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Supplement of *Saussurea Lappa* Declines Oxidative Stress and Augments Fertility Indicators of Male Rabbits Without Genotoxicity

Yousry E. El-Bolkiny[1], Nabila E. M. El-kassas[2], Omnia S. Ali[1], Ismail A. Elnady[3]* and Noura H. Gouda[4]

[1]*Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.*

[2]*Agricultural Research Center, Animal Production Research Institute, El-Dokki, Giza, Egypt.*

[3*]*Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*

[4]*Animal and Poultry Production Department, Faculty of Technology and Development, Zagazig University, Zagazig, Egypt.*

Abstract

CURRENTLY, the use of natural products in animal nutrition supplementation in replacement of chemical additives has increased; not only do they contain many active components, but they are also safer, which has led to interest in herbal and medicinal plants. Natural herbal plants enhance animal performance and meat quality for human consumption. Saussurea lappa (Costus) is one of herbal plant that possess bioactive ingredients of many medical applications. Aim: the present work aimed to investigate the effect of costus on blood parameters, DNA integrity, and fertility of Flemish Giant male rabbits. Forty male rabbits (3000-3200 g) of 6 months of age were randomly divided into four experimental groups (n = 10). The first group received normal rodent's diet to serve as a control (C). The other three groups received orally costus at doses of 50 (T1), 100 (T2) and 200(T3) mg/kg B.W for consecutive 8 weeks. The obtained results showed a significant increase in RBCs, WBCs, Hb and no changes in PCV and platelet's count with treated groups compared to the control group. Ejaculated volume of semen, live, total output, concentration, and viability of sperms were improved in a significant way. Additionally, serum testosterone level was enhanced in all treatments compared to the control. Whereas there was a decrease in cholesterol, triglycerides, and MDA with an increase in CAT compared to the control. Interestingly, there is no observed DNA fragmentation in the investigated tissues post all treatments. These results can recommend using costus in diet or water provided to rabbits safely for enhancing productivity of rabbits and other poultry.

Keywords: Saussurea, DNA, semen, testosterone, rabbit.

Introduction

Medical herbs have become one of the most popular forms of contemporary medicine in recent years. due to their safety, cheapness, and availability. In addition, the procedure of preparing and using them is free of trouble and is used to improve the state of immunity and production and treat many different disorders because they have bioactive molecules that improve a healthy state.

Saussurea lappa (Costus) is one of these herbal plants that is rich in many medicinal bioactive compounds such as flavonoids, steroids, terpenes, alkaloids sesquiterpenes, costunolide, dehydrocostus lactone, and others [1,2,3]. By these biomolecules, costus acts as an antioxidant that global extensively in traditional medical systems to treat several illnesses, including inflammation, tenesmus, diarrhea, dyspepsia, and vomiting [4,5]. In addition, it contains plenty of some essential vitamins and minerals that play an essential role in the metabolism pathway, such as vitamins A, C, and B complex, as well as Ca, Mg, Fe, Mn, Zn, Pb, Sr, Cu, Ni and Cr [6]. Costus has been screened and approved for several pharmacological activities like anti-hepatotoxic activities [7], treating or helping prevent diabetes [8,9], anti-fungal and anti-worms [10,11,12,13].

Other uses of costus were reported as anti-helminthic [14], anti-tumour [2,3,15], anti-inflammatory [16,17], anti-ulcer [18], anti-microbial [19,20] and immuno-stimulant activities [21]. It is also used for treating cardiovascular diseases [1] and

*Corresponding authors: Ismail A. Elnady, E-Mail.: ismailelnady@azhar.edu.eg, Tel.: 01288312471
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balancing of blood constituents [22] by protecting cells against oxidative damage [23,24,25].

Previous investigators showed that these bioactive molecules can react with ROS [26,27] to delay and neutralize their damaging effects on the organ's functions and thus they reduce oxidative stress and protect the body from many diseases. Moreover, the excessive production of ROS is very damaging to spermatozoa leading to male infertility. Achieving the highest level of production in rabbit farms depends primarily on improving fertility [28,29]. Results of [30] in male rabbits showed that the costus root ethanolic extract had protective effect against paracetamol-induced hepato-renal damage and ethephon-induced renal toxicity in male rats [31]. Costus root extract also had protective effect against testicular toxicity of Bisphenol A in mature male albino rats [32].

Accordingly, this study mainly aimed to assess the potential curative influence of Costus water extract on CBC, lipids, oxidative stress, and DNA fragmentation. In addition, it extended to check the fertility of male rabbits by investigating the testicular functions through semen characterization, and serum testosterone level.

Material and Methods

Experimental location:

The experiential was conducted at the El-Gemmaza experimental station, Gharbia governorate, Egypt, which belonged to the Animal Production Research Institute (APRI/132429/191214).

Animals and experiments:

In the present study, 40 male Flemish Giant rabbits at sexual maturity age with live body weight (3000 ± 200) g were randomly divided into four experimental groups. Group one received an unchanged pelleted diet as a control (C). The 2nd, 3rd and 4th experimental groups were orally given Costus at doses of 50, 100 and 200 mg/kg BW (T1, T2, and T3 respectively,) three times a week for consecutive 8 weeks. All animals were provided with normal food pellets and drinking water ad libitum. Fresh tap water was automatically available throughout the experimental period by stainless steel nipples fixed in each cage. The ingredients and chemical composition of the pelleted rations are according to the manufacturer trademark.

Costus extract preparation and treatment protocol:

In the present study, the powder of costus roots was purchased from the local market of Tanta city. Ten grams of costus powder were weighted and dissolved in I L of boiled distilled water and kept cooling as a stock. At treatment, I ml of stock solution was taken and extended with distilled water reaching to doses of 50, 100 and 200 mg/kg BW of rabbits. Oral treatment protocol was 3 times / week to male rabbits for consecutive 8 weeks.

Blood samples and biochemical analysis:

Rabbit blood samples were withdrawn from the ear veins of all groups. Each sample was placed in two different tubes: one containing EDTA for CBC analysis immediately and the other without anti-coagulant for later biochemical analysis. Seram were separated after centrifugation at 3000 rpm for 15 minutes and were aliquoted then kept at -20 °C until biochemical analysis.

Erythrocytes count (RBCs), hemoglobin concentration [Hb], leukocytes count (WBCs), hematocrit value (HCT) and Platelet count (PLT) were determined by Fully Automatic Body Cell Counter (NS-Biotec. Hema 21). Total serum cholesterol and triglyceride concentration were determined using commercial spectrophotometer kits (Diamond Diagnostics company, Egypt). Malondialdehyde (MDA) and catalase (CAT) were assayed using colorimetric kits (Bio-diagnostic, Cairo, Egypt). Testosterone was assayed using ELISA kits (PITKTT-5, 2006-12-29).

Semen collection and physical characteristics:

After 50 days, semen samples were collected from all male rabbits three times a week using an artificial vagina during early morning 7.00: 8.00 a.m. All the equipment used in handling the semen, including slides, cover slides, syringes, saline, stains, and needles were kept in an incubator at 37 °C. After being collected, samples were immediately transported to the laboratory that was temperature-controlled at 30 °C, removed gel from semen if it was present, and incubated at 37 °C in a water bath, then evaluated. Ejaculate volume was measured using the collection graduated glass tube.

Sperm concentration of (the number of sperm/ml) was measured by an improved hemocytometer [33]. Total output was calculated by multiplying concentration (the number of sperm/ml) by the ejaculate volume.

Both mass and forward motility of rabbit spermatozoa were evaluated according to [34].The percentages of live and abnormalities of rabbit spermatozoa were evaluated using nigrosine/eosin stain according to [35].

Total genomic DNA extraction:

DNA extraction from WBCs and testis tissues was carried out in line with the methodology of [36]. Some edits were inserted by [37] in which the straight staining of the DNA sample was ended. For characterization and separation of DNA fragments, agarose gel electrophoresis is used to resolve DNA fragments according to their molecular weight where smaller fragments migrate more quickly than larger ones. The movement space on the gel varies

inversely with the logarithm of the molecular weight. The size of the fragment can therefore be determined by calibrating the gel, using known size standards, and comparing the distance of the unknown one.

Data statistical analysis:

Data obtained from experiments were expressed as mean ± standard error, the results were analyzed statistically using one way ANOVA followed by Duncan t-test to distinguish significance among treatments based on SPSS program. Differences between means were considered significant at $p \leq 0.05$[38].

Results

The current data (Table 1) indicated that costus significantly improved RBCs, Hb and WBCs with treated groups of male Flemish Giant rabbits compared to control, but the fluctuations in HCT, and PLT induced by costus in all groups were insignificant. It is noted that the higher enhancing effect was for 100 and 200 mg/Kg followed by 50 mg/Kg.

As shown in (Table 2), total cholesterol and triglycerides were decreased by increasing the costus dose compared to the control. The best positive effect of costus was recorded in T3 (200 mg/Kg). Concerning the antioxidant state, the present data showed that in all treated groups, the concentration of MDA dramatically dropped while the CAT increased significantly. The gradual increase of CAT and decrease of MDA was uppermost following administration of 200 mg/Kg (T3). These results indicate that costus can reduce both lipids and oxidative stress biomarkers by increasing ROS scavenging enzyme (CAT) and decreasing the end product (MDA) of the oxidation process.

Serum testosterone levels (Fig.1) in male rabbits treated by costus were increased ($P < 0.05$) compared to the control. On the other hand, rabbits treated up to 200 mg/kg of costus showed a significant increase versus control but less than T1 and T3 (50 and 100 mg/Kg, respectively).

Table (3) shows the administration of costus at doses of 50,100 and 200 mg/kg BW led to significantly increased ejaculated volume, viability, concentration and total output of spermatozoa, 200 mg/Kg had a marked effect on ejaculated volume and total output (twofold the control). All doses of costus could not fluctuate the percentage of live spermatozoa, while significantly reducing the abnormalities % versus the control values.

The effects of costus treatments (50,100 and 200 mg/kg BW), administered orally 3 times a week for 8 weeks) on DNA fragmentation of WBCs, and testicular tissues, and the determination of genotoxicity was assessed using agarose gel electrophoresis for DNA fragmentation (Fig 2 and 3).

Figure (2) display intact DNA without any fragmentation in the WBCs of the control (lane 1), Likewise, the administration of costus had no effect on DNA as seen in lane 2, 3 and 4, respectively compared to lane 1 of the control group.

The same results were observed on the examination of the DNA fragmentation in the testicular tissue (Fig. 3), which displays an intact DNA band without any fragmentation observed in the control rabbits (lane 9). Similarly, the administration of costus different doses had no effect on DNA fragmentation as seen in lane 10,11 & 12, respectively compared to the control group (lane 9).

Discussion

The present results showed that costus in all examined doses, improved CBC and prevent anemia indicating by significant increases in the total numbers of erythrocytes (RBCs) and Hb concentration, while fluctuations in HCT and PLT concentrations were insignificantly increased. Also, costus led to increased WBCs count stimulating cellular immunity compared to the control. In the same trend [30,39] found that costus restored erythrocytes, leukocytes counts and Hb concentration in rabbits treated with Paracetamol. In the same line, [3] showed costus improved CBC elements in Ehrlish-bearing mice treated with cisplatin.

In addition, supplement of costus improved all concentrations of RBCs, WBCs, and Hb in rats treated with Chloropyrifos Ethyl or Triamcinolone Acetonide [22,23]. Dissimilarly, [40,41] noted that administration of costus extract markedly decreased WBCs count. A study by [2] noted that costus contain bioactive compounds include alkaloids, saponins, steroids, terpenes, polyphenol, flavonoids, sterols, tannins, and glycosides. Also, costus includes hemopoietics that stimulate the synthesis of blood cells, plus contains other antioxidant substances that inhibit free radical formation. These hemopoietic and antioxidant substances in costus extract can hinder hemolytic anemia and ameliorate blood components [42,43]. Present results regarding WBCs agree with a study by [30] who showed that co administration of costus increased WBC as compared to control rabbits. This WBCs increase is attributable to a bioactive component known as dehydrocostus lactone and costunolide that refers costus enhanced the immunity of rabbits.

All current doses of costus decreased cholesterol and triglycerides levels in rabbits serum in a significant manner indicating anti-lipidemic effect of costus. These outcomes are in consistence with results cited by many investigators [44,45] in hyperlipidemia, [46,47] in guinea pigs given CCL4 and [48] in diabetic rats. Maybe the protective properties of costus roots are attributable to the fact that they contain a lot of phytochemicals that have high antioxidant and anti-inflammatory characteristics [49]. The decreased

cholesterol level effect of costus might be due to the presence of bioactive components, especially phenolic elements [50].

In the current work, it was noticed that costus reduced the oxidative stress in male rabbits through significantly decreased MDA, the end product of oxidative process, and increased CAT serum levels as compared with control. These results might be caused by the antioxidant characteristics of costus in accordance with [27,51,52, 53,54,55]. Thus, by promoting the capacity of CAT to scavenge free radicals, costus extract appears to protect cellular organelles against reactive oxygen species [56]. Co-administration of costus is correlated with decreased MDA and an increased action of CAT. Current results in rabbits line up with those of [3,57,58] in rats.

In the same regard, costus significantly increased serum testosterone level these results agree with findings of [12,57]. Similarly, [59] found that costus extract improves reproductive performance by raising gonadotropin hormone and total testosterone levels. These findings were compatible with the results of [32] who postulated that costus had a protective effect against harmful lead influences by increased testosterone secretion in blood flow. The antioxidant properties of costus may be responsible for its protective effects on testes against damage or toxicity. Furthermore, it has been reported that antioxidants improve the synesis of steroid hormones, especially androgen, by enhancing Leydig cell function, and that reason increases testosterone production and enhances division of spermatogonia, i.e., costus improved semen quality [60].

The current findings regarding semen characteristics agree with the findings of [59] who noted that treatment of male rats with costus enhances sperm motility, viability, and concentration through protective testicular tissue from harmful ethephon impact, lead [57] and cyclosporine [61] in male rats. Costus root extract improved semen density, concentration, spermatozoa, and quality; then again, reduced abnormalities by the presence of many active antioxidant ingredients, including flavonoids, steroids, and chlorogenic acid [57]. The previous author mentioned that flavonoids are one of the most important ingredients in medical plants due to their strong ability to scavenge free radicals and bind metal ions. Thus, they may protect cellular organelles that contain highly unsaturated fatty acids, mainly plasma membrane, mitochondria, and nucleus, against free radicals and peroxidation. Moreover, costus can reduce xenobiotic-induced testicular DNA damage and support spermatogenesis; thus, costus may protect tissue against free radicals and lipid peroxidation.

Results were consistent with those of [62] who found that flavonoids in costus extract enhance oxidative stress reduction, a cell protective agent when exposed to activity scavenging free radicals that damage cell structures, thereby preventing DNA damage. Costus thus seems to possess anti-apoptotic qualities, as demonstrated by Ethephon-induced renal toxicity in male rats [63].

Conclusion

Through current observation, we can recommend that the oral intake of costus extract has beneficial enhancing effects on CBCs, antioxidants, and testicular functions without genomic alterations with reducing serum lipids and oxidative stress in male rabbits leading to augmentation of fertility and productivity indices.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study was approved by the research and ethics committee of the Animal Production Research Institute, Giza, Egypt (APRI/132429/191214).

TABLE 1. Effect of supplementation costus on CBC elements of male Flemish Giant rabbits.

Treatments	C	T1	T2	T3	Sig.
RBCs ($\times10^{12}$/L)	5.2^{b} ± 0.15	6.0^{a} ± 0.14	6.1^{a} ± 0.10	5.7^{a} ± .09	*
Hb (g/dl)	$11.5^{b\pm}$ 0.24	$13.5^{a\pm}$ 0.24	13.8^{a} ± 0.21	13.6^{a} ± 0.17	*
WBCs ($\times10^{9}$/L)	5.1^{b} ± 0.13	6.1^{b} ± 0.19	$9.7^{a\pm}$ 0.16	8.6^{a} ± 0.27	*
HCT (%)	43.62 ± 0.71	44.69 ± 0.97	42.50 ± 0.94	47.42 ± 0.63	NS
PLT ($\times10^{3}$/µl)	320 ± 16.3	290 ± 13.7	310 ± 17.4	325 ± 21.9	NS

All data are expressed as mean ± standard error (X̄ ± SE); a and b with different superscripts in the same row indicate a significant difference at $P < 0.05$.

TABLE 2. Effect of supplementation costus on serum lipid constituents and oxidative stress biomarkers of male Flemish Giant rabbits.

Treatments	C	T1	T2	T3	Sig.
T. Chol. (mg/dl)	$39.4^{a} \pm 0.65$	$38.1^{a} \pm 0.85$	$36.9^{b} \pm 0.76$	$31.5^{c} \pm 0.75$	**
Tri.G. (mg/dl)	$155.9^{a} \pm 4.2$	$123.4^{b} \pm 2.9$	$95.6^{bc} \pm 1.5$	$75.1^{c} \pm 1.1$	**
CAT (mmol/min/L)	$1063^{c} \pm 7.7$	$1336^{b} \pm 6.3$	$1326^{b} \pm 7.6$	$1463^{a} \pm 6.3$	**
MDA (mmol /L)	$37^{a} \pm 0.8$	$28^{b} \pm 0.7$	$25^{c} \pm 1.0$	$23^{c} \pm 0.9$	**

All data are expressed as mean ± standard error (X` ± SE); a and b with different superscripts in the same row indicate a significant difference at $P < 0.05$.

TABLE 3. Effect of supplementation on physical semen characteristics of male Flemish Giant rabbits.

Treatments	C	T1	T2	T3	Sig.
lated Volume/ml	$0.6^{b} \pm 0.04$	$1.0^{a} \pm 0.06$	$0.9^{a} \pm 0.04$	$1.0^{a} \pm 0.04$	*
Viability (%)	$73.2^{b} \pm 1.3$	$78.2^{a} \pm 1.3$	$73.2^{b} \pm 2.1$	$73.2^{b} \pm 1.3$	*
Con. / ml (x10^{6})	$425^{b} \pm 2.9$	$386^{c} \pm 4.3$	$505^{b} \pm 1.5$	$658^{a} \pm 1.1$	**
Total output (x10^{6})	$212^{c} \pm 17$	$393^{b} \pm 19$	$443^{b} \pm 16$	$645^{a} \pm 27$	**
Live sperm (%)	90.8 ± 1.4	90 ± 1.3	90.2 ± 1.0	89.2 ± 1.0	Ns
Abnormal sperm (%)	$5.0^{a} \pm 1.1$	$4.8^{a} \pm 4.3$	$2.8^{b} \pm 1.5$	$2.8^{b} \pm 2.9$	*

All data are expressed as mean ± standard error (X، ± SE); a and b with different superscripts in the same row indicate a significant difference at $P < 0.05$.

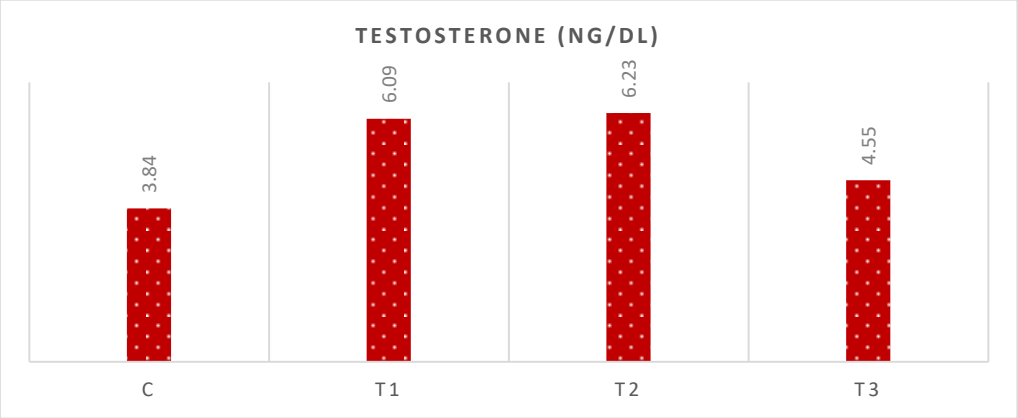

Fig. 1. Effect of supplementation costus on testosterone level of male Flemish Giant rabbits.

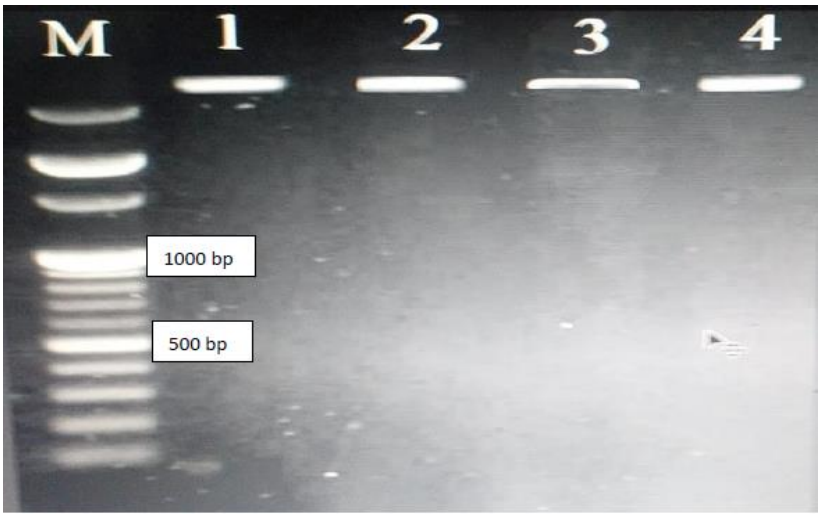

Fig.2. Representative digital photograph of WBCs DNA fragmentation in the control and experimental groups dosed costus (50, 100 and 200 mg/Kg) right Lanes (1-4) & left Lane M: DNA marker (50 bp DNA ladders).

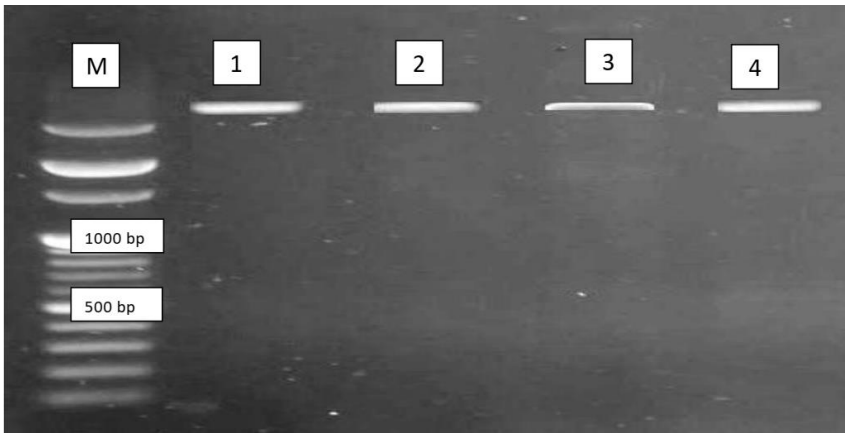

Fig. 3. Representative digital photograph of testicular DNA fragmentation in the control and experimental groups dosed costus (50, 100 and 200 mg/Kg) right Lanes (1-4) & left Lane M: DNA marker (50 bp DNA ladders).

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إضافة القسط لذكور الارانب يقلل من الإجهاد التأكسدي ويزيد من مؤشرات الخصوبة دون احداث سمية جينية

يسري السيد البلقيني[1]، نبيلة إلسيد القصاص[2]، أمنية صابر علي[1]، إسماعيل عبدالرؤف النادي[*3] ونورا حمدي جودة[4]

[1] قسم علم الحيوان، كلية العلوم، جامعة طنطا، طنطا، مصر.

[2] مركز البحوث الزراعية، معهد بحوث الإنتاج الحيواني، الدقي، الجيزة، مصر.

[*3] قسم الإنتاج الحيواني، كلية الزراعة بالقاهرة، جامعة الازهر، القاهرة، مصر.

[4] قسم الإنتاج الحيواني والداجني، كلية التكنولوجيا والتنمية، جامعة الزقازيق، الزقازيق، مصر.

الملخص

في الوقت الحالى زاد استخدام المصادر الطبيعية في تغذية الحيوانات بدلاً من الإضافات الكيميائية، لإنها تحتوي على العديد من المواد الفعالة بالإضافة لكونها أكثر أمانًا، مما أدى إلى الاهتمام بالنباتات الطبية والعطرية. تعمل النباتات الطبية على تحسين أداء الحيوان وجودة اللحوم للاستهلاك البشري. القسط الهندي هو أحد النباتات التي تمتلك مكونات نشطة بيولوجيًا تستخدم في العديد من التطبيقات الطبية. تهدف الدراسة الحالية الي معرفة تأثير مستخلص نبات القسط على مكونات الدم ومستوي الكوليسترول ومضادات الأكسدة وسلامة الحمض النووي وهرمون التستوستيرون وجودة السائل المنوي لذكور ارانب فلامش جانيت. تم تقسيم أربعين أرنبًا ذكرًا من نوع فلامش جانيت بمتوسط وزن (3000-3200 جم) بعمر 6 أشهر بشكل عشوائي إلى أربع مجموعات تجريبية (ن = 10). تلقت المجموعة الأولى العليقة الأساسية فقط بدون أي إضافات وتمثل المجموعة الضابطة. تلقت المجموعات الثلاث الأخرى العليقة الأساسية بالإضافة الي تجريعها مستخلص نبات القسط عن طريق الفم بجرعات 50 و100 و200 مجم / كجم وزن الجسم لمدة 8 أسابيع متتالية. أظهرت النتائج المتحصل عليها زيادة معنوية في عدد كرات الدم الحمراء وخلايا الدم البيضاء والهيموجلوبين في حين لم يكن هناك تغييرات معنوية في حجم خلايا الدم الحمراء وعدد الصفائح الدموية. كما أدت المعاملة بمستخلص القسط الي تحسين جودة السائل المنوي حيث زادت حجم القذفة المنوية وكذا تركيز الحيوانات المنوية بالإضافة الي زيادة حيوية الحيوانات المنوية. بالإضافة إلى ذلك، تم زيادة مستوى هرمون التستوستيرون في جميع المعاملات مقارنة بالمجموعة الضابطة. في حين كان هناك انخفاض معنوي في مستوي الكوليسترول والدهون الثلاثية و MDIمع زيادة في مستوي الكاتاليز مقارنة بالمجموعة الضابطة. ومن المثير للاهتمام ان المعاملة بمستخلص القسط لم يؤثر سلبا علي سلامة الحمض النووي في الأنسجة المدروسة. توصي الدراسة الحالة بان مستخلص القسط له تأثيرات إيجابية علي مقاييس الدم وكذا الخصوبة وجودة السائل المنوي لذكور الارانب لذا يمكن استخدام القسط في النظام الغذائي للأرانب بأمان لتعزيز إنتاجية الأرانب والدواجن الأخرى.

الكلمات الدالة: القسط، الحمض النووي، السائل المنوي، هرمون التستوستيرون، الارانب.