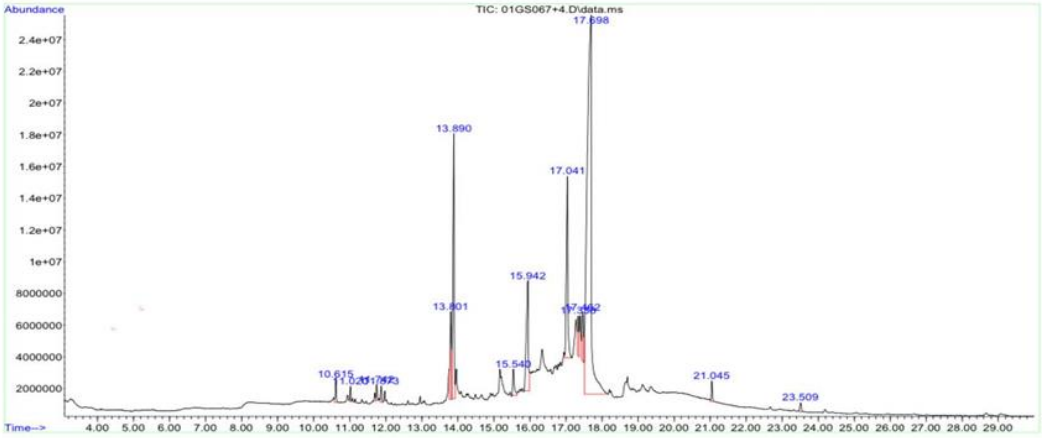

Fig. 1: Active chemical compounds in the alcoholic extract of *S. Costus.*

S. Costus contains many effective compounds that are well known for their wide therapeutic applications (17), such as flavonoids, alkaloids, phenols, coumarins, tannins, and essential oil contents that can kill hepatocellular carcinoma cells (16). It can also be used as an anti-cancer agent for treating various types of breast and colon cancer (18). It is considered a preventive treatment against reproductive toxicity as it prevents and treats the significant decrease in the follicle-stimulating hormone, luteinizing hormone, and testosterone and increases. The number of sperms and their vitality, and treats the distortion that occurs in the sperm and non-motile sperms, as the results of this study were consistent with (20).

Biochemical analysis: The study's findings demonstrated that the group receiving just Chlorpromazine treatment had an average decrease in testosterone, FSH, and LH levels, all dropping significantly. The drop rate was 3.62 mUl/ml, 2.340 mUl/ml, and 2.486 mUl/ml, compared to the control group. This is because the psychiatric medication chlorpromazine alters hormones, and altered hormones result in impotence and hypogonadism (21). According to (23), chlorpromazine also raises blood levels of prolactin while lowering LH and FSH hormones (Table 2).

Regarding the three groups that received the alcoholic extract of Saussurea costus' root, the group with a concentration of 1.25 mg/g showed a highly significant increase in testosterone, 5.330mUl/ml. The average levels of the hormones FSH and LH rose to 4.399mUl/ml and 3.240mUl/ml, respectively. According to (Table 1), the group with a concentration of 2.4 mg/kg also recorded a high rise in the average hormones, amounting to 6.408mUl/ml for Testosterone, 5.612mUl/ml for FSH, and 4.664mUl/ml for LH. In conjunction with Chlorpromazine, the group with a concentration of 4 mg/kg of the alcoholic extract of Saussurea costus recorded a highly significant increase in the mean of both Testosterone and FSH hormones, amounting to 7.535mUl/ml and 7.006mUl/ml, respectively. Table 1 shows LH was 5.527 mUl/ml compared to the control group. According to (22), the costus can prevent and treat the decrease in FSH, LH, and testosterone, consistent with the increase in hormones observed in the groups treated with the alcoholic extract of S. Costus root. It has been proven that S. costus extract can treat the decrease in the three hormones resulting from ethephon poisoning In addition, the group treated with Clomid at a concentration of 2 mg/kg recorded an increase in the three hormones LH, FSH, and Testosterone, respectively 8.103mUl/ml,8.758mUl/ml,8.495mUl/ml Because Clomid has a stimulating effect in the hypothalamus to secrete follicle-stimulating hormone, luteinizing hormone, and testosterone (19), which leads to an increase in the levels of these hormones.

Table 2: Shows the mean values and some statistical parameters of testosterone, FSH, and LH in male rats using different treatments.

Parameters	Treatments	Significant	L.S.D. 0.05	Variation	SD standard deviation	Mean
Control	Testosterone	NS	0.614	0.245	0.495	4.98
	FSH	NS	0.508	0.168	0.410	3.545
	LH	NS	0.661	0.285	0.534	2.966
Chlorpromazine	Testosterone	NS	0.811	0.428	0.655	3.62
	FSH	NS	0.640	0.267	0.517	2.340
	LH	NS	0.619	0.250	0.500	2.486
Clomid	Testosterone	S	0.932	0.566	0.752	8.495
	FSH	S	1.013	0.668	0.817	8.758
	LH	S	1.062	0.735	0.857	8.103
***S. costus* con. 1.25**	Testosterone	HS	0.540	0.190	0.436	5.330
	FSH	S	1.173	0.897	0.947	4.399
	LH	S	0.653	0.278	0.527	3.240
***S. costus* con. 1.25**	Testosterone	HS	0.577	0.217	0.466	6.408
	FSH	S	1.125	0.824	0.908	5.612
***S. costus con.* 4**	LH	S	0.836	0.455	0.675	4.664
	Testosterone	HS	0.891	0.517	0.719	7.535
	FSH	HS	0.812	0.429	0.655	7.006
	LH	S	0.852	0.473	0.688	5.527

Histological study: The study's findings revealed that the average diameter of the seminiferous tubules, Leydig cells, and Sertoli cells decreased significantly in the group treated with chlorpromazine, reaching 12.2 when compared to the control group and the three groups treated with alcoholic extract of S. costus, at a significant level of $P \leq 0.05$. Chlorpromazine causes suppression of testicular function and a decrease in fertility parameters, and this is consistent with (24). It also causes tissue damage to the testicle (28), as shown in Table 2.

The average diameter of the seminiferous tubules increased noticeably in the group that obtained treatment with the alcoholic extract of *S. costus* at a concentration of 4 mg/g, reaching 20.5 mm. Compared to the Chlorpromazine-treated group, which recorded 12.2 for the average tubule diameter, there was also a rise in the Leydig and Sertoli cells rate, as indicated in Table 3.

When compared to the control group and the two groups with a concentration of 1.25 and 2.4, the group treated with the alcoholic extract of *Saussurea costus* at a concentration of 4 mg/kg showed a significant increase in the average diameter of the seminiferous tubules of the testis at $P \leq 0.05$. The seminiferous tubules' average diameter for the two concentrations was 15 and 18.5, respectively. While as indicated in Table 2, the group with a concentration of 4 mg/g recorded the highest level of 20.5.

Table 3: Shows the effect of the three treatments on the average diameter of the seminiferous tubules in the testis (in micrometers) (P≤0.05).

Parameters Groups mg\g	Seminiferous tubules in the microscopic field (µm)Mean+SD	Seminiferous tubules in the microscopic field (10×) Mean+SD	Leydig cells (Number) Mean+SD	Sertoli cells (Number) Mean+SD
Control	A324±4.47	A23.452.97	A39.853.42	A27.852.59
Chlorpromazine	E212.6±15.08	D12.251.924	D21.852.39	C18.851.924
***S. costus* con. 1.25**	D256.2±16.1	C15.451.517	D2553.39	C20.452.7
***S. costus* con. 2.4**	C285.4±13.58	BC1852.55	C29.453.29	AB2551.581
***S. costus con.* 4**	BC301±14.34	B2052.24	B34.452.7	A26.852.39
Clomid	AB307.8±7.43	BC17.852.39	C29.452.88	B23.452.074
P-value	0.00043	0.00071	0.0026	0.00037
Sign.	Sign.	Sign.	Sign.	Sign.

The use of Chlorpromazine has a negative effect on the histological structure of the male testicle. The one that is responsible for the production of sperm and testosterone. Any damage to the testicular tissue can disrupt normal reproductive function. The following are some of the carcinogens' effects on the histological structure of the male testicle. Among Figure 4 is a defect in the tissue that may lead to disruption of the seminiferous tubules responsible for sperm production. It can lead to damage to the supporting cells within the tubules and loss of structural integrity, and this has to do with the hormonal imbalance shown in chemical findings, including testosterone.

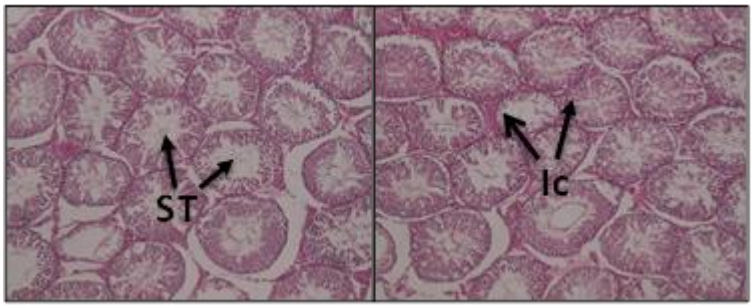

Fig. 2: Cross section of testis of a control animal showing normal appearance (ST) seminiferous tubules and (Ic) interstitial cells at (HandE) magnification (10x).

Tissue changes are one of the effects of taking the chemotherapy drug Chlorpromazine Side effects of this drug include abnormalities in the sperm and a difference in the concentration of hormones. Costus root extract improves drug-induced testicular damage in adult male rats, likely by reducing oxidative stress and testosterone levels. The extract also attenuated aberrations in antioxidant defense markers in mice treated with Chlorpromazine compared to those treated with the extract (25). These results indicate that the beneficial antioxidant effect of Costus extract may be related to its active components, such as flavonoids, anthraquinones, and terpenes. Moreover, in this study, chlorpromazine treatment induced a significant elevation in TNF-a, IL1- and testicular DNA damage with a significant decrease in the number of CD4 cells. These results are consistent with those reported in a previous study (26). These results show that administering the woody extract to rats alone improves fertility in mice. The extract's antioxidant properties may reflect its

protective effect against toxicity induced by Chlorpromazine treatment. Furthermore, this study showed that injecting Chlorpromazine into mice resulted in a significant decrease in blood CD4 cell levels compared to that observed in normal controls, consistent with results previously shown in another study (27).

The results in Fig. 4 and 5 also showed that Chlorpromazine alone resulted in significant degradation of interstitial tissue, as evidenced by the formation of cellular vacuoles and tissue bleeding, which is consistent with. The degradation of testicular tissue specimens may be due to residual Accumulation of cytoplasm and fluid retention in the lumen of some testicular tubules. Sertoli cell atrophy directly manifests decreased testosterone levels and Leydig cell atrophy induced by oxaliplatin treatment (29). The higher rate of germ cell apoptosis and the resulting sterility has been attributed to the atrophy of Sertoli cells (30). Costus extract can restore the typical structure of the seminiferous tubules of the testis and the interstitial spaces (31 and 32). The group treated with Clomid (2 mg/kg) showed a significant increase in the average diameter of the seminiferous tubules in the Clomid-treated group. There was also an increase in the number of primary and secondary spermatogonia cells and spermoblasts, as they appeared in the form of layers showing all stages of sperm and fully mature sperm and appeared densely in the Within the lumen of the seminiferous tubule compared to the control group.

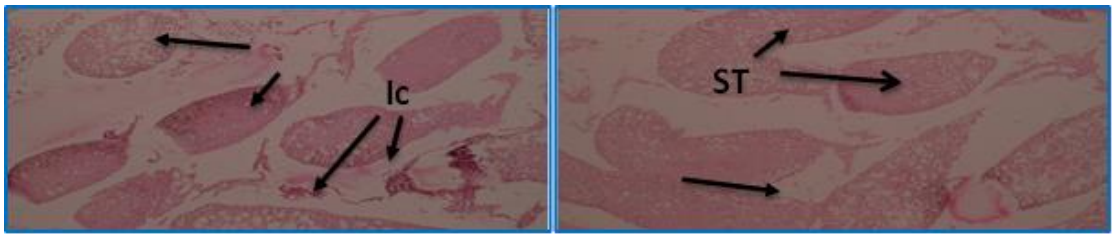

Fig. 3: Cross-sectional section of the testis of an animal treated with Chlorpromazine 2 mg/kg Note: a decrease in the average diameter of the tubules (ST) as well as a decrease in the number of interstitial cells (Ic), the thickness of the interstitial tissue and an increase in the distances between the seminiferous tubules at magnification (HandE) (10x).

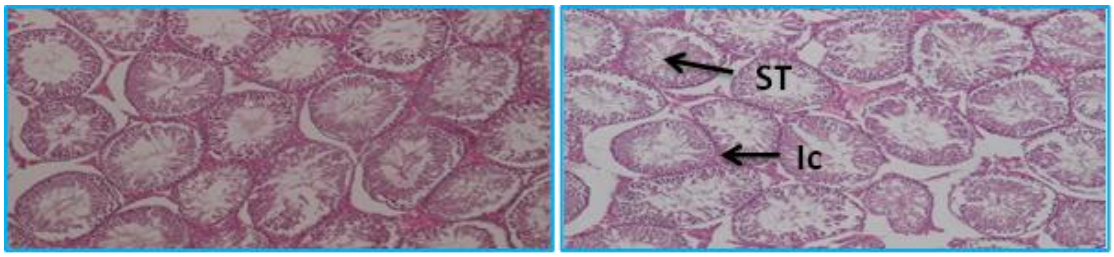

Fig. 4: Cross-section of the testis of an animal treated with alcoholic extract of the root of Indian costus 4 mg/kg. Note: an increase in the diameters of seminiferous tubules (ST), an increase in interstitial cells (Ic) at magnification (HandE) (10x).

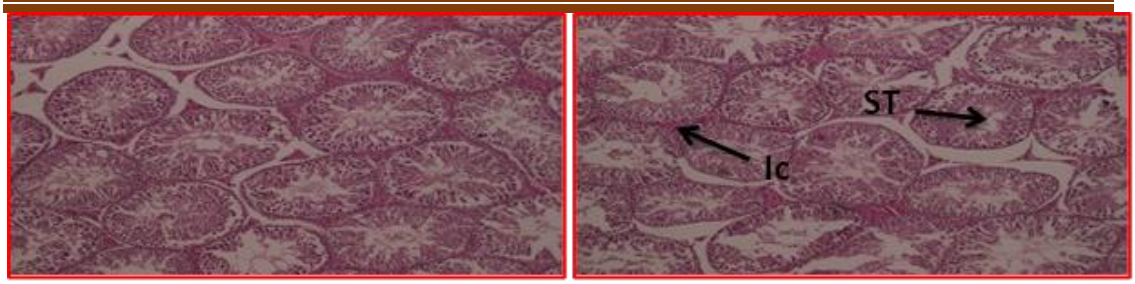

Fig. 5: Cross section of testis of an animal marked with alcoholic extract of the root of Indian costum 2.4 mg/g Note: an increase in the diameters of the seminiferous tubules (ST), an increase in interstitial cells (Ic) at magnification (HandE) (10x).

Conclusions

The increase in testosterone and sperm activity showed that the extract lessened the drug's negative impact on testicular tissue samples. Additionally, the extract promoted the regeneration of the testicular histological structure. Also, it altered testicular oxidative stress, hormone analysis, spermatogenesis, and lead-induced pathological alterations and a dose of 4 mg/kg of alcoholic extract of the root of Indian costum had beneficial effects in raising sexual hormones and increasing the concentration of sperms, and it had similar results to the group that was dosed with Clomid.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author R. R. Abdul Kareem; methodology, writing—original draft preparation, Author Sh. H. Sayer writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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References

1. Abd Eldaim, M. A., Tousson, E., El Sayed, I. E. T., and Awd, W. M. (2019). Ameliorative effects of Saussurea lappa root aqueous extract against Ethephon-induced reproductive toxicity in male rats. Environmental toxicology, 34(2): 150-159. https://doi.org/10.1002/tox.22669.
2. Abd El-Rahman, G. I., Behairy, A., Elseddawy, N. M., Batiha, G. E. S., Hozzein, W. N., Khodeer, D. M., and M. Abd-Elhakim, Y. (2020). Saussurea lappa ethanolic extract attenuates triamcinolone acetonide-induced pulmonary and splenic tissue damage in rats via modulation of oxidative stress, inflammation, and apoptosis. Antioxidants, 9(5): 396. https://doi.org/10.3390/antiox9050396.
3. Abdulwahid, A. S., Mohammed, A. B., and Al-Mjbel, A. A. (2022). Onion (Allium cepa) and sumac (Rhus coriaria) powder as dietary supplements for Japanese quail (Coturnix japonica): effect on egg production, blood parameters and antioxidant activity. https://doi.org/10.17957/IJAB/15.1964
4. Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., ... and Shah, R. (2021). Male infertility. The Lancet, 397(10271): 319-333. https://doi.org/10.1016/S0140-6736(20)32667-2.
5. Al-Mukhtar, K. A., and Abdel-Hakim, A. (2000). Histology. 2nd ed. Ministry of Higher Education and Scientific Research University of Baghdad.
6. Al-Mushhadani, T. M., Arteen, H. E., and Jumaa, H. J. (2020). Effect of Plant Seeds Peganum harmala L-Evaporation on Liver and Heart Enzymes Activity and Total Protein in Male White Rats (Rattus norvegicus) Treated with Chlorpromazine. Rafidain Journal of Science, 29(1): 68-79. https://doi.org/10.33899/rjs.2020.164482.
7. Alotaibi, A. A., Bepari, A., Assiri, R. A., Niazi, S. K., Nayaka, S., Rudrappa, M., and Bhat, M. P. (2021). Saussurea lappa exhibits anti-oncogenic effect in hepatocellular carcinoma, HepG2 cancer cell line by Bcl-2 mediated apoptotic pathway and mitochondrial cytochrome C release. Current Issues in Molecular Biology, 43(2): 1114-1132. https://doi.org/10.3390/cimb43020079.
8. Al-Rawi, Kh. M., and Abdel-Aziz, K. A. (2000). Designing Agricultural Experiments, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul, Mosul, 2nd ed.
9. Ashry, M., Galal ELSahra, D., Abdel-Wahhab, K. G., Abdelsalam, M. E., Mourad, H. H., El-Bitar, A. M., and Gomaa, H. F. (2022). Saussurea Costus Extract Has Anti-inflammatory, Antioxidant, and Hormonal Effects Against Testicular Toxicity Induced by Oxaliplatin in Male Albino Rats. Iranian Journal of Toxicology, 16(2): 83-90. DOI: 10.32598/IJT.16.2.894.1.
10. Babakhanzadeh, E., Nazari, M., Ghasemifar, S., and Khodadadian, A. (2020). Some of the factors involved in male infertility: a prospective review.

International journal of general medicine, 5(13): 29-41. DOI: 10.2147/IJGM.S241099.

11. Bakhshwin, D., Faddladdeen, K. A. J., Ali, S. S., Alsaggaf, S. M., and Ayuob, N. N. (2022). Nanoparticles of Costus speciosus ameliorate diabetes-induced structural changes in rat prostate through mediating the pro-inflammatory cytokines IL 6, IL1β and TNF-α. Molecules, 27(3): 1027. https://doi.org/10.3390/molecules27031027.
12. Balboa, E. M., Soto, M. L., Nogueira, D. R., González-López, N., Conde, E., Moure, A., ... and Domínguez, H. (2014). Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. Industrial Crops and Products, 58: 104-110. https://doi.org/10.1016/j.indcrop.2014.03.041.
13. Bauer, A., and Brönstrup, M. (2014). Industrial natural product chemistry for drug discovery and development. Natural product reports, 31(1): 35-60. https://doi.org/10.1039/C3NP70058E.
14. Chen, L., Shi, G. R., Huang, D. D., Li, Y., Ma, C. C., Shi, M., ... and Shi, G. J. (2019). Male sexual dysfunction: A review of literature on its pathological mechanisms, potential risk factors, and herbal drug intervention. Biomedicine and Pharmacotherapy, 112: 108585. https://doi.org/10.1016/j.biopha.2019.01.046.
15. Elkhateeb, S. Z., Ebraheem, M. O., and Ahmed, I. A. (2024). The Impact of Environmental Exposure Duration on Natural Detoxification and Physiological Health in Domestic Sheep Via Glucuronide Pathways. Journal of Life Science and Applied Research, 5(1): 27–33. https://doi.org/10.59807/jlsar.v5i1.95.
16. Ganeshpurkar, A., and Saluja, A. K. (2017). The pharmacological potential of rutin. Saudi pharmaceutical journal, 25(2): 149-164. https://doi.org/10.1016/j.jsps.2016.04.025.
17. Ghafel, H. H., and AL-Rahawi, A. H. (2016). Effectiveness of infertile women's commitment to implement in vitro fertilization (long/short) protocols. Journal of Contemporary Medical Sciences, 2(5):9-14. DOI: 10.22317/jcms.v2i5.61.
18. Hussein, D. M., and Alzubaidi, B. A. (2022). Effect of dostenix and origanum majorana l. leaf extract in regulating the prolactin and sex hormones in females rats chlorpromazine-induced Hyperprolactinemia. International Journal of Drug Delivery Technology, 12: 395-400.
19. Katz, D. J., Nabulsi, O., Tal, R., and Mulhall, J. P. (2012). Outcomes of clomiphene citrate treatment in young hypogonadal men. BJU international, 110(4): 573-578. https://doi.org/10.1111/j.1464-410X.2011.10702.x.
20. Karim, K. K., and Abdulla, N. R. (2024). Use of various sources of calcium in the diets of broiler and its effects on carcass and some meat quality. Tikrit Journal for Agricultural Sciences, 24(1), 45–56. https://doi.org/10.25130/tjas.24.1.5.
21. Jassim, A. A., and I. Al-Jugifi, W. (2023). Gradually Increasing in Lighting Intensity on Characteristics of Ross 308 Broiler Carcass and Some Internal Organs. Anbar Journal of Agricultural Sciences, 21(1): 205-213. doi: 10.32649/ajas.2023.179730.

22. McKenzie, A. (2011). The history of anaesthesia society. Proceeding of the summer scientific meeting held jointly with Lad o Pairts at The Royal College of Surgeons of Edinburgh 3rd and 4th. 44: 1360-6891.
23. Mohammed, A. B., Hamad, O. K., and Khttab, T. A. (2023). Effect of zinc oxide nanoparticles in drinking water on growth rate, biochemical parameters, and intestinal histology of broilers. Advances in Agriculture, 2023(1), 8523516. https://doi.org/10.1155/2023/8523516.
24. Oyovwi, M. O., Ben-Azu, B., Agbonifo-Chijiokwu, E., Moke, E. G., Ajayi, A. M., Wilson, J. I., ... and Igweh, J. C. (2022). Possible mechanisms involved in the prevention and reversal of chlorpromazine-induced testicular damage by taurine and coenzyme-Q10 in rats. Nutrire, 47(2): 31. https://doi.org/10.1186/s41110-022-00180-w.
25. Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. International journal of herbal medicine, 4(4): 59-64.
26. Sharma, A. (2018). Gene expression analysis in medicinal plants under abiotic stress conditions. In Plant metabolites and regulation under environmental stress, pp. 407-414. https://doi.org/10.1016/B978-0-12-812689-9.00023-6.
27. Shati, A. A., Alkahtani, M. A., Alfaifi, M. Y., Elbehairi, S. E. I., Elsaid, F. G., Prasanna, R., and Mir, M. A. (2020). Secondary metabolites of Saussurea costus leaf extract induce apoptosis in breast, liver, and colon cancer cells by caspase-3-dependent intrinsic pathway. BioMed Research International, 2020. https://doi.org/10.1155/2020/1608942.
28. Siriwardhene, M. A., Sabalingam, S., and Goonetilleke, A. (2023). Antidiabetic activity of methanol extract and fractions of Costus speciosus leaves in normal and alloxan induced diabetic rats. Journal of Drug Delivery and Therapeutics, 13(5): 5-12. https://doi.org/10.22270/jddt.v13i5.6045.
29. Ventimiglia, E., Capogrosso, P., Boeri, L., Serino, A., Colicchia, M., Ippolito, S., ... and Salonia, A. (2015). Infertility as a proxy of general male health: results of a cross-sectional survey. Fertility and sterility, 104(1): 48-55. https://doi.org/10.1016/j.fertnstert.2015.04.020.
30. Vijayalakshmi, M., Umamaheswari, A., Agalya, B., Jegasubramaniam, S. N., and Prabu, S. L. (2022). Foresight on phytoconstituents and associated pharmacological activities of traditional medicinal plant: Saussurea costus (Falc.) Lipschitz. Current Pharmacology Reports, 8(4): 281-289. https://doi.org/10.1007/s40495-022-00287-8.
31. Wisdom, G. B. (1976). Enzyme-immunoassay. Clinical chemistry, 22(8): 1243-1255. https://doi.org/10.1093/clinchem/22.8.1243.
32. Ye, R. J., Yang, J. M., Hai, D. M., Liu, N., Ma, L., Lan, X. B., ... and Yu, J. Q. (2020). Interplay between male reproductive system dysfunction and the therapeutic effect of flavonoids. Fitoterapia, 147: 104756. https://doi.org/10.1016/j.fitote.2020.104756.