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Impact of Ginseng, *Panax ginseng*, and Sandalwood, *Santalum album*, against the Effect of Ibuprofen on Male Fertility of Rats

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Abstract | The use of ibuprofen as a chemotherapy drug has an adverse effect on male fertility. By altering hormonal profiles, ibuprofen causes a state of compensatory hypogonadism. Consequently, the purpose of this study was to assess the potential effects of ginseng or sandalwood, as a natural remedy, on the previously documented negative effects of ibuprofen on male fertility. For this study, twenty-four male were employed. Four groups of six rats each were used: the control group received no medication, while the other three groups received oral doses of ibuprofen (40 mg/kg/day), ginseng (500 mg/kg b.wt/day) with ibuprofen (40 mg/kg/day), and sandalwood oil (100 mg/kg b.wt/day) with ibuprofen (40 mg/kg/day) for a total of 14 days. Serum samples were taken one and two weeks post-dosage to measure levels of testosterone, luteinizing hormone and follicle stimulating hormone. Furthermore, samples from the testis, epididymis, and seminal vesicles were taken at the second week of treatment to perform a histopathological analysis, analyze the semen profile and measure the activities of the enzymes glutathione-s-transferase and nitric oxide in the testicles. Testicular GST and NO activities, as well as the level of testosterone and sperm profile, were all significantly reduced while taking ibuprofen. Significant changes were also brought about in the testicular, epididymal, and seminal vesicle histological architectures. Important components of male fertility, such as testosterone, LH, and FSH levels, sperm profile, and testicular GST and NO activities were significantly increased when ginseng and ibuprofen were combined. Ginseng supplementation improved the histological structures of the testis, epididymis, and seminal vesicles. The effects of sandalwood oil were favorable. To decrease the negative effects of ibuprofen on the sperm profile, testosterone level, and spermatogenesis of treated males, it is advisable to use ginseng in conjunction with ibuprofen.

Keywords: Ibuprofen, Ginger, Sandalwood oil, Male fertility.

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INTRODUCTION

Fertility is known as the capability of the male and female to produce live offspring by normal mating process. The male fertility is defined as the percentage of males that produced a successful pregnancy versus the total number of males that were cohabited. Spermatogenesis, the process by which the testis produces healthy, mature spermatozoa, is what determines male fertility (De Jonge *et al*., 2022).

The effects of drug therapy on male fertility are often not well evaluated for each drug. Certain medicines have the potential to impact male fertility through libido reduction, sexual dysfunction, hypothalamic-pituitary-gonadal axis disruption, and direct gonadal toxicity. On the other hand, while being taken, several small molecule drugs may positively guard against the genesis of germ cells and the preservation of fertility. Consequently, before pharmaceuticals are approved for therapeutic use, their effects on reproduction must be assessed (Peiting *et al*., 2023). Male endocrine disturbance has been proposed as a major contributing factor to the rise in male reproductive problems reported worldwide. In general, androgens which are mostly produced by testicular Leydig and Sertoli cells as well as other hormones are necessary for male reproduction and overall health (Kristiansen *et al*., 2017).

The well-known non-steroidal anti-inflammatory medicine (NSAID) ibuprofen ((RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid) is used extensively to treat pain, fever, and inflammation. Ibuprofen affects semen quality and changes hormonal profiles by selectively suppressing gene expression, which results in a condition of compensated hypogonadism. Ibuprofen may have an effect on semen quality through chelating zinc ions, decreasing the formation of nitric oxide, and reducing the synthesis of prostaglandins and testosterone (Saleem, 2019; 2021).

Worldwide, the use of herbal remedies to treat male infertility is growing. As a result, their therapeutic applications are more suited to enhancing male reproductive health (Sengupta, 2014). Male infertility can result from a variety of factors, including neurohormonal imbalances, abnormalities in the reproductive tissue, decreased semen quality and quantity, and issues with sexual behavior. The state of male reproductive health is rapidly declining worldwide, and current treatments for male infertility are costly, less widely available, require lengthy course of therapy, and have a number of adverse consequences. On the other hand, herbal remedies are better suited to provide more comprehensive methods of enhancing male reproductive health. A unique family of herbs known as aphrodisiacs exists in Ayurvedic pharmacology; they are said to nourish and stimulate the sexual tissues.

Aphrodisiacs are agents that used to arouse sexual desire and performance, when derived from herbs, have been shown to directly stimulate male sexual libido, support reproductive activities, restore healthy tissue functioning, and support neuroendocrine regulation—all of which are necessary for exhibiting the necessary sexual strength in a contented state of mind and body (Low and Tan, 2007).

Known as the "King of Herbs," ginseng has shown great promise as a means of enhancing overall health. Ginseng is used to improve sexual behavior and treat sexual dysfunction. It is also said to be an aphrodisiac. The primary pharmacologically active ingredients in ginseng, ginsenosides, are primarily responsible for these effects. It has been demonstrated that ginsenosides, an active ingredient in Panax ginseng, increase male libido, copulatory function, and sexual satisfaction (Leung and Wong, 2013).

Commercially speaking, sandalwood (*Santalum album*) is referred to as East Indian sandalwood. Sandalwood oil has been used for its therapeutic properties since ancient times (Soundararajan *et al*., 2017). As an aphrodisiac, sandalwood can boost libido, enhance sexual desire, and even help impotent men (Axe, 2023).

Thus, the purpose of this study was to assess how well ginger and sandalwood oil counteracted the effects of ibuprofen on male fertility in rats by looking at the following: testicular oxidative stress and antioxidant defense markers (Nitric oxide, glutathione S-transferase, and malondialdehyde), sperm picture (sperm cell concentration, percentage of live and dead sperm, and assessment of total sperm abnormalities) and histopathological examination of the testes, epididymis, and seminal vesicle.

MATERIALS AND METHODS

Drug: Ibuprofen (Brufen®)

The supplier of Ibuprofen (Brufen®) was Unipharma Company, located in Al Obour City, Cairo, Egypt. According to AL Mackey *et al*. (2016), ibuprofen is nearly insoluble in water but highly soluble in the majority of organic solvents, including ethanol (66.18g/100mL at 40°C for 90% EtOH), methanol, acetone, and dichloromethane.

Dosage: Ibuprofen is taken orally at a therapeutic dose of 40 mg/kg/day. (Jonah and others, 2014)
Molecular formula: $C_{13}H_{18}O_2$

Medicinal Plants

Panax ginseng: or ginseng, was acquired from Sigma Company in Giza, Egypt.
Dosage: Male rats were given the test product orally once a day at a therapeutic dose of 500 mg/kg/day by gavage (Park *et al*., 2013).
Chemical Formula: $C_{30}H_{52}O_2$

Sandalwood oil: The supplier of sandalwood oil was Sigma Company, located in Giza, Egypt.
The recommended dosage for sandalwood oil is 100 mg/kg/day for therapeutic purposes (Younis, 2020).

Experimental Animals

Twenty-four male Sprague-Dawley rats, weighing between

225 and 250 gm and 8 to 10 weeks old, in good clinical condition. They were bought from the Experimental Research Center, Faculty of Veterinary Medicine, Zagazig University, and during the experiment period, they were kept in the Pharmacology Department's laboratory at the Faculty of Veterinary Medicine, Mansoura University, under standard laboratory conditions (12 hours of light, 12 hours of darkness, and 24±3°C). They were kept in metal cages, fed a well-balanced diet devoid of any medication, and given unlimited water. Before the experiment, they spent two weeks being accommodated.

Rat Grouping

A total of twenty-four clinically sound male Sprague-Dawley rats, weighing between 225 and 250g, and aged between 8 and 10 weeks, were divided into four equal groups, each consisting of six rats, which is adequate to identify any changes between the rats after treatment.

Group 1: These rats were used as controls, receiving no treatment.
Group 2: ibuprofen 40mg/kg/day was given orally to the rats through a stomach tube over a period of 14 days.
Group 3: For 14 consecutive days, rats were given ginseng (500 mg/kg b.wt/day) and ibuprofen (40 mg/kg/day) orally through a stomach tube.
Group 4: ibuprofen (40 mg/kg/day) and sandalwood oil (100 mg/kg b.wt/day) were given orally to the rats via stomach tubes over a period of 14 days.

Blood Sampling

Per Donovan and Brown (2005), two heart punctures were used to obtain two blood samples from five rats in each group during the first and second weeks following therapy. In order to examine the blood picture, one blood sample was obtained in test tubes containing EDTA, and the second blood sample was taken in test tubes devoid of EDTA. The blood samples were allowed to clot at room temperature before being centrifuged for 15 minutes at 3000 r.p.m. to produce clear sera. The collected sera were kept at -20°C in a deep freezer until the serum total testosterone, FSH, and LH were tested.

Measurement of Serum Total Testosterone, FSH, and LH Levels

Using the Enzyme Linked Immunosorbent Assay (ELISA) method, the levels of total testosterone, follicle stimulating hormone, and luteinizing hormone in the serum of control and treated rats were determined in accordance with Tietz (1995), Fiman *et al.* (1975), and Howland (1972), respectively.

Testicular, Epididymal, and Seminal Vesicle Collection

At the conclusion of the first and second week following treatment, five rats from each group were rendered unconscious with chloroform in order to collect the testes, epididymis, and seminal vesicles. These specimens were then grossly inspected and weighed in accordance with the procedure outlined by Crawford (2008).

Examination of Epididymal Sperm (Sperm Picture)

Epididymal sperm content were collected by diffusion method, as mentioned by Seed *et al.* (1996) for examination of epididymal sperm abnormalities according to Bearden and Fuquay (1980), and Ramnik (2006), as well as examination of epididymal sperm cell count and percentage of live sperm.

Test of Antioxidant Defense Markers

Testicular tissue homogenate was made using Fernandez-Botran *et al.* (2002) methodology. Following the first and second weeks of medication, the removed testis was homogenized in 9 milliliters of cold-iced buffer (phosphate buffered saline solution, pH 7.4) per gram of tissue after being cleaned with cold 0.85% NaCl. The homogenate was put into a centrifuge once it had finished homogenizing. The supernatant was collected carefully and used for assessment of testicular glutathione s- transferase (GST) activity (Habig *et al.*, (1974), testicular nitric oxide (NO) activity (Montgomery and Dymock, 1961).

Histopathological Technique

Five rats per group were used to obtain specimens from the testes, epididymis, and seminal vesicle glands at the two-week mark following ibuprofen, ginseng, and sandalwood oil administration. For the histological analysis, every specimen was preserved in 10% neutral buffered formalin fixation. Following appropriate fixation, the tissue samples were cleaned in xylene, dehydrated using ethyl alcohols in increasing concentrations, and then rinsed under running water. For two hours, the tissues were immersed in paraffin wax. After the tissue was finally embedded in hard paraffin, slices measuring five µ in thickness were cut out of the blocks and stained with hematoxylin and eosin (H&E) (Bancroft and Stevens, 1990).

Statistical analysis (Snedecor and Cochran, 1981)

The null hypothesis (HO) for this study assumed that "there is no effect or difference in male fertility outcomes between the rats treated with ginseng, sandalwood, or ibuprofen. "To test this hypothesis' One- way analysis of variance (ANOVA) was employed to assess the variance of the results from the five rats in each group. Subsequently, Tukey's Honestly Significant Difference (HSD) test was utilized to compare the means of the treatments using SPSS software (version 20; Inc., Chicago, IL, Unite States). Statistical significance

was values deemed significant if the p-value was equal to or less than 0.05. The presence of different superscript letters above each column in the charts indicates a significant difference.

RESULTS AND DISCUSSION

Four groups of six adult male rats each were used in this investigation; however, samples were obtained from five rats in each group to look into the effects of ibuprofen on several male reproductive indices, either by itself or in conjunction with ginseng or sandalwood oil. The variance of each group's results was evaluated using one-way analysis of variance (ANOVA).

Impact of Ibuprofen, Ginseng, and Sandalwood on the Appearance of Sperm

Impact on the Concentration of Epididymal Sperm Cells

The effects of Ibuprofen, Ginseng with Ibuprofen, and Sandalwood oil with Ibuprofen on the concentration of epididymal sperm cells in male rats were displayed in Figure 1. In male rats, the epididymal sperm count was considerably lower in the first and second week post-treatment when ibuprofen 40 mg/kg was administered orally as opposed to the control group. In the meantime, when ginseng and ibuprofen were administered together, the epididymal sperm count significantly increased (P>0.05) in the first and second weeks after treatment, respectively, as compared to the ibuprofen-treated group. In a similar vein, the drop in epididymal sperm count that ibuprofen alone was able to achieve was enhanced when sandalwood oil was combined with it.

Impact on the Percentage of Live Sperms

The findings shown in Figure (2) demonstrated the impact of Ibuprofen, Ginseng with Ibuprofen, and Sandalwood oil with Ibuprofen on the percentage (%) of live sperms in treated male rats following their administration. In comparison to the control group (93.36 ± 0.68 and 92.89 ± 1.06), the recorded data showed a substantial (P>0.05) drop in the percentage of viable sperms in the ibuprofen-treated group, which were 58.80 ± 1.56 and 62.12 ± 1.55 at the first and second week, respectively, post treatment. However, compared to the ibuprofen-treated group, there was a substantial increase (P>0.05) in the percentage of viable sperms in male rats given ginseng together with ibuprofen at the one-week (84.17 ± 1.22) and two-week (78.83 ± 2.07) post-treatment. The usage of sandalwood oil in conjunction with ibuprofen at the first (76.09 ± 1.92) and second (73.71 ± 1.68) weeks after therapy likewise showed an ameliorated effect, according to the data.

Impact on the Percentage of Total Sperm Abnormalities

Figures 3, illustrate the effects of Ibuprofen, Ginseng with Ibuprofen, and Sandalwood oil with Ibuprofen on the percentage of total sperm abnormalities in mature, healthy Sprague Dawley rats. When male rats were given ibuprofen, the percentage of total sperm abnormalities increased significantly (P>0.05) from 4.57 ± 0.10 and 4.57 ± 0.12 in the control group to 24.86 ± 0.42 and 24.85 ± 0.37 at the first and second week, respectively, following treatment. Rats treated with ginseng in addition to ibuprofen had a significantly higher % of total sperm abnormalities (P>0.05) than rats treated with ibuprofen alone. Following ginseng and ibuprofen treatment, the percentage

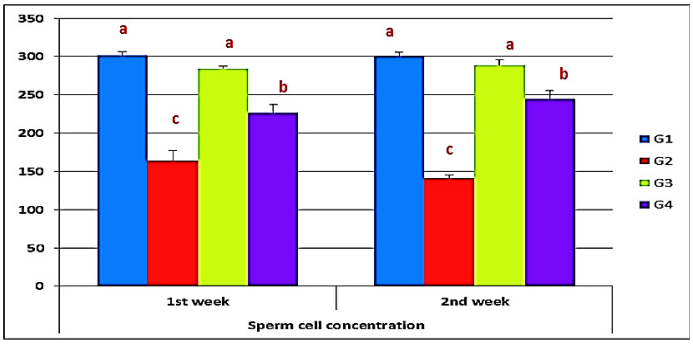

Figure 1: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on epididymal sperm cell concentration of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05)

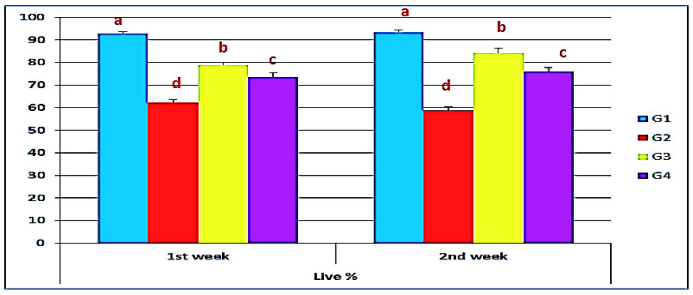

Figure 2: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on live sperms percent (%) of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05)

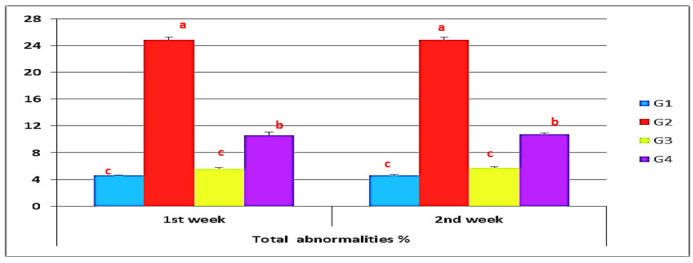

Figure 3: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on percent of total sperm abnormalities of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05).

of total sperm abnormalities was 5.73 ± 0.20 and 5.58 ± 0.16, respectively. Furthermore, at the first and second weeks after treatment, the percentage of total sperm abnormalities in the sandalwood oil and ibuprofen treated group was considerably greater (10.70 ± 0.49 and 10.60 ± 0.24, respectively) than in the ibuprofen treated group. Spermatogenesis and fertilization may be impacted by the previously established negative effects of ibuprofen on sperm cell concentration, total sperm abnormalities, and living sperms. In the meanwhile, ginseng has been found to have positive effects on spermatogenesis, motility, and count of sperm.

Impact on the Sperm Profile

Figures 4, 5, 6, and 7 show the effects of ibuprofen administered orally, ginseng with ibuprofen, and sandalwood oil with ibuprofen on the sperm profile of treated male rats. Ibuprofen lowered the number of normal sperm, changed the shape of the sperm tail, caused the sperm head to become detached, reduced the number of motile sperm, and decreased sperm production (low concentration). The obtained results demonstrated that the addition of either ginseng with ibuprofen (which demonstrated normal sperm morphology with high concentration) or sandalwood oil with ibuprofen (which demonstrated moderate concentration and abnormalities) alleviated the effects of ibuprofen on the percent of sperm mass motility, sperm abnormalities, and sperm live/dead ratio. According to Bo Yun (2018), a number of ginsenosides—the primary components of ginseng—have demonstrated augmenting effects on sperm motility and count as well as spermatogenesis by raising testicular cyclic adenosine monophosphate in male rats and glial cell-derived neurotrophic factor expression in Sertoli cells.

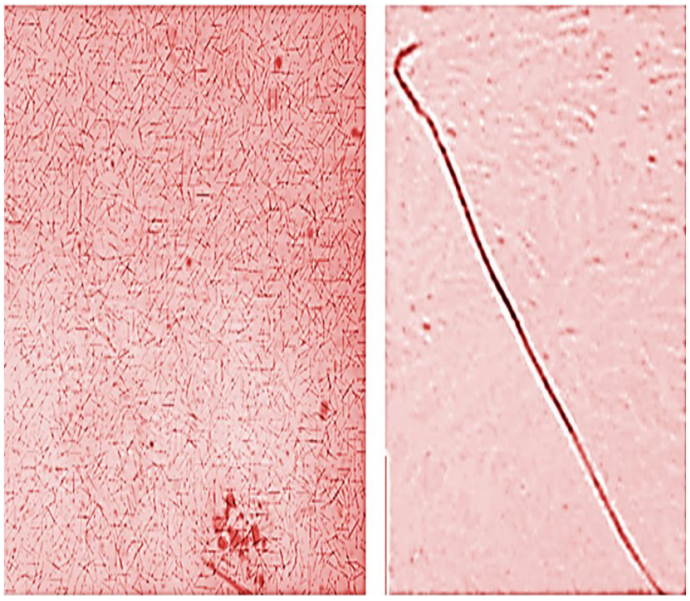

Figure 4: Control group showing normal sperms with proper concentration. X10 and X40.

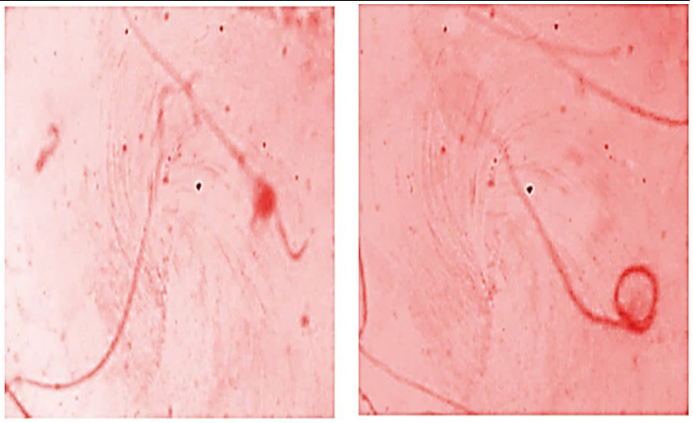

Figure 5: Ibuprofen treated group showing coiled tail and dethatched head abnormalities. X40.

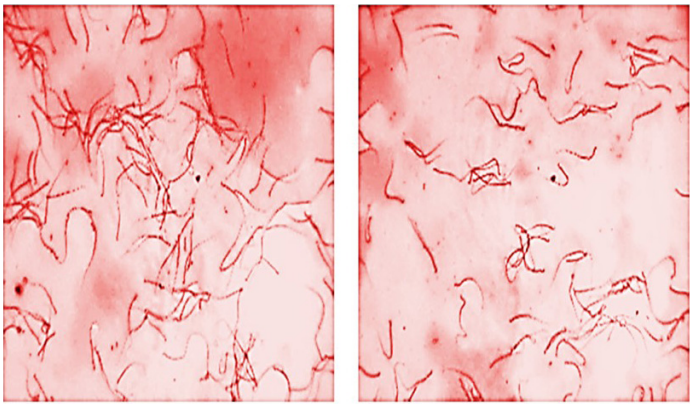

Figure 6: Ginseng with Ibuprofen treated group showing normal sperms with high concentraion. X10.

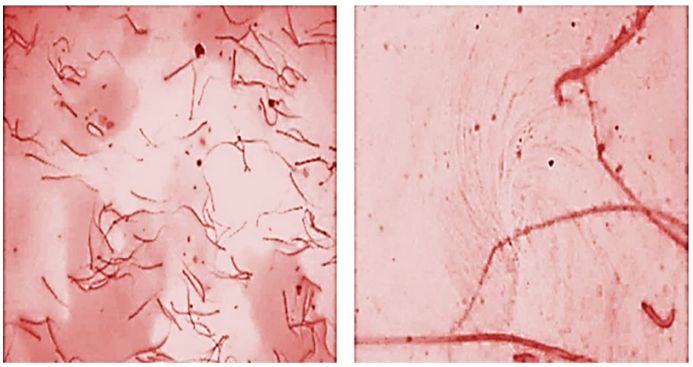

Figure 7: Sandalwood oil with Ibuprofen treated group showing some sperms abnormalities. (X10 and X40)

Impact of Ibuprofen, Ginseng, and Sandalwood on Total Serum Testosterone Hormone Level.

The data presented in Figure (8) demonstrated the impact of oral administration of ibuprofen (40 mg/kg), ginseng (500 mg/kg), and sandalwood (100 mg/kg) on the total serum testosterone hormone level in adult male rats in good health. In comparison to the control group (4.23 ± 0.23 and 4.01 ± 0.19, respectively), the obtained data showed a substantial (P>0.05) reduction in total serum testosterone hormone levels in the ibuprofen treated groups at the 1st (2.04 ± 0.15) and 2nd (1.97 ± 0.26) week after dose. The

decrease brought on by ibuprofen alone was reversed by the addition of either sandalwood oil or ginseng. In the first and second weeks following therapy, the results were 7.11 ± 0.39 and 6.45 ± 0.39 (ginseng with ibuprofen) and 4.94 ± 0.28 & 4.75 ± 0.27 (sandalwood oil with ibuprofen).

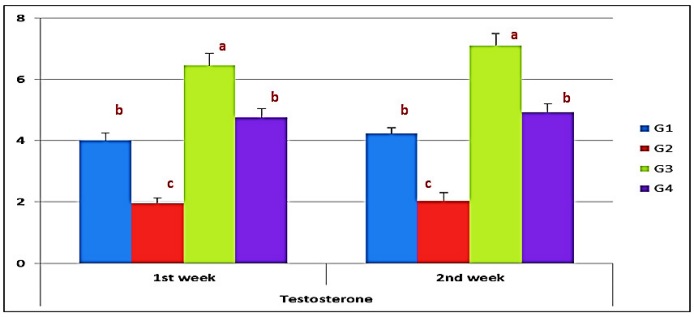

Figure 8: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on total serum testosterone level of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05).

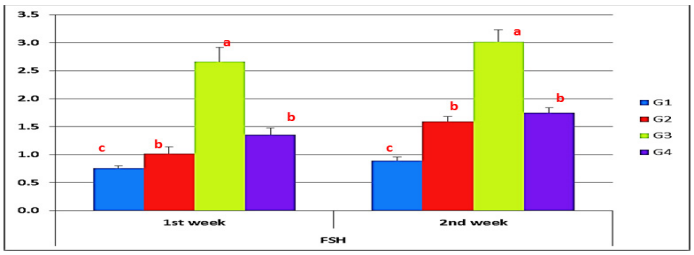

Figure 9: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on total serum follicle stimulating hormone level of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05).

Impact of Ibuprofen, Ginseng, and Sandalwood oil on Serum Follicle Stimulating Hormone Level

Figure 9, depicted the effects of oral ibuprofen, ginseng with ibuprofen, and sandalwood with ibuprofen on serum follicle stimulating hormone level in mature male rats in a healthy treatment group. The results showed that the group treated with ibuprofen had significantly higher levels of serum follicle stimulating hormone (P>0.05) throughout the first and second week after treatment as compared to the normal control group (0.90 ± 0.05 and 0.76 ± 0.06). Ibuprofen caused an increase that was 1.59 ± 0.07 and 1.02 ± 0.17, in that order. Male rats receiving treatment saw an improvement in serum levels of follicle stimulating hormone during the first and second weeks after ginseng or sandalwood oil was added along with ibuprofen. When ginseng and ibuprofen were combined, the serum levels of follicle stimulating hormone were 3.02 ± 0.26 and 2.66 ± 0.21, respectively, however when sandalwood oil and ibuprofen were combined, the levels were 1.75 ± 0.12 and 1.36 ± 0.09.

Impact of Ibuprofen, Ginseng, and Sandalwood oil on The Level Of Total Serum Luteinizing Hormone

The data are displayed in Figure 10, which also shows the effects of oral ibuprofen, ginseng with ibuprofen, and sandalwood with ibuprofen. The results showed that, at the first and second week after medication, all treated rats had significantly higher levels of total serum luteinizing hormone as compared to the normal control group. The group receiving ginseng and ibuprofen had the greatest levels of serum testosterone hormone (5.42 ± 0.32 and 4.46 ± 0.31 after the first and second week post treatment, respectively), while the control group's levels were 1.78 ± 0.09 and 1.79 ± 0.16.

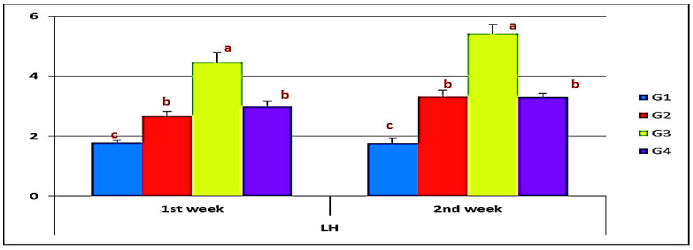

Figure 10: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on total serum luteinizing hormone level of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05).

Impact of Ibuprofen, Ginseng, and Sandalwood oil on Indicators of Antioxidant Defense in the Testicles

Glutathione-s-transferase (GST) activity: Figure (11) shows the effects of ibuprofen (40 mg/kg), ginseng (500 mg/kg), and sandalwood (100 mg/kg) on male rats' testicular glutathione-s-transferase activity after they were administered. Testicular GST activity was shown to be significantly lower (P > 0.05) in the ibuprofen-treated group at the one- and two-week mark after treatment (0.87 ± 0.07 and 0.98 ± 0.03, respectively) than in the control group (2.09 ± 0.29 and 2.08 ± 0.16, respectively). Table 10 demonstrates that the testicular GST levels were considerably (P > 0.05) higher in the ginseng with ibuprofen group (4.00 ± 0.28 and 3.65 ± 0.28, respectively) than in the control group and sandalwood oil and ibuprofen-treated group (2.79 ± 0.22 and 2.43 ± 0.09, respectively).

Testicular nitric oxide (NO) activity: Figure (12) displays the findings that were collected on the effects of ibuprofen (40 mg/kg), ginseng (500 mg/kg), and sandalwood (100 mg/kg) on the testicular nitric oxide activity of male rats that were treated. Testicular nitric oxide activity was shown to be lower in the group that received ibuprofen treatment. Testicular nitric oxide activity levels were 9.55 ± 0.25 and 8.99 ± 0.36 during the first and

second weeks after treatment, respectively, in comparison to the normal control group (12.33 ± 0.93 and 12.57 ± 0.32). Additionally, the testicular nitric oxide levels of rats administered ginseng with ibuprofen during the first and second weeks after treatment showed a substantial (P>0.05) increase (40.00 ± 1.19 and 38.09 ± 1.02) when compared with the other groups. Sandalwood oil added to ibuprofen reduced its effect on male treated rats' testicular nitric oxide levels, which were measured at 20.74 ± 0.27 and 20.79 ± 0.45 during the first and second weeks of therapy.

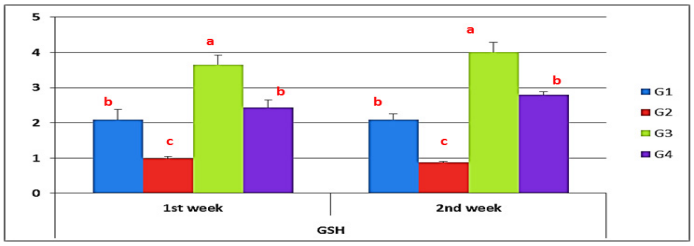

Figure 11: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on testicular glutathione-s-transferase activity of male rats. *Column with different letters indicate statistically significant differences (P≤0.05).

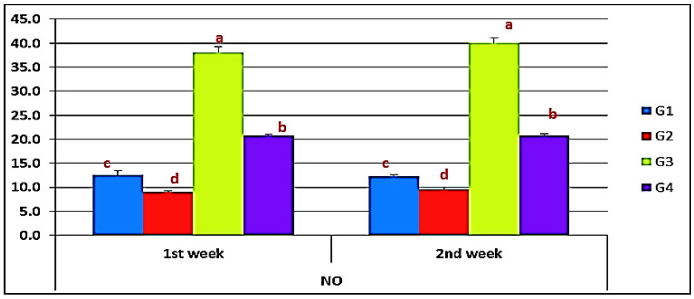

Figure 12: Effect of ibuprofen (G2), ginseng with ibuprofen (G3) and sandalwood with ibuprofen (G4) on testicular nitric oxide activityl of male rats.
*Column with different letters indicate statistically significant differences (P≤0.05).

Histological results: Figures 13, 14 and 15, show the histological effects of ibuprofen (40 mg/kg), ginseng (500 mg/kg), and sandalwood (100 mg/kg) on the testes, epididymis, and seminal vesicle of treatment groups after their administration.

Everywhere, reproduction is regarded as a fundamental component of production. Significant financial losses in animal production result from any decline in male fertility. The primary objective of animal farming operations is to maintain a high reproductive rate. Numerous factors including medicine, diet, hormone imbalances, certain illnesses, and genetics influence male fertility (Yimer and Rosnina, 2014).

Ibuprofen is a derivative of propionic acid. It is the NSAID that is prescribed the most frequently. It is a non-selective inhibitor of both COX-1 and COX-2, or cyclo-oxygenase-1 and cyclo-oxygenase-2. It is used to treat rheumatoid arthritis, pain, inflammation, and fever. Ibuprofen lowers testosterone levels in mature males and has a deleterious influence on reproduction (Barbosa *et al.*,2020).

Ginseng has been considered a tonic and used to improve male libido, copulatory performance and sexual satisfaction (Leung and Wong, 2013).

Sandalwood Oil (EISO) is the volatile essential oil usually obtained from the heartwood of Santalum album L. trees. It has been utilized for thousands of years in traditional folk medicine to treat a variety of human illnesses due to its many therapeutic benefits. As an aphrodisiac, sandalwood can boost libido, enhance sexual desire, and even help men experiencing impotence (Axe, 2023).

Impact of ibuprofen, ginseng and sandalwood oil on the concentration of epididymal sperm cells, the percentage of living sperms, and the percentage of total sperm abnormalities

When compared to the control group, the oral administration of 40 mg/kg of ibuprofen greatly decreased the number of epididymal sperm, the percentage of living sperm, and the percentage of total sperm abnormalities in male rats receiving therapy during the first and second weeks after treatment. In the meantime, giving ginseng along with ibuprofen caused a substantial increase (P>0.05) in the number of epididymal sperm, the percentage of living sperm, and the percentage of total sperm abnormalities in the group receiving ibuprofen treatment. In a similar vein, taking sandalwood oil in addition to ibuprofen enhanced the reduction in the specified parameters that ibuprofen by itself produced.

Our observed decrease in nitric oxide and testosterone levels may be a reflection of the detrimental effect ibuprofen has on the quality of semen. The findings of Saleem (2019), who suggested that ibuprofen may have changed the quality of semen by lowering the synthesis of nitric oxide and testosterone, lend weight to this view. Ibuprofen can also lower treated mouse sperm parameters and sperm chromatin/DNA integrity. (Fatemeh *et al.*, 2015). Additionally, Ibuprofen impairs sperm function, particularly motility, which compromises the ability of sperm to fertilize (Banihani, 2018). Moreover, Melissa *et al.* (2024) reported that the use of analgesics like ibuprofen can affect the synthesis of testosterone, lowering its concentration, reducing the number of germ cells, and causing oxidative stress, which in turn causes spermatocyte apoptosis in men and male infertility.

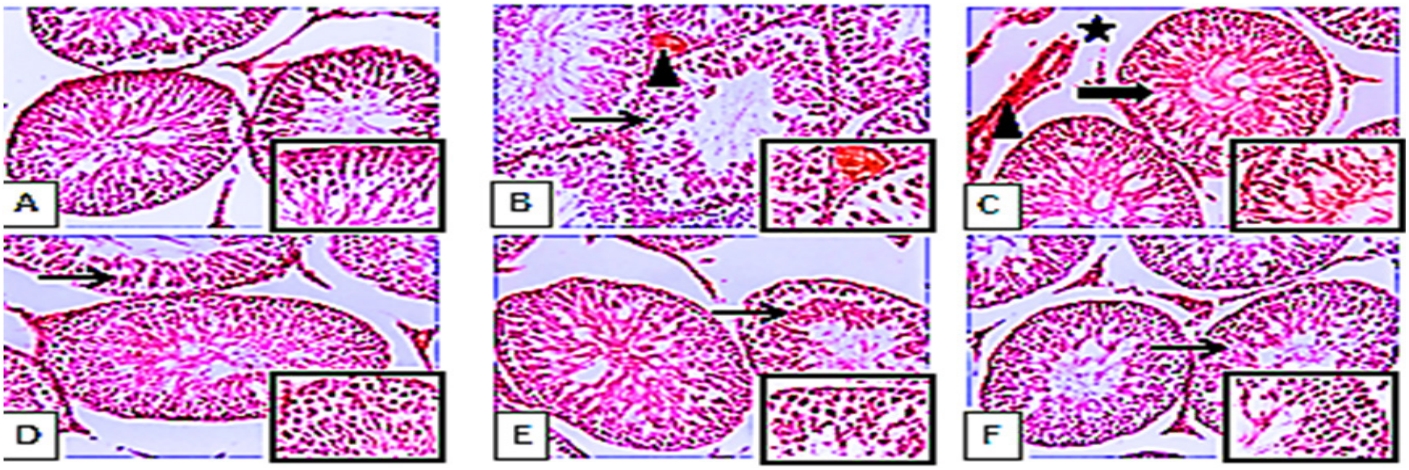

Figure 13: Representative photomicrograph of testicular sections from control and treated groups. A). Control group showing normal seminiferous tubular architecture. B) Ibuprofen treated group showing diffuse degenerative seminiferous tubules with loss of germ cells and mild interstitial congestion, inset decrease spermatocytes with partial spermatocyte necrosis, disorganized spermatid with distorted intraluminal spermatozoa. C) Ibuprofen treated group showing interstitial congestion and edema separated tubular vacuolation, inset, vacuolated seminiferous tubular germ epithelium with necrotic spermatids. D) Ginseng with ibuprofen treated group showing restoration of most tubular architecture with few tubular disorganization showed decrease the germ cell lining, inset, high power on the preserved semineferous tubules with normal germ cell lining. E) Ginseng with ibuprofen treated group showing moderate irregular semineferous tubular arrangement with necrotic, clumped spermatids, see inset image. F) Sandalwood with ibuprofen treated group showing restoration of most semineferous tubular architecture with occasional spermatocytes necrosis, see inset image.
Thin arrow = necrotic semineferous tubules with loss of germ epithelial lining, thick arrow = vacuolation, star = edema, arrowhead = interstitial congestion, Image magnification = 100x, inset= 400x.

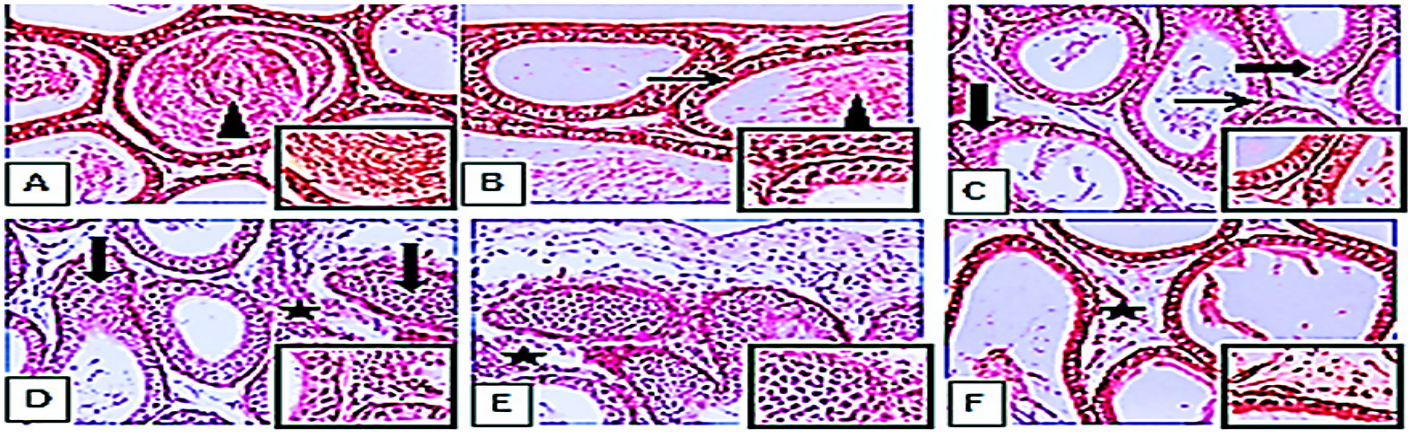

Figure 14: Representative photomicrograph of epididymis from control and treated groups. A) Control group showing normal architecture of epididymal duct with dense intraluminal spermatozoa, inset, normal architecture of ciliated pseudostratified epithelial lining with dense intraluminal spermatozoa. B) Ibuprofen treated group showing diffuse ductal epithelial degeneration with mild intraluminal spermatozoa, inset, epithelial vacuolation with occasional necrotic cells and duplication of most epithelial nucleus. C) Ibuprofen treated group showing mild tubular vacuolation with partial proliferation of ductal epithelial lining, inset, tubular vacuolation with occasional necrotic cell and inflammation. D,E) Ginseng with ibuprofen treated group showing partial to complete ductal proliferation lined either with 2-3 layers of cells or completely filled ductal lumen, mild focal to multifocal periductal inflammation, insets, mild interstitial aggregations of lymphoplasmacytic cells, macrophages and few neutrophils surrounded a highly proliferated ductal epithelial cells replacing the ductal lumen. F) Sandalwood with ibuprofen treated group showing restoration of ductal epithelial architecture with mild interstitial edema admixed with few inflammatory cells, inset; interstitial edema admixed with few lymphocytes and neutrophils.
Thin arrow = ductal vacuolation, thick arrow = ductal proliferation, star = edema with inflammation, arrowhead = intraluminal spermatozoa, Image magnification = 100x, inset = 400x.

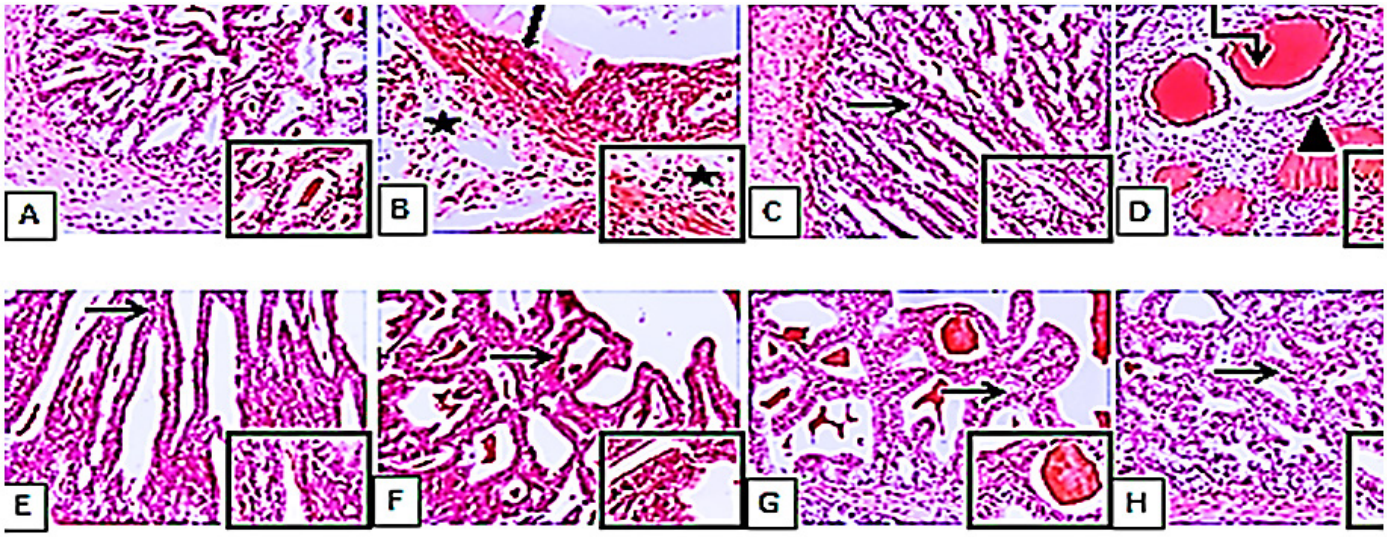

Figure 15: Representative photomicrograph of seminal vesicles from control and treated groups. A) Control group showing normal architecture of seminal vesicles with pseudostratified epithelial lining and normal muscularis layer, see inset image. B) Ibuprofen treated group showing diffuse severe edema admixed with mild hemorrhage and scattered inflammatory cells widely compressed and separated the fused and necrotic seminal vesicle mucosa and muscularis layers, inset, edema admixed with hemorrhage and lymphoplasmacytic cells separated the muscularis layer. C) Ibuprofen treated group showing adenomatous hyperplasia with intraluminal papillary projections, inset the proliferated acinar epithelium showed duplication of the nuclei. D) Ibuprofen treated group showing cystic fibrosis with cystic dilation of acinar lined with either attenuated flattened epithelium or 2-3 cell layers and surrounded with dense fibrosis, see inset image. E, F) Ginseng with ibuprofen treated group showing proliferated acinar epithelium with intraluminal papillary projection. G, H) Sandalwood with ibuprofen treated group showing mild acinar epithelial hyperplasia.
Thin arrow = proliferated acinar, arrowhead = fibrosis, twisted arrow = cystic dilation, thick arrow = fused and necrotic acinar epithelium, star = edema with inflammation, Image magnification = 100x, inset= 400x

ssessment of sperm morphometric measures, including total sperm count, total motility, and progressive motility, revealed that the ginseng-treated group outperformed the sandalwood-treated group in terms of improving the aforementioned metrics. These effects are consistent with the findings of numerous authors (Dahlberg, 1988; Hwang *et al*., 2004; Shelbaya, 2016; Sanad *et al*., 2021; Mhaibes *et al*., 2023) who reported that ginseng elicited a significant decrease in dead and abnormal sperm of treated males as well as a significant increase in total sperm counts, alive sperms, and individual sperm motility. Moreover, Bo Yun (2018) noted that via raising testicular cyclic adenosine monophosphate in male rats and glial cell-derived neurotrophic factor expression in Sertoli cells, different ginsenosides have demonstrated boosting effects on sperm count, motility, and spermatogenesis.

Impact of ibuprofen, ginseng, and sandalwood oil on total serum testosterone hormone, FSH, and LH levels

Hormones play a key role in all physiological process of any living being. Testosterone, LH, and FSH are the three hormones that most affect male reproductive. Changes in these hormone levels have the potential to profoundly alter the physiology of reproduction.

When compared to the control group, the ibuprofen treated groups' total serum testosterone hormone levels decreased significantly (P>0.05) in the first and second weeks after dosage. Although it has been seen that ibuprofen reduces testosterone synthesis, the precise mechanism by which it does so remains unclear. Research has shown that ibuprofen can reduce the production of testosterone by inhibiting the suppression of transcription in Leydig cells (Kristensen *et al*., 2018). The decrease brought on by ibuprofen alone was reversed by the addition of either ginseng or sandalwood oil. At the first and second week after dosing, the recorded data showed a significant increase in the levels of follicular stimulating hormone and total serum luteinizing hormone in all treated rats as compared to the normal control group.

Ibuprofen's effects are consistent with those of Ben Maamar *et al*. (2017), who observed a decrease in the testosterone/LH ratio, mainly due to the drug's elevation in LH levels. The findings showed that the addition of sandalwood or ginseng oil to ibuprofen resulted in a statistically significant rise in the treated groups' levels of FSH, LH, and testosterone. Our findings are consistent with those of several authors. According to Linjawi (2015), male patients treated with ginseng had higher levels of testosterone, FSH, and LH. Similar findings were made by Shelbaya (2016),

Sanad *et al*. (2021), and Muhammad *et al*. (2024) who discovered that ginseng administration significantly raised testosterone, FSH, and LH levels. Herbs can improve male reproductive functions, such as the development of secondary male sexual organs, exaggerate pubertal changes, and boost overall male fertility, by controlling the sex hormones (testosterone, LH, FSH, and interstitial hormones) (Dohle *et al*.,2003).

Impact of ibuprofen, ginseng and sandalwood oil on testicular glutathione-s-transferase (GST) activity and Testicular nitric oxide (NO) activity

The study examined the effects of 40 mg/kg of ibuprofen, 500 mg/kg of ginseng, and 100 mg/kg of sandalwood on testicular glutathione-s-transferase activity and testicular nitric oxide (NO), in male rats following their administration.

In the first and second weeks following therapy, there was a substantial ($P > 0.05$) drop in testicular GST and NO activities in the ibuprofen-treated group when compared to the control group. However, when male rats were given ibuprofen along with ginseng or sandalwood oil, the decrease in testicular GSH and NO activities caused by ibuprofen alone was positively corrected. As a well-known vasodilator, nitric oxide (NO) can improve blood flow to the penis, causing a penile erection, as well as to other male reproductive organs, enabling improved hormonal accessibility to support healthy reproductive functioning (Achike and Kwan, 2003).

Similar to the findings of aspirin (a nonsteroidal anti-inflammatory drug), which caused a significant decrease in the level of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione-s-transferase (GST), and catalase activities (Nair *et al*., 2006), the recorded results regarding the declined activities of GST and NO in rats given ibuprofen.

In contrast to the ibuprofen group, the ginseng-treated group showed an increase in the activity of the antioxidant enzymes GSH and NO in the testicles. These results are consistent with those of Kim and Park (2003) and Parker *et al*. (2010), who found that treated males exhibited increased activity of the testicular total antioxidants SOD, malondialdehyde, and (GSH) antioxidant enzymes.

In comparison to the ibuprofen group, sandalwood oil additionally enhanced the activities of testicular GST and NO. Glutathione S-transferase (GST) activity was observed to increase in adult male albino mice upon oral treatment of sandalwood oil, as demonstrated by Banerjee (1993). Furthermore, Hegde *et al*. (2014) found that the group treated with sandalwood oil had higher levels of glutathione, catalase, and superoxide dismutase.

Histopathological Observations

Impact on testis, epididymis, and seminal vesicles: The testicular, epididymal, and seminal vesicle sections from the control and treated groups were photomicrographed. The results showed that the control group had normal architecture of the pseudostratified epithelial lining, normal architecture of the seminal vesicles with pseudo stratified epithelial lining, and normal muscularis layer. The epididymal duct and seminal vesicle architecture were all normal (see inset image).

Disorganized spermatid with distorted intraluminal spermatozoa and interstitial congestion and edema separated tubular vacuolation, inset, and vacuolated seminiferous tubular germ epitheliums with necrotic spermatids were observed in the group treated with ibuprofen. Diffuse degenerative seminiferous tubules with loss of germ cells and mild interstitial congestion were also observed in this group. Ibuprofen caused broad ductal epithelial degradation, inset, epithelial vacuolation with sporadic necrotic cells, and duplication of the majority of epithelial nuclei on the epididymis. It also caused minor intraluminal spermatozoa. Inset: edema mixed with hemorrhage and lymphoplasmacytic cells separated the muscularis layer; diffuse severe edema admixed with mild hemorrhage and scattered inflammatory cells widely compressed and separated the fused and necrotic seminal vesicle mucosa and muscularis layers. A similar result was observed by Bilal (2014), who reported that aspirin caused a considerable drop in the diameter of seminiferous tubules, which was linked to an increase in the gaps between them, a decrease in the thickness of seminiferous tubules, and a decrease in the diameter of the epididymal tubules.

The majority of the testis' tubular architecture was restored in the ginseng and ibuprofen-treated group, with minimal tubular disarray and a moderately irregular semineferous tubular arrangement with necrotic, clumped spermatids (see inset image). The epididymis displayed mild focal to multifocal periductal inflammation, insets, mild interstitial aggregations of lymphoplasmacytic cells, macrophages, and a few neutrophils surrounding a highly proliferating ductal epithelial cells that replaced the ductal lumen. Partial to complete ductal proliferation was lined with either two to three layers of cells or completely filled ductal lumen. The ginseng and ibuprofen-treated group displayed proliferating acinar epithelium with intraluminal papillary projection on seminal vesicles. Previous histological research on rats treated with ginseng revealed increased thickness of the germinal layer, the basement membrane of the seminiferous tubule, and the primary and secondary spermatogenesis (Mandaki *et al*., 1998). Additionally, taking ginseng

regularly offers strong defense against injury to the testicles (Kim, 2007). Furthermore, ginseng supplementation was reported by Eskandari *et al*. (2016) to decrease apoptosis in testicular tissue.

The majority of the seminiferous tubular architecture of the testis was restored in the sandalwood with ibuprofen-treated group, while there was sporadic spermatocyte necrosis (see inset image). In a similar vein, the group treated with sandalwood and ibuprofen demonstrated the restoration of ductal epithelial architecture along with a minor form of interstitial edema and a low number of lymphocytes, neutrophils, and inflammatory cells. In the seminal vesicle, the sandalwood group treated with ibuprofen displayed modest acinar epithelial hyperplasia. These results are consistent with testicular and epididymal index weight and reproductive hormone (FSH, LH, and TSH) levels seen in this treated group.

CONCLUSIONS AND RECOMMENDATIONS

According to the findings, using ginseng or sandalwood oil along with ibuprofen improved the evaluated male fertility markers in a promising way. Because there have been few research using consistent methodology, it is still unknown exactly how ginseng affects male fertility. Further research is necessary to fully understand the association between sandalwood oil and male fertility. The effect of ibuprofen on male fertility is still unclear because there aren't enough studies on the subject and different approaches have been used. In order to fully comprehend the effects of ibuprofen on semen profile and, consequently, on the ability of fertilization, adequate in vitro and in vivo study is essential. When using non-steroidal anti-inflammatory drugs (NSAIDs), particularly ibuprofen, over an extended period of time, caution should be exercised.

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NOVELTY STATEMENT

This study compiles the protective effects of ginseng on male reproductive function, and also focuses on its role that may represent novel therapeutic strategies for the treatment of male reproductive diseases or disorders.

AUTHOR'S CONTRIBUTION

Magdy Amer conceived of the presented idea. All authores developed the theory and performed the computations. Shaimaa Hamam and Nani Nasr El-Deen verified the analytical methods. Magdy Amer supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors affirm that they do not have any competing financial interests or personal relationships that may have influenced the findings presented in this paper.

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