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Original Article

Effects of *Hibiscus Sabdariffa* Aqueous Extract on permatogenesis and Sperm Parameters of Mice

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Abstract

Background and Aim: Some medicinal plants are conventionally used for treatment of different aspects of male infertility. This study was conducted to evaluate the effects of *Hibiscus Sabdariffa* aqueous extract on spermatogenesis and sperm parameters of mice.

Materials and Methods: Forty-eight were divided into 4 groups with 3 replications and 3 animals in per replication and received doses of 0, 100, 200 and 300 mg/kg of *Hibiscus Sabdariffa* aqueous extract for 28 days. Levels of testosterone, luteinizing hormone (LH), follicular stimulating hormone (FSH), total antioxidant status (TAS), superoxide dismutase (SOD), glutathion peroxidase (GPx), malondialdehyde (MDA) blood variables including total protein, albumin, urea, glucose, urea, triglycerides, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C), testis weight, sperm abnormality and viability, motility and sperm concentration were evaluated.

Results: Oral administration of *Hibiscus Sabdariffa* aqueous extract reduced levels of FSH and LH in comparison to control group ($p<0.05$). The level of TAS was significantly lower in level of 300 mg/kg of the extract in comparison to control group ($p<0.05$). The levels of SOD and MDA were significantly higher and lower in comparison to control group, respectively. The levels of triglycerides and cholesterol were significantly lower in control group in comparison to other groups ($p<0.05$). Testicular weights were significantly lower in 100 and 200 mg/kg extract groups in comparison to 300 mg/kg extract group ($p<0.05$). Normal sperm count was significantly higher in mice treated with 100 mg/kg of the extract. Defected sperms were significantly lower in mice treated with extract.

Conclusion: It cannot be stated to use of clear dose of *Hibiscus Sabdariffa* aqueous extract in order to improve spermatogenesis and sperm parameters in mice.

Keywords: Antioxidant parameters, Hibiscus Sabdariffa, Lipid profile, Male Mice, Sexual Hormones, Testis Weight

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Introduction

Infertility is defined as inability in conceive after one year of sexual action without application of contraception (1). It is known as one of the challenges in human society. The different factors including environmental, behavioral, genotoxic and genetic can default spermatogenesis and infertility in various stages (2). Spermatogenesis is known as process that germ cells perform three phases of development: mitosis (spermatogonial proliferation), meiosis (spermatocyte DNA recombination, reduction and division), and spermatogenesis (spermatid differentiation); resulting in the transformation of the undifferentiated spermatogonia into highly specialized spermatozoa (3).Spermatogonial population is classified into two major categories in rodents including undifferentiated and differentiated (4). Antioxidant parameters are known to have importance in spermatogenesis because of their effects in improving spermatogenesis, steroidogenesis in male reproductive function (11).

Medicinal plants are used for treatment the different diseases. Medicinal plants are efficient, cheap, safe and available. Some medicinal plants are empirically used for treatment of different aspects of male infertility including sexual asthenia, libido (sexual desire), erectile and ejaculatory disorders, and sperm abnormalities (azoospermia, oligospermia). *In vitro* and/or *in vivo* studies have shown biological activities for some of medicinal plants (5). The roselle (*Hibiscus sabdariffa*)contains major compounds such as gossypetin, glucoside, hibiscin and hibiscus anthocyanin. *Hibiscus sabdariffa* seed is traditionally used to increase the lactation in case of poor milk production in human (6). Thus, this study aimed to evaluate the effects of aqueous extract *Hibiscus Sabdariffa*on spermatogenesis and sperm parameters of mice.

Materials and Methods

In this study, 48 adult mice (25-45 g) were purchased from Laboratory Animals Center, Tehran University. All the animals were maintained under temperature controlled rooms (24˚C) with 12h/12h light/ lighting cycle on basis the experimental protocols. All mice were treated as recommended by Principles of Laboratory Animal Care. Animals had free access to feed and water and monitored for 1 week before experiment start. Animals were divided into 4 groups with 3 replications and 3 animals in per replication. Animals received the extract in gavage form. Experiment was lasted for 28 days. Animals were treated with *Hibiscus Sabdariffa* aqueous extract in doses of 0, 100, 200 and 300 mg/kg. Experimental groups were as follows;

1) Animals received distilled water (Control group).
2) Animals received extract in level of 100 mg/kg (100 mg/kg extract group).
3) Animals received extract in level of 200 mg/kg (200 mg/kg extract group).
4) Animals received extract in level of 300 mg/kg (300 mg/kg extract group).

Preparation of Hibiscus Sabdariffa aqueous extract

The plant was prepared from local market. The extract was achieved as reported by Emelike and Dapper (2013) with minor modification. Firstly, 30 g of leaf dry powder was weighted and 100 ml sterilized distilled water was added it and solution was placed in bain-marie in 60˚C. We dissolved it in small volume of water for obtaining a concentrated extract. The achieved extract was filtered. The extract was stored in 4˚C.

Sperm collection and blood sampling

Mice were anesthetized by ether and weighted by digital scale. In order to prepare the serum for hormonal experiments, 1 ml blood was collected from animal heart and centrifuged. After abdominal surgery, testes and epididymis were separated. Levels of FSH, LH and testosterone were assessed by commercial kits of Monobind Company (USA). The serum concentrations of total protein, albumin, urea, glucose, urea, triglycerides, LDL-C and HDL-C were assessed by Pars Azmoon commercial kits (Tehran-Iran).

Antioxidant parameters

Part of blood was used as whole blood for measurement of glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant status (TAS). Malondialdehyde (MDA) were measured by ZELLbio commercial kit (Germany). The levels of GPx, SOD (439108), total antioxidant status (TAS) were measured by specified commerical kits (Randox

Laboratories, Ardmore, Crumlin, UK) as recommended by producer Company.

Sperm survivability and number of sperms

Testes were separated and weighted. Epididymis was separated and incubated in 37 ˚C for 20 minutes. 10 µl of solution was placed on lamella. Motility (%), viability (%) and sperm abnormality were assessed were measured as described by Varisli et al. (2013).

Statistical analysis

Statistical comparisons were performed by the ANOVA test for comparison of data in the control group and the experimental groups. The results were reported as mean ± S.D (standard deviation).

Results and Discussion

Effects of experimental treatments on levels of testosterone (Figure 1A), LH (Figure 1B) and FSH (Figure 1) are shown. Results indicated that treatment with *Hibiscus Sabdariffa* aqueous extract decreased levels of FSH and LH in comparison to control group ($P<0.05$). There was not significant difference between treatments of 200 and 300 mg/kg ($p>0.05$). The level of testosterone was significantly higher in 100 mg *Hibiscus Sabdariffa* aqueous extract and lower in 200 and 300 mg *Hibiscus Sabdariffa* aqueous extract in comparison to control group ($p<0.05$). There was not significant difference between treatments of 100 and 200 mg/kg of extract for testosterone level ($p>0.05$).

Effects of experimental treatments on antioxidant parameters are presented in Figure 2. The level of TAS (Figure 2.A) was significantly lower in level of 300 mg/kg of the extract in comparison to control group ($p<0.05$). It was not observed significant difference between extract treatments ($p>0.05$). The levels of SOD (Figure 2.B) and MDA (Figure 2.C) were significantly higher and lower in the treated groups in comparison to control group, respectively ($p<0.05$), but significant difference was not observed among extract treatments for mentioned parameters ($p>0.05$). The level of GPX was not influenced by experimental treatments (Figure 2.D).

Effects of experiments treatments on blood biochemical parameters are shown in Figure 3. Level of total protein was significantly higher in 200 mg/kg extract in comparison to control group (Figure 3 A) ($p<0.05$), but significant difference was not observed among extract treatments for total protein ($P>0.05$). Albumin concentration was significantly higher in 100 and 200 mg/kg extract compared with control group (Figure 3 B) ($p<0.05$), but it had not significant difference with 300 mg/kg extract ($p>0.05$). Animals in 100 mg extract group showed lower urea in comparison to other groups (Figure 3 C) ($p<0.05$), but it was not observed significant difference between other groups ($p>0.05$). The levels of triglycerides and cholesterol was significantly lower in control group in comparison to other groups (Figure 3 E and F) ($p<0.05$), but it was not observed significant difference between extract groups ($p>0.05$). Glucose (Figure 3 D), LDL (Figure 3 G) and HDL (Figure 3 H) were not affected by experimental treatments ($p>0.05$).

Effects of experimental treatments on testis weight are presented in Figure 4. Left testis+ epididymis weight (Figure 4A) was significantly lower in 100 mg/kg extract group in comparison to control group ($p<0.05$). Right testis+ epididymis weight (Figure 4B) was significantly lower in 100 mg/kg extract group in comparison to control group ($p<0.05$), while it was significantly higher in 200 and 300 mg/kg extract groups in comparison to control group ($p<0.05$). Left and Right weights (Figure 4C, D) were significantly lower in 100 and 200 mg/kg extract groups in comparison to 300 mg/kg extract group ($p<0.05$).

Effects of experimental treatments on sperm abnormality are shown in Figure 5. There was no significant difference between groups for pin head (Figure 5A) and main piece defect (Figure 5D). Defect neck (Figure 5B) and end piece defects (Figure 5E) sperms were significantly lower in animals treated with extract compared to control group. There were not significant differences between extract groups for mentioned parameters ($p>0.05$). Normal sperms (Figure 5F) was significantly higher in 200 mg extract compared with control group ($p<0.05$). We did not observe significant difference between extract groups for mentioned parameter ($p>0.05$).

Effects of experimental treatments on viability, motility and concentration of sperm are shown in Figure 6. Progressive motility (Figure 6A) and concentration (Figure 6D) was significantly smaller in the treated groups in comparison to control group ($p<0.05$). There was not significant difference between extract groups for progressive motility ($p>0.05$). Unprogressive

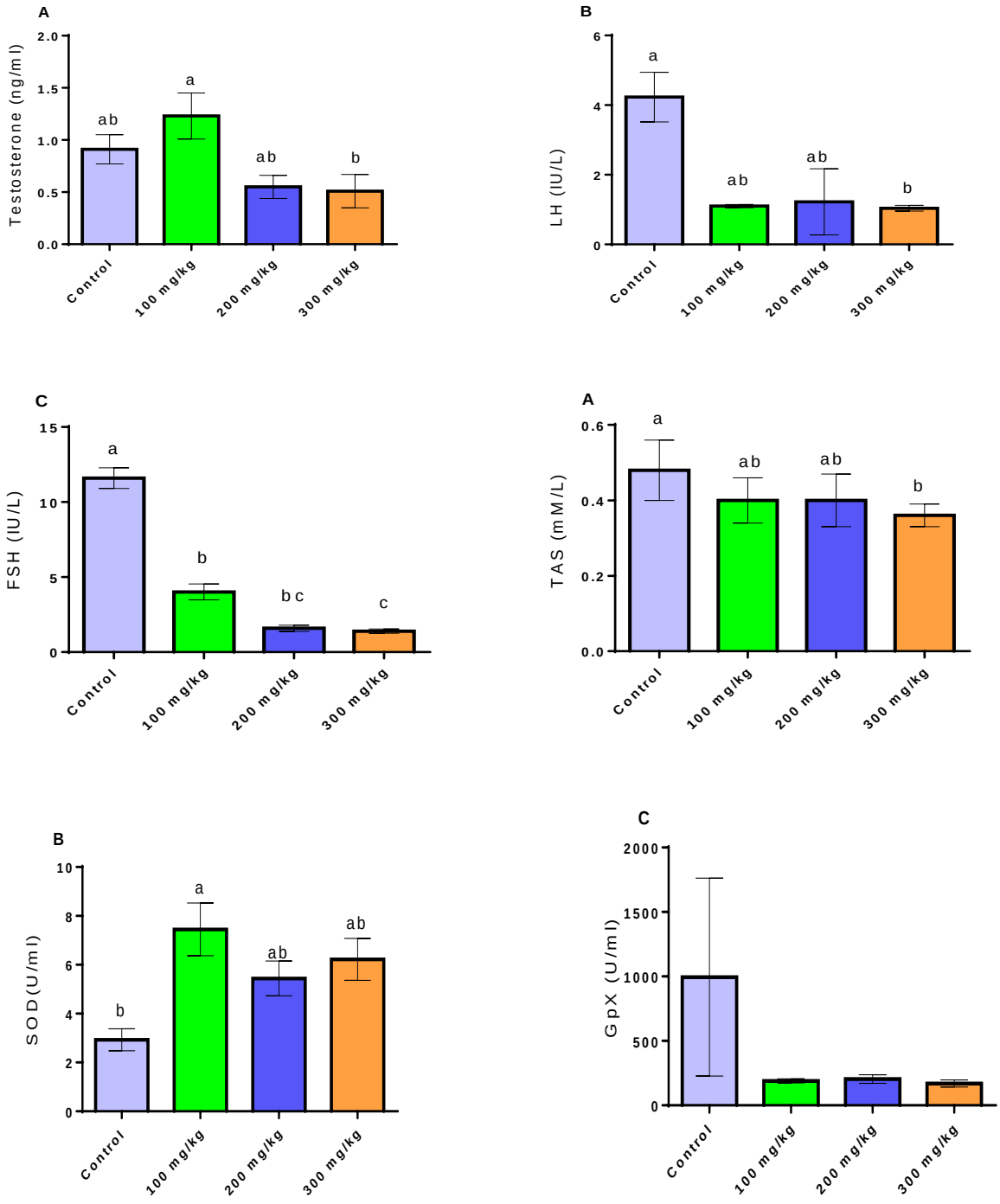

Figure 1. Effects of experimental treatments on levels of testosterone (A), LH (B) and FSH (C). Superscripts (a-c) show significant differences between groups.

motility (Figure 6B) was significantly higher in 100 and 200 mg/kg extract compared with 300 mg/kg

(P<0.05). Immobility percentage (Figure 6C) was significantly higher in 100mg/kg extract compared with

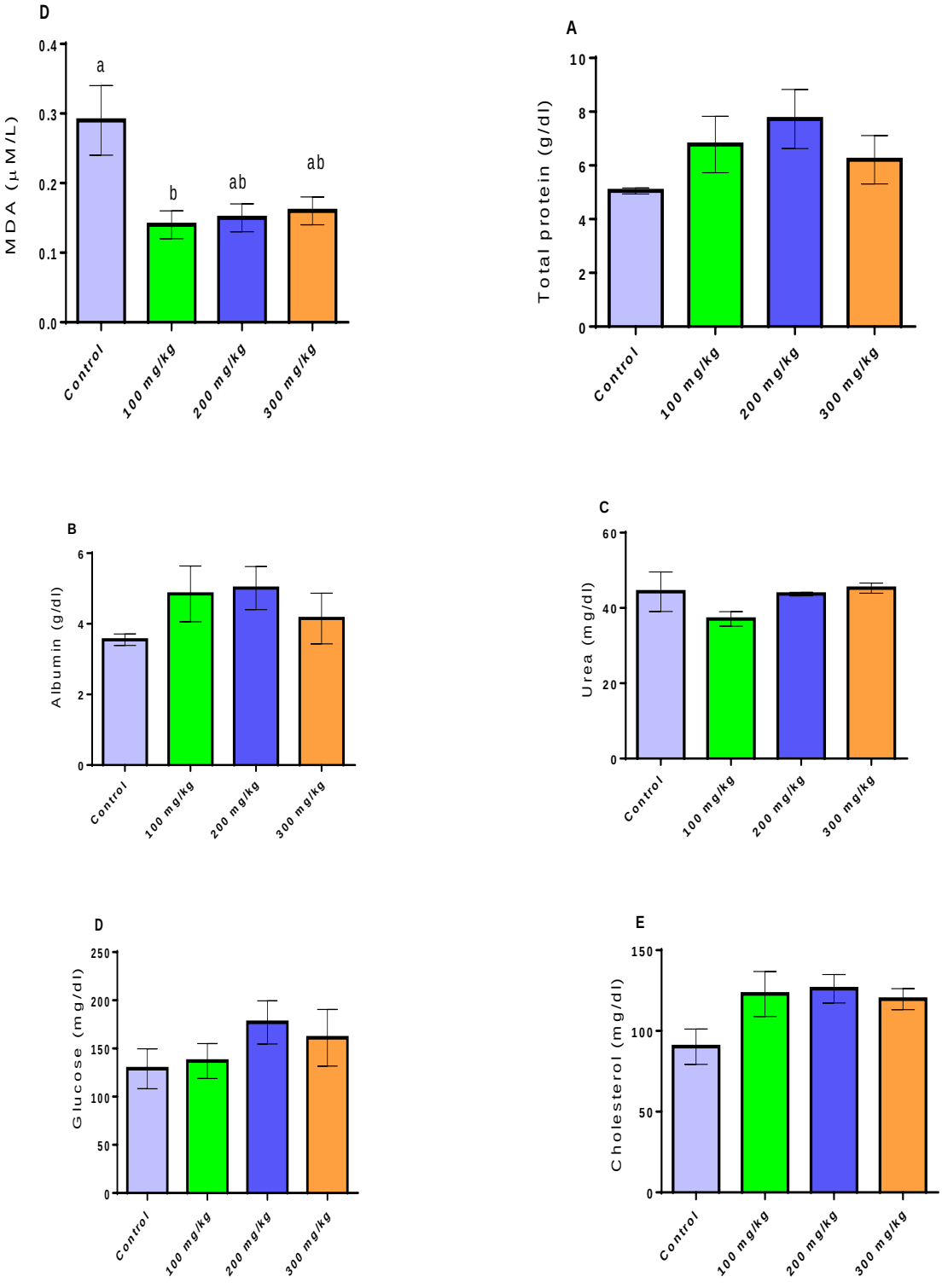

Figure 2. Effects of experimental treatments on levels of TAS (A), SOD (B), GPx (C) and MDA (D). Superscripts (a-c) show significant differences between groups.

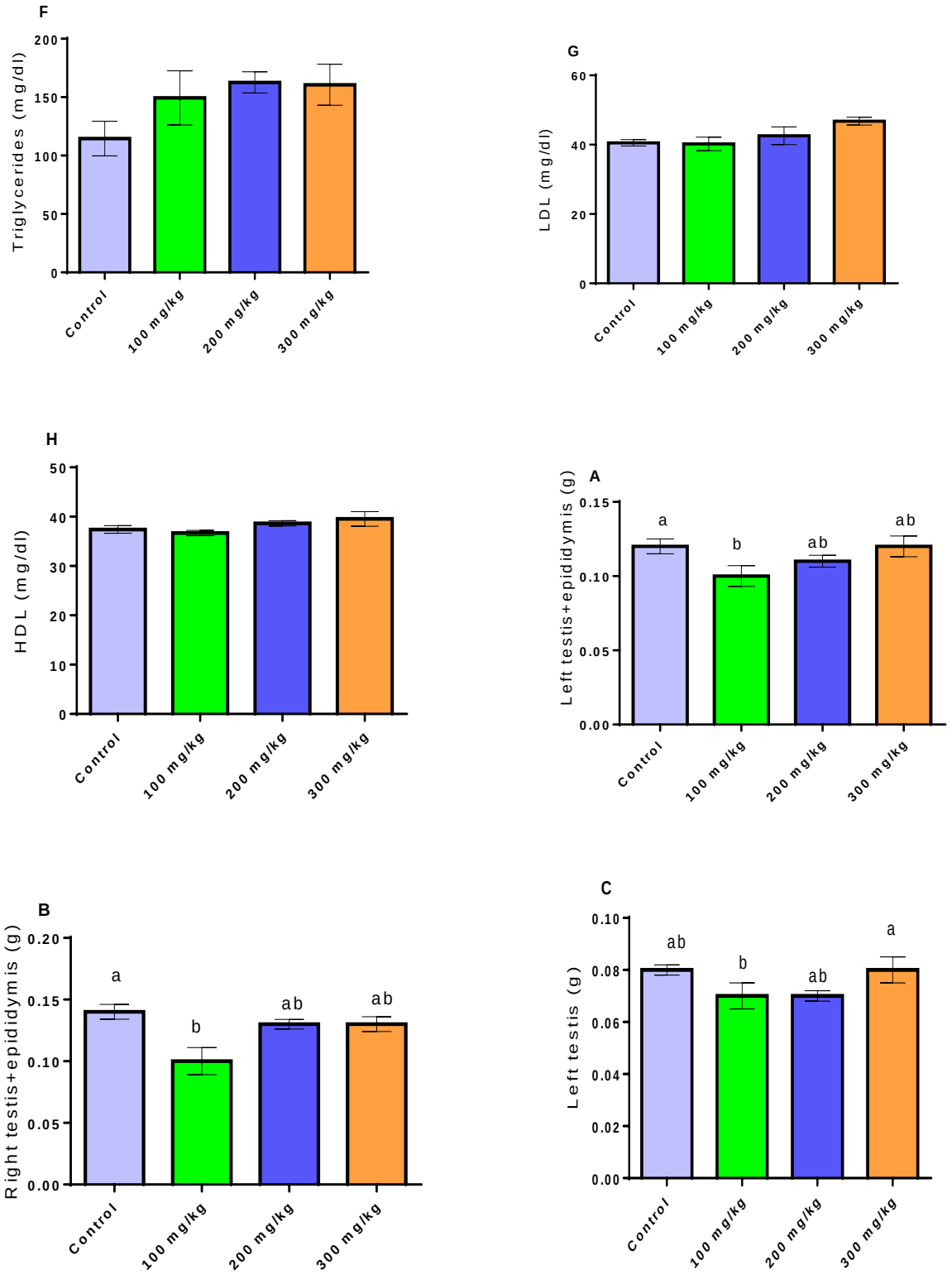

Figure 3. Effects of experimental treatments on blood biochemical parameters.

other groups ($p<0.05$). Dead sperm percentage was significantly lower in 300 mg/kg extract in comparison

to other groups (Figure 6F) ($p<0.05$).

Medicinal plants contain some active substances which

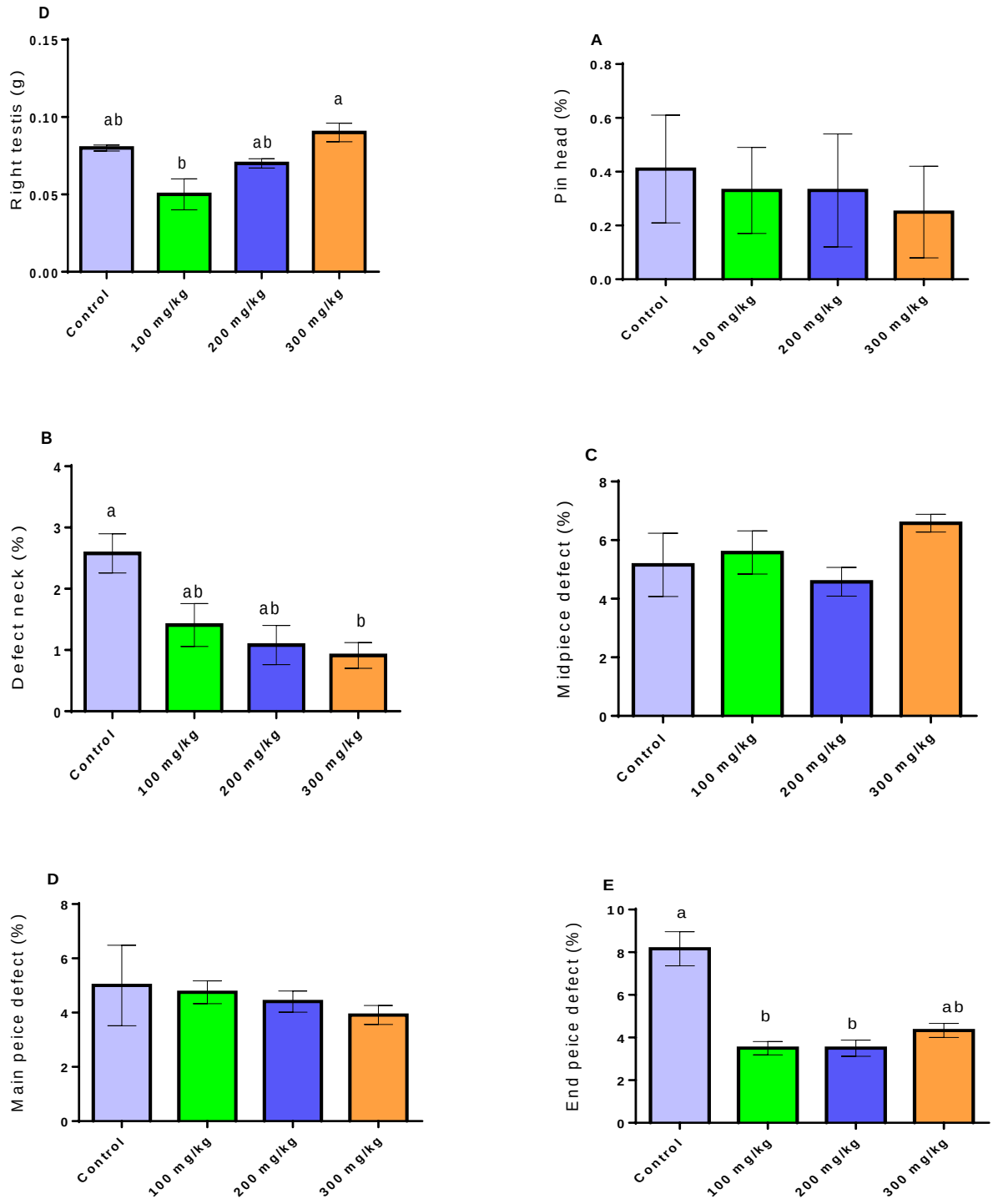

Figure 4. Effects of experimental treatments on testis weight.

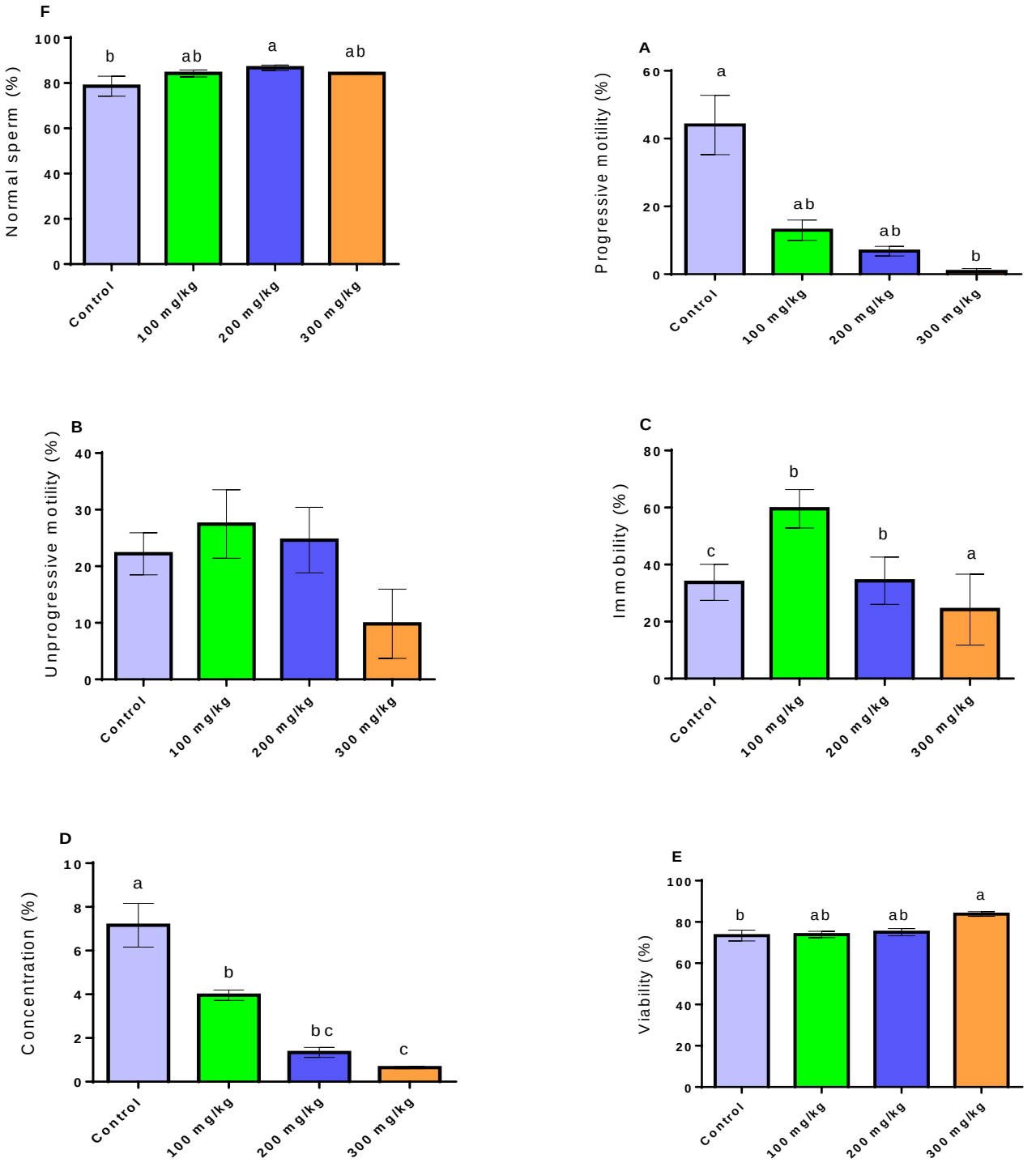

Figure 5. Effects of experimental treatments on sperm abnormality.

can encourage health and decrease illness (7). Oral administration of *Hibiscus Sabdariffa* aqueous extract

lowered levels of FSH and LH in comparison to control group. Regarding to previous studies, Al-Qarawi (2005) showed that administration of *Rutachalepensis* aqueous extract in levels of 0.5 up to 2 g/kg could increase the levels of testosterone and FSH in rats. Testosterone and its active conversion product promote erection through protecting the nitric oxide level. Some medicinal plants are conventionally used in order to treat of male infertility and reported promoting effects on testosterone production. The level of testosterone was also higher in 100 mg *Hibiscus Sabdariffa* aqueous extract and lower in 200 and 300 mg *Hibiscus Sabdariffa* aqueous extract in comparison to control group (8). Oral administration of *Hibiscus macranthus* and *Basellaalba* stimulates testosterone production in the level of 720 mg/kgin male adult rats after 15 days of treatment (9). Other study showed that treatment with *Saturejakhuzestanica* essential oil (75, 150 and225 mg/kg) increased serum concentrations of FSH and testosterone in adult male rats for 45 days (10). The differences between our findings and previous studies could be attributed to active compounds in the extract, animal's types, experiment condition, etc. Testosterone and FSH are known to have stimulatory effects on spermatogenesis. It seems that *Hibiscus Sabdariffa* aqueous extract does not stimulate testosterone and FSH.

The level of TAS was significantly lower in level of 300 mg/kg of the extract in comparison to the control group. The levels of SOD and MDA were significantly higher and lower in comparison to the control group, respectively. Antioxidants are known to have promoting effects on spermatogenesis, steroidogenesis in male reproductive function (11). In one in vivo study, it is reported that administration of *Peganumharmala* (50 mg/kg) extract had a protective antioxidant effect in order to protection of the reproductive activities against the negative effects of reactive oxygen species generated in large amounts after six months of administration (12). Estrogens are known to have essential roles for regulation of male reproductive activity (13). Oral administration of *Punicagranatum* increased antioxidant enzymes activity and decreased malondialdehyde level (14). It was expected that the extract could improve antioxidant capacity but such result was not observed.

Blood parameters were assessed in order to evaluate the metabolic changes in the body. Level of total protein was significantly higher in 200 mg/kg extract in comparison to control group, but albumin concentration was significantly higher in 100 and 200 mg/kg extract compared with control group. The levels of triglycerides and cholesterol were significantly lower in control group in comparison to other groups. Previous studies have shown that green tea extract could decrease

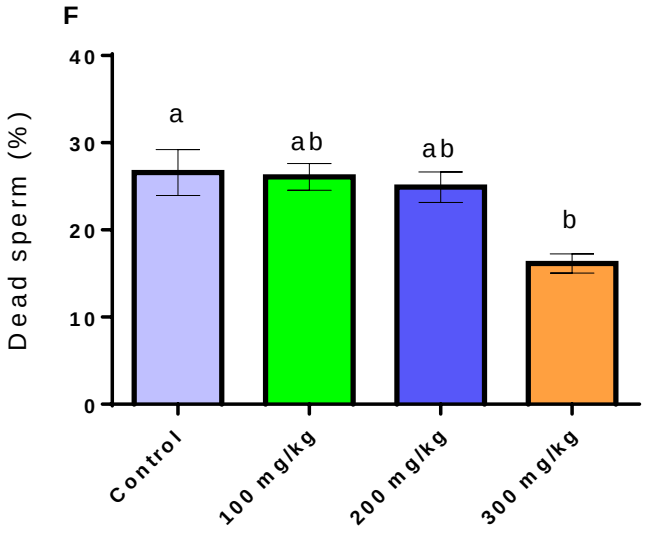

Figure 6. Effects of experimental treatments on viability, motility and concentration of sperm.

the production of lipid peroxidation products level (15). Other study has shown that oral administration of black tea for 3, 6, 9 and 12 months decreased levels of thiobarbituric acid reactive substances (16). It is reported that catechin existing in tea balances lipid peroxidation in rat testis (17). In the current study, *Hibiscus Sabdariffa* aqueous extract could decease levels of MDA, thus it was expected that *Hibiscus Sabdariffa* aqueous extract decrease lipid parameters. However, such result was not observed.

Right testis+ epididymis weight was significantly higher in 200 and 300 mg/kg extract groups in comparison to control group. Left and Right weights were significantly lower in 100 and 200 mg/kg extract groups in comparison to 300 mg/kg extract group. Qarawi (2005) have reported that administration of *Rutachalepensis* aqueous extract in levels of 0.5 up to 2 g/kg could increase testis weight in rats (8). Oral administration of *Saturejakhuzestanica*essential oil (75, 150 and225 mg/kg) could increase testis weightin adult male rats for 45 days (10). It cannot be certainly stated that higher and lower levels could increase testies weight.

Normal sperms percentage was significantly higher in 200 mg extract compared with control group ($P<0.05$). Defect neck and end piece defects sperms were significantly lower in animals treated with extract compared to control group. Reproductive capability in the male sex have the production of semen containing normal spermatozoa (quality) in the sufficient count (quantity), together with the desire and ability to mate (18). Studies have shown that administration of pumpkin decreased sperm count with primary and secondary abnormalities through production of zinc and protein (19). It seems that active compounds in extracts increases normal sperms.

Progressive motility and concentration was lower compared with control group ($p<0.05$). Unprogressive motility was higher in groups of100 and 200 mg/kg extract in comparison to 300 mg/kg. Immobility percentage was increased in 100mg/kg extract rather than other groups. Dead sperm percentage was significantly lower in 300 mg/kg extract in comparison to other groups. Qarawi (2005) have reported that treatments with *Rutachalepensis* aqueous extract in levels of 0.5 up to 2 g/kg could improve the sperm motility and viability in rats. Another study showed that administration of the ethanol extract of *Croton zambesicus* in levels of 5 and 10 mg/kg in vivo could increase the sperm number and motility (20). Oral treatment of *Nigella sativa* oil (0.5 ml/day) to healthy or hyperlipidemic animals for 60 days could increase sperm motility and count (21). Oral supplementing of *Phoenix dactylifera*date palm pollen suspensions in levels of 120 mg/kg for 5 weeks could improve the sperm count, motility and morphology (22). Researchers related increased sperm concentration to the increased testosterone and FSH levels in testicular tissue, because of hormones role in spermatocytogenesis and spermiogenesis in seminiferous tubules and epididymal function in maturation of sperms (23). However, FSH level was not higher in animals treated with extract in comparison to control group.

Conclusion

In conclusion, level of GPx and testosterone was significantly higher and level of MDA was significantly lower in animals treated with 100 mg/kg of the extract. Normal sperm count was significantly higher in mice treated with 100 mg/kg of the extract. Defected sperms were significantly lower in mice treated with extract. Future studies are needed to evaluate the effects of *Hibiscus Sabdariffa* aqueous extract on spermatogenesis and sperm parameters of mice.

Acknowledgment

This study was extracted from the thesis written by one of the authors, R. Beheshti.

Conflict of Interest

The authors declare that they have no conflict of interest.

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