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Effects of the alcoholic extract of ginseng roots and carob fruits in comparison with vitamin E in improving the efficiency of the male reproductive system of albino rabbits

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ABSTRACT

Scientists have been interested in human reproduction for a long time, since on the one hand, it is related to human life, and on the other hand, it ensures the transmission of genetic traits from parents to children, as low fertility is considered one of the social problems leading to psychological disorders in couples who are unable to have children or what is called infertile. Therefore, this study aimed to find out the protective effects of the alcoholic extract of ginseng roots and carob fruits on the level of oxidative balance in semen and improving reproductive efficiency by examining the histological parameters of testes in albino male rabbits, and comparing these effects with the effects of vitamin E known for its antioxidant properties. This study included using forty adult albino male rabbits in eight groups, with five rabbits in each group and convergent weights. All groups were fed a standard diet throughout the five-month experimental period. The treatments were as follows: The first group (T_1) were gavaged by distilled water, the second (T_2) by 200 mg kg^{-1} alcoholic extract of ginseng roots, the third (T_3) by 400 mg kg^{-1} alcoholic extract of ginseng roots , the fourth (T_4) by 200 mg kg^{-1} alcoholic extract of carob fruits, the fifth (T_5) by 400 mg kg^{-1} alcoholic extract of carob fruits, the sixth (T_6) by 100 mg kg^{-1} alcoholic extract of ginseng roots + 100 mg kg^{-1} carob fruits, the seventh (T_7) by 200 mg kg^{-1} alcoholic extract of ginseng roots + 200 mg kg^{-1} carob fruits, and finally, the eighth (T_8) by 400 mg kg^{-1} vitamin E. Our results showed a significant drop ($p < 0.05$) in the level of MDA concentration and a significant upraise in the level of CAT, GSH and TAC concentration in the semen. The results also revealed the normal shape of the seminiferous tubules and an improvement in reproductive efficiency through the elevation in spermatogenesis percentage (SP), primary spermatocytes percentage (PSP), secondary spermatocytes percentage (SSP), spermatozoa percentage (SpP) and mature sperm percentage (MSP) for groups dosed by ginseng root extract + carob fruits and by vitamin E compared to the normal control group. Ginseng roots extract at a dose of 200 mg kg^{-1} + carob fruits at a dose of 200 mg kg^{-1} (T_7) were superior to all treatments, followed by vitamin E (T_8). Thus, we concluded from the current study that the effect of T_7 reduces the level of free radicals and raises the level of antioxidants in the semen, and increases reproductive efficiency through the high percentage of sperm in the semen.

Keywords: Ginseng, Carob, Vitamin E, Oxidation balance, Testes.
Article type: Research Article.

INTRODUCTION

There are various causes of male infertility, including disorders in the process of spermatogenesis (Babakhanzadeh *et al.* 2020). Azoospermia, which affects 10%–20% of people with infertility, is one of the most severe forms of male infertility (Kumar 2013). Erectile dysfunction and lack of sexual desire are among the significant side effects of hormonal and pharmaceutical therapy for azoospermia (Hu *et al.* 2018). In addition, anti-estrogen drugs that

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are prescribed to patients with azoospermia during the treatment period have harmful effects, since they cause cardiovascular events and accelerate the growth of prostate cancer (Isidori *et al.* 2017). Therefore, drugs that lack harmful effects and stimulate spermatogenesis should be developed in patients with azoospermia and using synthetic drugs should be avoided. Oxidative stress brought on by free radicals has a substantial impact on the function of the male reproductive system. High levels of unsaturated fatty acids in the testis are linked to the rapid spermatozoa reproduction and a greater rate of oxygen consumption in the mitochondria, making it extremely vulnerable to oxidative damage (Asadi *et al.* 2017). Antioxidant supplements should be taken from external sources to avoid oxidative stress and maintain a healthy balance between free radicals and antioxidants, since the antioxidant system alone cannot reduce the effects of oxidative stress on the testis (Schreiber *et al.* 2019). Plants have exhibited a significant impact on human health through their positive benefits, and used to treat many diseases since ancient times (Santacroce *et al.* 2021). Due to its antioxidant characteristics or ability to stimulate sex hormones, medicinal herbs have recently attracted increased attention for their therapeutic uses and enhancement of reproductive efficiency (Ahmed *et al.* 2020; Ahmed *et al.* 2021; Ahmed *et al.* 2022; Ahmed *et al.* 2022). So, studies in pharmacology aim to discover medicines from natural sources including plants (Süntar 2020; Annooz & Areaaer 2022; Kadhim & Younis 2023; Abdul Hussein *et al.* 2023; Hamad *et al.* 2023). Ginseng has been used in traditional Chinese medicine since ancient times, as it contains nitrogenous compounds, polysaccharides, phytosterols, organic acids, essential oils, amino acids, and peptidoglycans in addition to ginsenosides (Mohamad *et al.* 2019). Ginseng is used as a growth stimulant, immune booster, anti-inflammatory, anti-diabetic, anti-tumour, anti-obesity, anti-bacterial, antioxidant, and aphrodisiac (Sun *et al.* 2019; Al Abbasi *et al.* 2022). Carob is one of the most important food crops and is currently grown in many regions of the world, which makes herbal substances seem like alternatives to industrial medicines, since these phytotherapeutic agents have less negative side effects compared to synthetic treatments (Gugliuzzo *et al.* 2019; Azab 2020). Carob has historically been used to treat male infertility as one of the natural remedies, since it stimulates and improves spermatogenesis by significantly affecting Sertoli and Leydig cells. Carob also plays an anti-apoptotic role and stimulates the expression of genes regulating the cell cycle (Faramarzi *et al.* 2019; Ghorbaninejad *et al.* 2023). In addition, there is a study of the antioxidant activities of carob, as it clarifies the biological relationship between oxidative stress and illnesses that affect the nervous system (Lakkab *et al*. 2018; Zhu *et al.* 2019). Tococromanol, another name for vitamin E, is a fat-soluble antioxidant that is only made by plants (Niu *et al.* 2022). Vitamin E deficiency has been linked to a number of illnesses, including cancer, Alzheimer's, and cardiovascular disease (Sozen *et al.* 2019). Therefore, the purpose of the current study was to establish a scientific rationale for using ginseng root, carob fruit, and vitamin E to enhance male reproductive potency.

MATERIALS AND METHODS

Materials

The roots of the Indian ginseng *Withania somnifera* were obtained from China, and the root extract was extracted using methanol alcohol at a concentration of 60%. The fruits of the carob *Ceratonia siliqua* were also obtained from Turkey, and the fruit extract was extracted using methanol alcohol at a concentration of 70%. Vitamin E was also obtained from China.

The animals

Forty albino male rabbits at the age of 5-6 months and 1300 ± 100 g in weight were used. The rabbits were placed in special plastic cages for rabbit breeding, then they were subjected to laboratory conditions including a temperature of 22 ± 2 °C and the hall was lit for 16 h per day. Afterward, the animals were left for two weeks to adapt to the conditions, with the daily diet being provided in two meals for all treatments, and free access to water.

Experiment Design

In this study, the rabbits were divided into eight groups, each with five rabbits of the same weight. Then the experimental groups were divided as follows:

1. The first group (T_1): Control was fed on a standard diet and then gavaged by distilled water throughout the experimental period of five months.
2. The second group (T_2), ginseng (200 mg): They were fed on a standard diet, then gavaged by 200 mg kg^{-1} alcoholic extract of ginseng roots every day throughout the five-month experimental period.

3. The third group (T_3), ginseng (400 mg): They were fed on a standard diet, then gavaged by 400 mg kg^{-1} alcoholic extract of ginseng roots every day throughout the five-month experimental period.
4. The fourth group (T_4), carob (200 mg): They were fed on a standard diet, then gavaged by 200 mg kg^{-1} alcoholic extract of carob fruits every day throughout the five-month experimental period.
5. The fifth group (T_5), carob (400 mg): They were fed on a standard diet, then gavaged by 400 mg kg^{-1} alcoholic extract of carob fruits every day throughout the five-month experimental period.
6. The sixth group (T_6), ginseng (100 mg)) + Carob (100 mg): They were fed on a standard diet, then they gavaged by100 mg kg^{-1} alcoholic extract of ginseng roots + 100 mg kg^{-1} alcoholic extract of carob fruits every day throughout the five-month experimental period.
7. The seventh group (T_7), ginseng (200 mg) + Carob (200 mg): They were fed on a standard diet, then gavaged by 200 mg kg^{-1} alcoholic extract of ginseng roots and 200 mg kg^{-1} alcoholic extract of carob fruits every day throughout the five-month experimental period.
8. The eighth group (T_8), Vitamin E (400 mg): They were fed on a standard diet, then gavaged by 400 mg kg^{-1} vitamin E every day throughout the five-month experimental period.

Excision of the testicles and semen collection

The animal's abdomen was opened using a dissection kit to obtain the testes to which the epididymis is connected. Afterward, the epididymis was separated, cut into parts and normal saline was added to it using a graduated medical syringe to find out the dilution ratio (1:5) and with continuous stirring by Wood Stick. The semen was obtained, and kept at a temperature of -20 °C until biochemical tests were performed, while the rest of the fluid was used to directly conduct sperm parameters tests.

Biochemical examinations of semen

The concentration of Malondialdehyde (MDA) was estimated according to the method of (Rao *et al.* 1989), the activity of Catalase(CAT) enzyme according to the equation (Goth 1991), and the level of Glutathione (GSH) according to the modified Elman reagent method (Al Zamely *et al.* 2001), total antioxidant capacity (TAC) using the Ferric Reduction Ability of Plasma method (Benzie & Strain 1996).

Histological preparations

The testicles were extracted after the dissection of the animals, then the steps were taken to prepare the histological sections, staining with hematoxylin and eosin (H & E). Afterward, light microscopy was used to analyse the slides after completion of the preparation and photographs were taken.

Statistical analysis

Significant differences were determined according to Test of multiple ranges by Duncan, with a significant level of $p < 0.05$ using Analysis of Variance (ANOVA) test.

RESULTS

Concentrations of MDA, CAT, GSH, and TAC

The outcomes of the present study in Table 1 revealed a substantial reduction of MDA concentration and a substantial rise of the CAT, GSH with TAC concentrations in the semen of albino male rabbits including the groups treated with ginseng, carob and Vitamin E, especially in comparison with the healthy control group which used the usual diet. So that, the treatments using 400 mg of each of ginseng and carob were superior to those using 200 mg. In addition, T_7 (the treatment using 200 mg ginseng + 200 mg carob) exhibited the best effects, followed by T_8 (vitamin E), then T_8 (100 mg ginseng + 100 mg carob).

Histological study of testes and sperm

The results of the current study for the healthy control group showed the normal shape of the testis section, as it showed spermatogenesis percentage (SP), primary spermatocytes percentage (PSP), secondary spermatocytes percentage (SSP), spermatozoa percentage (SpP) and mature sperm percentage (MSP) in normal proportions (as shown in the Fig. 1 and Table 2). In the case of the group treated with200 mg alcoholic extract of ginseng roots (T_2), an elevation in SP, PSP, SSP, SpP and MSP was appeared to a low degree (+; as shown in Fig. 2 and Table 2). While in the group treated with 400 mg alcoholic extract of the roots of ginseng (T_3), an upraise in SP, PSP, SSP, and SpP to a medium degree (++), and in MSP with a low score (+; as shown in Fig. 3 and Table 2).

In the case of the group treated with 200 mg alcoholic extract of carob, an elevation was observed in SP, PSP, SSP, SpP and MSP to a low degree (+; as shown in Fig. 4 and Table 2), while in the group with 400 mg, an upraise was observed in SP, PSP and SSP to a moderate degree (++), and also an increase in SpP and MSP with a low score (+; as shown in Fig. 5 and Table 2). In the case of the group treated with 100 mg alcoholic extract of ginseng + 100 mg alcoholic extract of carob (T_6) an elevation was observed in SP and PSP to a high degree (+++) and an increase in SSP, SpP and MSP with a medium degree (++; as shown in Fig. 6 and Table 2), while in the group treated with 200 mg alcoholic extract of ginseng + 200 mg alcoholic extract of carob, an elevation was found in SP, PSP, SSP, SpP and MSP with a high degree (+++; as shown in Fig. 7 and Table 2). In the case of the group treated with 400 mg vitamin E, there was an increase in SP, PSP, SSP with a high degree (+++), and an elevation in SpP MSP with a moderate degree (++; as shown in Fig. 8 and Table 2).

Table 1. The effect of Ginseng, Carob and Vitamin E on the concentration of MDA, CAT, GSH and TAC in the semen of male rabbits in the experimental groups.

Parameters / **Groups**	**MDA (nmol mL^{-1})**	**CAT (IU mL^{-1})**	**GSH (µm mL^{-1})**	**TAC (mmol L^{-1})**
Control	144.0 ± 2.983^{a}	128.0 ± 2.627^{d}	139.8 ± 1.772^{ef}	0.5256 ± 0.00496^{g}
Ginseng (200 mg)	144.8 ± 2.354^{a}	131.8 ± 2.154^{cd}	145.4 ± 1.913^{d}	0.5638 ± 0.00278^{e}
Ginseng (400 mg)	135.6 ± 1.990^{b}	147.4 ± 1.691^{b}	154.8 ± 1.562^{c}	0.5882 ± 0.00235^{c}
Carob (200 mg)	147.4 ± 1.208^{a}	127.4 ± 1.503^{d}	137.8 ± 1.428^{f}	0.5520 ± 0.00241^{f}
Carob (400 mg)	136.6 ± 1.631^{b}	137.4 ± 0.927^{c}	144.4 ± 1.435^{de}	0.5758 ± 0.00338^{d}
Ginseng (100 mg) + Carob (100 mg)	132.4 ± 1.631^{b}	137.2 ± 1.497^{c}	153.0 ± 1.871^{c}	0.5832 ± 0.00246^{cd}
Ginseng (200 mg) + Carob (200 mg)	108.0 ± 2.983^{d}	166.8 ± 1.985^{a}	176.4 ± 1.887^{a}	0.6344 ± 0.00354^{a}
Vitamin E (400 mg)	121.0 ± 2.429^{c}	150.2 ± 2.853^{b}	162.6 ± 1.631^{b}	0.6098 ± 0.00408^{b}

Note. The letters that are vertically different indicate a significant difference at the probability level ($P < 0.05$), while the values show the arithmetic mean with standard error.

Table 2. Effect of Ginseng, Carob and Vitamin E on histological parameters of testes and sperm of male rabbits in the experimental groups.

Parameters / **Groups**	**Spermatogenesis percentage (SP)**	**Primary Spermatocytes Percentage (PSP)**	**Secondary Spermatocytes Percentage (SSP)**	**Spermatozoa Percentage (SpP)**	**Mature Sperm Percentage (MSP)**
Control	Normal	Normal	Normal	Normal	Normal
Ginseng (200 mg)	+	+	+	+	+
Ginseng (400 mg)	++	++	++	++	+
Carob (200 mg)	+	+	+	+	+
Carob (400 mg)	++	++	++	+	+
Ginseng (100 mg) + Carob (100 mg)	+++	++	++	++	++
Ginseng (200 mg) + Carob (200 mg)	+++	+++	+++	+++	+++
Vitamin E (400 mg)	+++	+++	+++	++	++

• Abbreviations: (+) for low-grade elevation, (++) for medium-grade elevation, and (+++) for high-grade elevation.

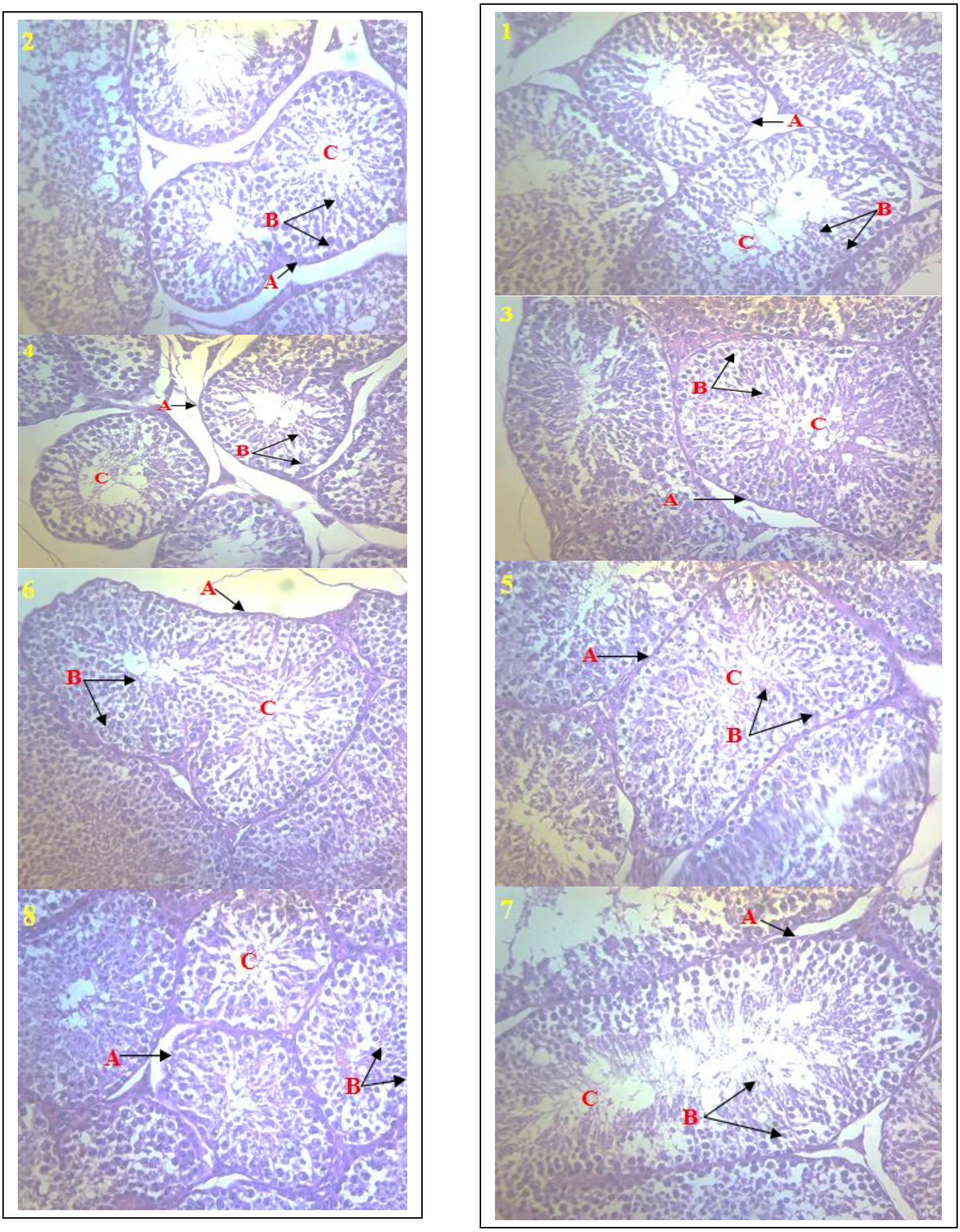

Fig.1-1. Testes section of a healthy control group; Fig.1-2: Testes section of the treated group by Ginseng (200 mg); Fig. 1-3: Testes section of the treated group by Ginseng (400 mg); Fig. 1-4: Testes section of the treated group by Carob (200 mg); Fig. 1-5: Testes section of the treated group by Carob (400 mg); Fig.1-6: Testes section of the treated group by Ginseng (100 mg) + Carob (100 mg). Fig. 1-7: Testes section of the treated group by ginseng (200mg) + carob (200mg); Fig. 1-8: Testes section of the treated group by vitamin E (400 mg); Staining with H & E; 200X.

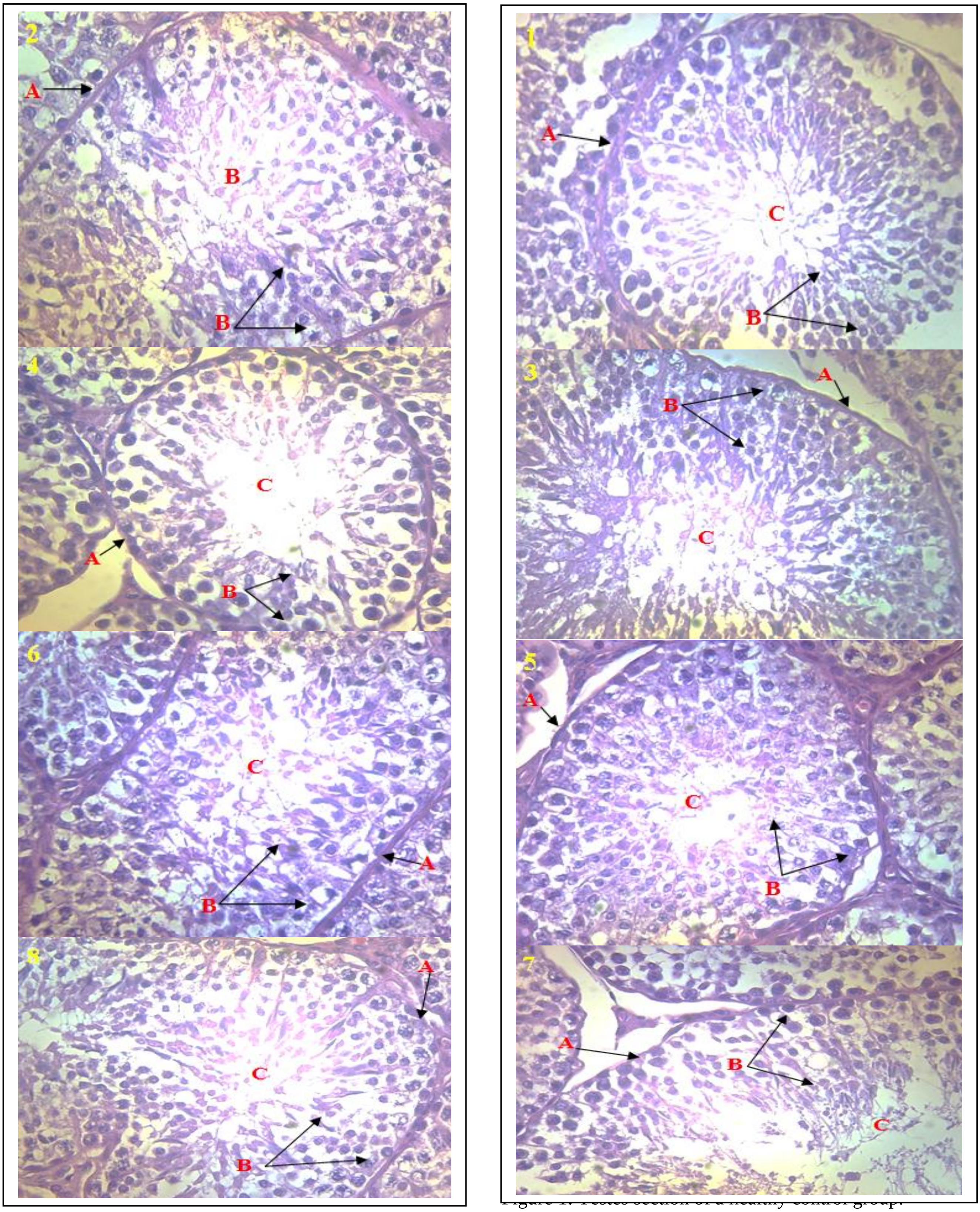

Fig. 2-1. Testes section of a healthy control group; Fig. 2-2: Testes section of the treated group by Ginseng (200 mg); Fig. 2-3: Testes section of the treated group by ginseng (400 mg). Fig. 2-4: Testes section of the treated group by carob (200 mg); Fig. 2-5: Testes section of the treated group by Carob (400 mg); Fig. 2-6: Testes section of the treated group by ginseng (100 mg) + carob (100 mg); Fig. 2-7: Testes section of the treated group by ginseng (200 mg) + carob (200 mg). Fig. 2-8: Testes section of the treated group by vitamin E (400 mg); Staining with H & E; 400X.

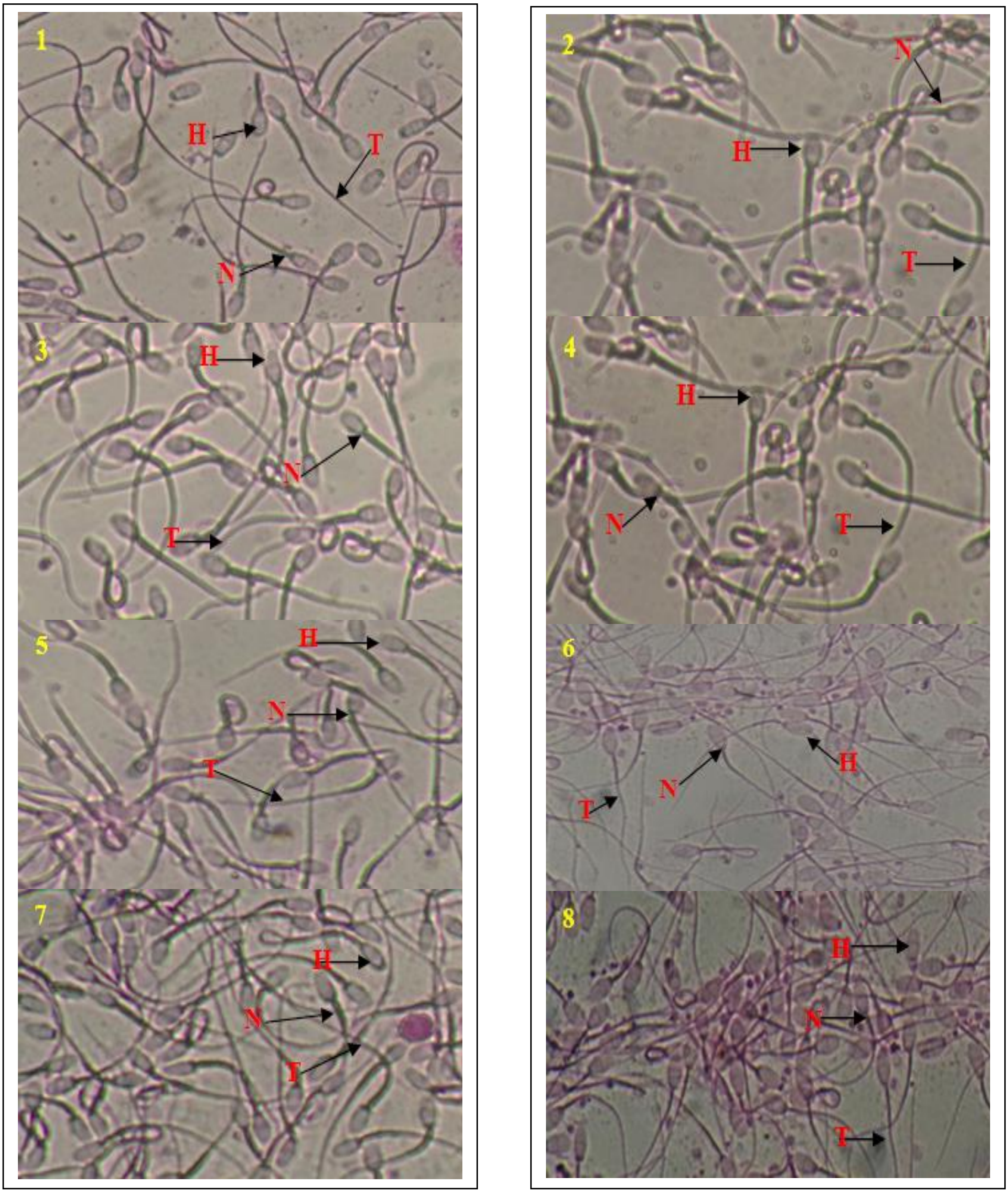

Fig. 3-1. Sperm in a healthy control group; Fig. 3-2: Sperm in the group treated with Ginseng (200 mg); Fig. 3-3: Sperm in the group treated with ginseng (400 mg); Fig. 3-4: Sperm in the group treated with carob (200 mg); Fig. 3-5: Sperm in the group treated with carob (400 mg); Fig. 3-6: Sperm in the group treated with Ginseng (100 mg) + Carob (100 mg); Fig. 3-7: Sperm in the group treated with ginseng (200 mg) + carob (20 0mg); Fig. 3-8: Sperm in the group treated with Vitamin E (400mg), Staining with H & E; 400X.

DISCUSSION

Oxidative stress results from the continuous proliferation of germ cells and spermatogenesis in the testes. However, the excessive formation of reactive oxygen species (ROS) associated with a high rate of metabolic activities in the testes can be reduced by the increased production of antioxidant enzymes endogenously and introducing dietary antioxidants exogenously (Schreiber *et al.* 2019). Dietary antioxidants can delay lipid peroxidation by inhibiting the initiation or propagation phase of redox chain reactions by scavenging free radicals (Huang *et al.* 2019). Given the role of ginseng root extract in reducing the level of MDA, raising the level of CAT, GSH, and TAC, and raising the reproductive efficiency of the testes and increasing the number of sperms in the present study, it may be due to the effect of ginseng extract on the function of the male reproductive system, and improving semen quality as well as fertilization rate (Sonmez *et al.* 2019). Ginseng supplementation exhibits a significant upraise in the level of luteinizing and testosterone hormone as well as an improvement in the level of oxidative stress through a decline in the level of free radical and an elevation in the level of antioxidant. The results showed the histological parameters of the testes naturally, by an elevation in the percentage of active sperm, thus improving the efficiency of the male reproductive system (Mansour *et al.* 2022). In the past, ginseng was used to treat cardiovascular and reproductive disorders due to its anti-aging, stimulant, and antioxidant properties (Potenza *et al.* 2022). The powerful antioxidants present in ginseng help fight the signs of aging. It has also been shown to aid the body in adapting and recovering from the negative effects of stress, illness, and overwork, since ginseng contains trace vitamins and minerals, as well as polysaccharides, volatile oils, pectin, and sterols (Yang *et al.* 2021). Ginseng enhances antioxidants such as ascorbic acid, and alpha-tocopherol activities, while declines lipid oxidation in rat testes (Kopalli *et al.* 2015). Also, elevating its activity in the body, leads to the increased expression of antioxidants (Sun *et al.* 2018). The activity of ginseng in promoting antioxidants in the current study may be due to the presence of ginsenosides that control extracellular kinase pathways, and the expression of several antioxidant enzymes in the testes (Ok *et al.* 2016). In the case of the role of carob extract in reducing the level of MDA, raising the level of CAT, GSH, and TAC, and improving the histological parameters of testes and reproductive efficiency in rabbits, it may be due to the antioxidant, anti-inflammatory, and metabolic syndrome activity of carob alcoholic extract (Rico *et al.* 2019). Carob aqueous extract showed anti-infertility activity in rats, and improved sperm quality and biochemical parameters related to fertility including testosterone, in addition to positive alterations in sperm concentrations (Ata *et al*. 2018; Vafaei *et al*. 2018). Treatment of infertile men with a 1500 mg capsule of carob extract resulted in an improvement in sperm count (Mahdiani *et al.* 2018). The aqueous extract of carob leaves and fruits showed an improvement in the level of spermatozoa and chromatin when it was given to elderly men who suffer from low sperm count in semen (Faramarzi *et al.* 2020). The addition of ethanolic extract of carob pods also improved sperm counts, sex hormones, and other biochemical parameters of fertility factors when induced reproductive toxicity with lead in mice (Soleimanzadeh *et al.* 2020). The aqueous extract of dried carob pods at a dose of 200 mg kg^{-1} of body weight in mice showed significant effectiveness in increasing the reproductive efficiency of the testes and sperm numbers, and in reducing the level of oxidative stress. In addition, when rats suffering from estrogen deficiency were given an aqueous extract of carob pods, they showed an increased level of estrogen (Sadat *et al.* 2019; Ammari *et al.* 2020). Oxidative stress is an important intrinsic factor that causes DNA damage in spermatogonia by inducing alterations in chromatin remodelling during spermatogenesis and in germ cells during meiosis (Wyck *et al.* 2018). Increased levels of free radicals cause negative effects on sperm reproduction, function, and fertility, and therefore levels of free radicals should be regularly adjusted in order to obtain normal sperm (Wagner *et al.* 2018). Previous studies have reported significantly reduced implantation and pregnancy rates in animals with severe sperm DNA damage (Zheng *et al.* 2018). The results of several studies on DNA damage also indicated significant and inverse relationships between sperm DNA damage along with fetal development and pregnancy (Sedó *et al.* 2017). Therefore, it can be concluded that carob promotes the production of sperm containing healthy DNA through its antioxidant function, which reduces oxidative stress due to the antioxidants present in carob extract such as some flavonoid derivatives responsible for maintaining the quality of DNA in sperm (Qin *et al.* 2016). The role of vitamin E in reducing oxidative stress represented by MDA, along with the increased level of antioxidants represented by CAT, GSH, and TAC, as well as the extent to which the histological properties of the testicles improve, may be due to the role of vitamin E as being one of the fat-soluble vitamins, and necessary for stabilization in the biological membrane, removing reactive oxygen species from the body, and also eliciting antioxidant effect. Vitamin E also reduces levels of total cholesterol and triglycerides, and many studies on human have been conducted on the effects of vitamin E supplementation on reducing oxidative stress and boosting body immunity (Lee & Han 2018; Otomaru *et al.* 2022). Supplemental vitamin E usage also reduced the level of total cholesterol, triglyceride, LDL, ALT, AST, and increased the level of HDL (Abdulazeez & Abdulrahman 2022; Kim *et al.* 2022). In addition, dietary vitamin E supplementation significantly reduces biomarkers related to oxidative stress by normalizing MDA levels (Kim *et al.* 2022). Furthermore, a previous study reported that vitamin E supplementation reduces interleukin-6 (Santos *et al.* 2016). Treatment of infertile men between the ages of 25 and 40 with a mixture of carob syrup and vitamin E increased sperm counts and sex hormones (Aghajani *et al.* 2019).

CONCLUSION

Our research's findings demonstrated that the alcoholic extract of ginseng roots, carob fruits and vitamin E played an important role in reducing oxidative stress in the testes formed as a result of the increased production of sperm by decreasing the level of MDA and raising the level of CAT, GSH and TAC in the semen. Our study also revealed an elevation in the reproductive ability of the treated groups by maintaining the normal shape of the seminiferous tubules and upraising the spermatogenesis percentage (SP), primary spermatocytes percentage (PSP), secondary spermatocytes percentage (SSP), spermatozoa percentage (SpP), and mature sperm percentage (MSP).

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