Journal homepage www.jzs.univsul.edu.iq
Journal of Zankoy Sulaimani
Part-A- (Pure and Applied Sciences)
ISSN:1812-4100

The effects of ginger and black seed extracts on albino rat spermatogenesis induced with hyperuricemia

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Article info

Original: 25 May 2021
Revised: 20 June 2021
Accepted: 24 June 2021
Published online:
20 December 2021

Key Words: *Natural plant metabolite, hyperuricemia, antioxidant enzymes, animal model, male reproductive system*

Abstract

Both ginger and black seed are promising nutraceutical natural plant products with reported diverse therapeutic properties. However, there is limited research on their effects on spermatogenesis in animals, especially rats. Thus, the current manuscript investigates the role of ginger and black seed on male albino rats regarding testicular function and spermatogenesis capacity. For this purpose, 24 animals were divided into 6 groups; each group was induced experimentally with hyperuricemia using intraperitoneal injections of oxonic acid for 1 week, except the first group that was used as a negative control group and received intraperitoneal injection of 0.9% saline solution without any further treatment. Then, the second group acted as hyperuricemia control rats and not received any treatment. In contrast, the third, fourth, fifth, and sixth groups were treated daily with ginger extract, black seed extract, allopurinol (control positive), and a mixture of ginger + black seed extract, respectively. Results demonstrated that ginger administered orally to rats at a dose of 25 mg/kg was superior to the administration of black seed extract and a mixture of both of them, particularly in enhancing the percentage of spermatozoa normalization, normalization of serum sexual hormones (testosterone, luteinizing hormone (LH) and follicular stimulating (FSH)) level, as well as normalization of oxidative parameters (GPX, SOD, and CAT) and improving histopathological alterations. In conclusion, I realized that supplementation of ginger extract to hyperuricemia rats for two weeks was significantly ($P < 0.05$) normalized the majority of abnormalities in the male reproductive system induced by hyperuricemia.

Introduction

Even though uric acid was first identified approximately two centuries ago, certain pathophysiologic aspects of hyperuricemia are still not clearly understood. Hyperuricemia or excessive circulating urate concentrations, which is the final product of purine metabolism, is an important risk factor for gout and plays a significant role in the pathogenesis of this condition ***[1, 2]***.

Oxidative stress is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues, as well as the inability of a biological system to detoxify these reactive products. ROS can play several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics such as anticlastic drugs contribute to greatly increase ROS production ***[3]***.

Evidence proposes that oxidative stress contributes to about 30 – 80% of sperm deformities cases and peroxidative damage of spermatozoa, hence resulting in male infertility ***[4]***.

Recently, several plant metabolites with antioxidant properties such as polyphenols, flavonoids, vitamins, and carotenoids have been exploited for their actual or supposed beneficial effect against oxidative stress ***[5]***. Thus, nowadays, several researchers worldwide have focused on the beneficial effects of using various active dietary ingredients, such as phytochemicals in counteracting multiple diseases and ailments related to oxidative stress ***[6]***.

Ginger and black seed are powerful antioxidants with anti-inflammatory, anti-apoptotic, and antitoxic properties ***[7, 8]***, which has been proven effective in improving the fertility issues in the male by increasing semen quality, reducing total abnormalities, increasing male fertility rates, as well as affecting sperm parameters such as percentages of motility and sperm motion characteristics ***[9]***.

To the authors' knowledge, only very few papers have investigated the potential effects of ginger and black seed on improving the animal fertility rate, but none of them has tested whether it could counteract the adverse infertility issues induced by hyperuricemia. Therefore, the current study aimed to explore the beneficial effects of using these natural plant products alone and in combination form to counteract the adverse effect of oxonic acid-induced hyperuricemia by oral administration to male albino rats regarding testicular abnormalities and declined spermatogenesis.

Materials and Methods

A. Materials

All chemicals, including oxonic acid; 97% ($C_4H_3N_3O_4$), allopurinol, glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), kits were purchased from high-quality companies. While both ginger and black seed were obtained directly from a local market as both are very well-known natural plant materials among people in our community.

B. Animals

In this current study, 24 male albino rats aged 8 - 10 weeks and weighed 200 - 250 g were obtained from the animal house of the Department of Biology, College of Science and Education Science, Sulaimani University, Republic of Iraqi. Animals were acclimatized for a week before commencing the study under a 12-hour light/dark cycle and a temperature of 23 ± 2℃. The rats were also supplied with a standard pellet diet and water *adlibitum* until the end of the study.

C. Experimental design and animal treatment

The protocol was approved by the Research Ethics Committee of the Department of Biology, College of Science, University of Sulaimani in accordance with relevant international guidelines and regulations. Animals (30) were divided into 6 groups of 4 animals each, in which all groups were induced with hyperuricemia using intraperitoneal injections of oxonic acid (250 mg/kg body weight) for 1 week, except the first group that was used as a negative control group and received intraperitoneal injection of 0.9% saline solution without any further treatment. Starting from day 8 of the experiment, the 2nd group acted as hyperuricemia control rats and not received any treatment, while the 3rd, 4th, 5th, and 6th groups were treated daily with ginger extract (25 mg/kg body weight), black seed extract (50 mg/kg body weight), allopurinol (control positive) (100 mg/kg body

weight) and a mixture of ginger + black seed extracts (25 mg ginger extract + 50 mg black seed extract/kg body weight), respectively for exactly 2 consecutive weeks.

D. Animal sacrificing

On the last day of the experiment (22nd day), all groups fasted overnight, and blood samples were collected for hormone (testosterone, LH and FSH) determination and oxidative enzyme (GPX, SOD, and CAT) investigation. Rats were then sacrificed by deep anesthesia using a mixture of xylazine and ketamine, and the tests (seminiferous tubules) were gently separated for histopathological examination. The seminal supernatant plasma was separated and stored at -20°C until being assayed.

E. Statistical analysis

Obtained data analyses were analyzed using SPSS software (version 25.0), at a level of significance $P < 0.05$. Results were expressed as mean ± standard error (SE).

Results

A. Semen quality study

Semen quality in all animal groups was evaluated immediately after collection for sperm abnormality, including the broken head, broken tail, without a head, without tail, and without a hook. There were significant differences ($P < 0.05$) between hyperuricemia control with all other groups (Table 1).

Table 1: Shows semen quality in terms of abnormality for each group directly after collection.

Animal Group	Broken head	Broken tail	Without head	Without tail	Without hook
Hyperuricemia control	7 ± 0.2	10 ± 0.3	8 ± 0.5	9 ± 0.1	6.5 ± 0.45
Control negative	1 ± 0.3*	3 ± 0.12*	1 ± 0.55*	3 ± 0.1*	2 ± 0.4*
Ginger extract	3 ± 0.22*	1 ± 0.5*	2 ± 0.6*	1 ± 0.2*	3 ± 0.21*
Black seed extract	3 ± 0.11*	2 ± 0.3*	3 ± 0.45*	3 ± 0.4*	4 ± 0.54*
Alloperonill (Control positive)	1 ± 0.32*	1 ± 0.23*	3 ± 0.35*	2 ± 0.5*	2 ± 0.5*
Black seed + Ginger extracts	1 ± 0.45*	1 ± 0.45*	3 ± 0.55*	2 ± 0.7*	2 ± 0.32*

*: Significant difference ($P < 0.05$) between hyperuricemia control with all other groups using T-test.

B. Serum hormone levels (ng/ml)

Serum testosterone (Rat Testosterone ELISA kit, Fortrees Diagnostic Limited, UK®), LH, and FSH (ELISA kit by CLIA Elabscience®) levels were quantified in compliance with the manufacturer's instructions. There was a significant difference ($P < 0.05$) between hyperuricemia control and other groups for testosterone hormone. In contrast, for LH and FSH hormones, there was a significant difference ($P < 0.05$) only between hyperuricemia control and negative control, but no other animal groups (Table 2).

Table 2: Shows the level of sexual hormones in various animal groups.

Animal Group	Testosterone	LH	FSH
Hyperuricemia control	3.49 ± 0.15	0.37 ± 0.35	0.68 ± 0.22
Control negative	0.387 ± 0.5*	2.74 ± 0.5*	2.7 ± 0.15*
Ginger extract	0.39 ± 0.7*	0.54 ± 0.45	0.47 ± 0.6
Black seed extract	0.56 ± 0.11*	0.198 ± 0.4	0.43 ± 0.4
Alloperonill	0.98 ± 0.35*	0.48 ± 0.11	0.68 ± 0.23
Black Seed + Ginger extracts	0.47 ± 0.25*	0.43 ± 0.2	0.37 ± 0.25

*: Significant difference ($P < 0.05$) between hyperuricemia control with all other groups using T-test. LH: Luteinizing hormone, FSH: Follicular stimulating hormone.

D. Serum oxidative stress enzymes

Serum oxidative enzymes for all animal groups were determined using the commercially available kit for spectrophotometric analysis. It was found that there was a significant difference ($P < 0.05$) between hyperuricemia control with all other groups (Table 3).

Table 3: Shows the level of serum oxidative enzymes in various animal groups.

Animal Group	GPX	SOD	CAT
Hyperuricemia control	7.2 ± 0.5	102.5 ± 0.22	6.5 ± 0.3
Control negative	9.1 ± 0.1*	87.8 ± 0.5*	12.3 ± 0.4*
Ginger extract	8 ± 1.1*	95.2 ± 0.25*	8.1 ± 0.22*
Black seed extract	8.8 ± 0.3*	98.1 ± 0.5*	9.2 ± 0.11*
Alloperonill	9.4 ± 0.11*	90.2 ± 0.2*	10.9 ± 0.32*
Black Seed + Ginger extracts	8.8 ± 0.7*	94.6 ± 0.1*	6.8 ± 0.55*

*: Significant difference ($P < 0.05$) between hyperuricemia control with all other groups using T-test.
GPX: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase.

E. Histopathological investigation of testicular tissues

Testes samples were fixed in diluted formalin (10%) for 48 hours, processed and stained using hematoxylin and eosin (H & E) stain, and microscopically examined using a light microscope (Olympus, Japan) as described elsewhere ***[10]*** (Figure 1).

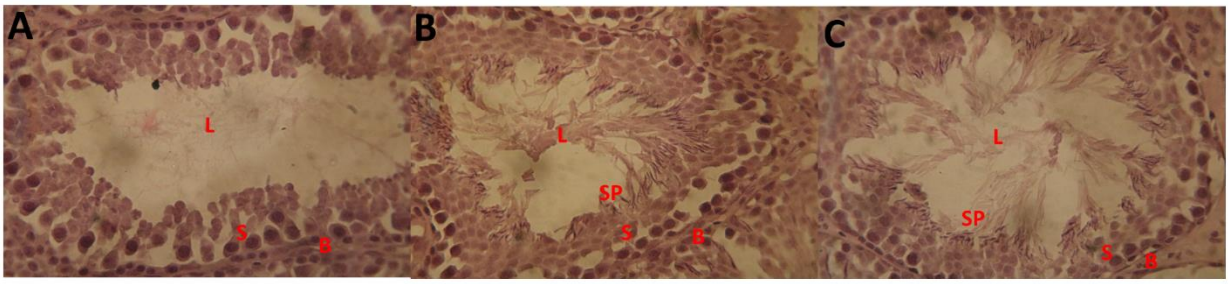

Figure 1: Showing effects of ginger and black seed extract on spermatogenesis in hyperuricemia albino rats after 2 weeks' treatment, H&E staining (400 X). (A) Hyperuricemia control group showing lumen of seminiferous tubule (L) contains only Sertoli cells (S) on basement membrane (B) and no spermatogonia, (B) Ginger and black seed treated group showing Sertoli cells (S) on basement membrane (B) of the lumen of seminiferous tubules (L) that contains spermatids (SP), and (C) allopurinol treated group appears to be in the more active state showing a higher number of spermatids compared to group B.

Discussion

Plants have long folklore of use in aiding human/animal fertility through improving spermatogenesis/ovulation, normalization of sexual hormones, and reducing oxidative stress ***[11]***. Thus, the aim of this study was to investigate the possible protective effects of ginger and black seed extracts against the development of oxonic acid-induced hyperuricemia in rats through measuring sexual hormones, serum enzymes, and testicular histopathological changes.

In this study, it was realized that the animals in the hyperuricemia group showed significant changes in their sperm morphology, such as broken head/tail, without head/tail, and without a hook. In contrast, these sperm abnormalities were not found in treated groups. This result confirms that natural medicinal plant products can improve spermatogenesis and protect sperm morphology/motility. Furthermore, this result is in agreement with the results of other studies which are done on the same topic but using different plant products ***[10 -12]***.

Regarding the effect of ginger/black seed extracts treatment on sexual hormones, it was found that only the level of testosterone was significantly improved and retained back to normal range compared to hyperuricemic rats, while LH and FSH were not normalized after ginger/black seed extracts treatment compared to hyperuricemic animals. This finding was in tune with other studies that reported oxonic acid induced a significant decline in serum testosterone, LH, and FSH, which in turn will be reflected on spermatogenesis as well as on the structural morphology of the seminiferous tubules ***[13 -15]***.

It has been reported that oxidative stress plays a crucial role in testicular damage. Consequently, in this study, it was found that ginger/black seed extracts treatment produced a significant decrease in the level of GPX, SOD, and CAT compared to hyperuricemic rats. The primary mechanism of oxonic acid involvement in oxidative damage and free radical generation is attributed to its ability to interact with cellular macromolecules that disrupt cell growth, mitotic activity, and differentiation. These findings agree with other studies using various plant products and active compounds ***[14, 16, 17]***.

Additionally, the histopathological effects of ginger/black seed extracts also were investigated in all animal groups. It was realized that in the mixture treatment group, the seminiferous tubules had a standard shape, the inner cells were regular and closely connected with the inner membrane, and the seminiferous cells were present in all stages and arranged in order. Numerous active spermatozoa were observed in the seminiferous tubules, almost similar to the positive control group. The spermatogenic cells in the testicular seminiferous tubules showed shrinkage, disordered in arrangement, decreased in the number of layers, and

unclear in their levels. Relatively inflated and loose cells and few sperm are also noted in the tube cavity. These findings are similar to other studies working on the effect of natural products in oxonic acid-induced hyperuricemia ***[18-20]***.

Conclusion

It is concluded that supplementation of ginger and black seed extracts to hyperuricemic rats for 14 days was successfully ameliorated the adverse effects of hyperuricemia through normalization of the parameters done on male reproductive tissues, hormones, and serum enzymes.

Acknowledgment

I appreciate the support and assistance from the staff of the Department of Biology, College of Science and Education Science, University of Sulaimani.

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