

The Effects of Rosemary Extract on Spermatogenesis and Sexual Hormones of Mice under Heat Stress

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Abstract Heat stress can affect reproduction potential as an environmental factor. This Study was carried out to investigate the effects of rosemary extract on spermatogenesis and sexual hormones of laboratory mice under heat stress. 50 male mature mice were examined in five groups including a control group and four experimental groups [0, 100, 200 and 400 mg/kg of rosemary extract]. Samples were kept under heat stress four hours a day and received the extract doses for 30 days. At the end of the experiment, the amount of testosterone, LH, and FSH hormones plus the number of spermatogenic cells were measured. Obtained data were analyzed using the SPSS program. Heat stress in zero doses reduced testosterone, LH, and FSH significantly whereas rosemary extract increased testosterone and LH in 200 and 400 doses and FSH in 100,200, and 400 doses. Primary spermatocytes were decreased in zero doses significantly but increased significantly in other experimental groups [p<0.05]. In general, Heat stress reduces male sex hormones and spermatogenic cells but rosemary extract compensated this reduction dose-dependently and improved sexual potential under heat stress.

Keywords: *Rosemary extract, Sexual hormones, Spermatogenic cells, Heat stress, Mice*

Introduction

Reproduction is an important indicator of community health. Low reproduction rate reduces reproduction efficiency of community. Therefore, having knowledge about reproduction processes is becoming important more and more. This importance is because of necessity of protecting generations and preparing suitable situations for population increasing plans. Low abilities of male sex leads to reduced populations by affecting reproduction and decreasing fertility rate. Two aspects of male reproduction performance are important: sperm production which is known as spermatogenesis and production of pituitary hormones and androgen which supports the male reproduction system and causes secondary male sex characteristics.

The effect of seasons on different aspects of reproduction performance is obvious and considering that the largest population of world is in regions with seasonal stressors which affect production ability, it is important to know that heat stress can cause problems in reproduction efficiency, management plans and community health [1]. Heat stress can increase the body temperature

directly. Even a little increase in body's temperature can reduce pregnancy amount. Increase in temperature affects reproduction system and fetus and these changes affect fertility under heat stress [2].

Previous studies showed that heat stress affects sperm quality and produces abnormal sperms. Heat shortens Duration of estrus and reduces the severity of estrus signs by affecting hormone patterns [1].

Humans have long recognized the value of medicinal plants and have searched nature for curing every pain. Studies have shown that various herbal extracts are effective on organic performances of body especially reproduction system and testis tissue [3].

Last researches about herbal drugs and food complements indicates that extant compounds including fibers, vitamins, flavonoides, strols, and other anti oxidants can reduce blood fat, inhibit the oxidation of LDL and remove free oxygen radicals and are effective in curing diseases by affecting immune system and improving metabolism [4].

In Europe, rosemary is often used to help treat indigestion. In fact, Germany's Commission E has approved rosemary for the treatment of indigestion. However, it should be noted that there is currently no meaningful scientific evidence to support this claim. Rosemary is a rich source of antioxidants and anti-inflammatory compounds, which are thought to help boost the immune system and improve blood circulation. Laboratory studies have shown rosemary to be rich in antioxidants, which play an important role in neutralizing harmful particles called free radicals.

Several studies have found some beneficial effects for rosemary leaves such as anti-inflammatory, anti-viral, cytoprotective and anti-tumoral effects [5]. Rosemary contains numerous compounds including diterpenes, triterpenes, flavones and steroids. The main compounds, which cause RE efficacy, are two phenolic diterpenes: carnosic acid [CA] and carnosol [about 90% of antioxidant activity] [6].

The goal of this study was investigating the effects of rosemary hydro alcoholic extract on sexual hormones and spermatogenesis of male mice under heat stress.

Materials and methods

Fifty male mature laboratory mice weighing 30 ± 5 g were divided in five groups with 10 members in each group. Mice were kept for 15 days to adapt to environment. Samples had free access to food and water and normal light. Treatment groups were under heat stress [30-36 °C] four hours a day for 30 days.

Treatment groups were:

- Control group including 10 mature mice to obtain basic concentrations of FSH, LH and testosterone hormones and also observing testis tissue slides, spermatogenesis and fertility. This group was kept in situation similar to treatment groups without adding rosemary extract or heat stress.

- Zero group which was used to study the effects of heat stress on sexual parameters.

- Three experimental groups: these groups which were kept under heat stress received 100, 200 and 400 mg/kg of hydro alcoholic extract of rosemary in drink water.

Animals were kept according to national health institute manual.

After 30 days, blood samples were taken from all groups and sexual hormones were evaluated using immune enzymatic method. After biopsy, testes were removed and histological slides were prepared using hematoxylin eosin method for counting spermatogenic cells.

Obtained data were analyzed using SPSS program in a complete randomized design with 5 groups and 10 replications. Mean comparisons were done using LSD test at 5% probability level.

Results

FSH concentration of zero dose was decreased significantly in proportion to control group while experimental groups [100, 200, and 400 mg/kg of extract] increased it significantly [$p < 0.05$] [Figure 1].

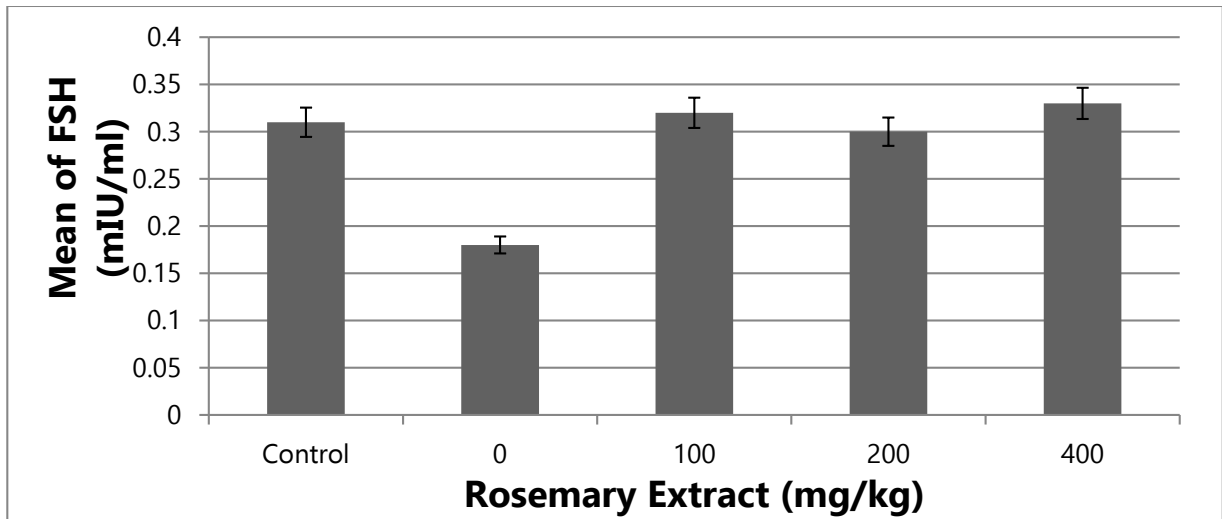


Figure 1. The mean of FSH concentration of various treatment groups

Mean comparison of LH concentration of treatment groups showed that zero group had lower LH than control group whereas 200 and 400 groups [$p < 0.05$] increased LH amount significantly [Figure 2].

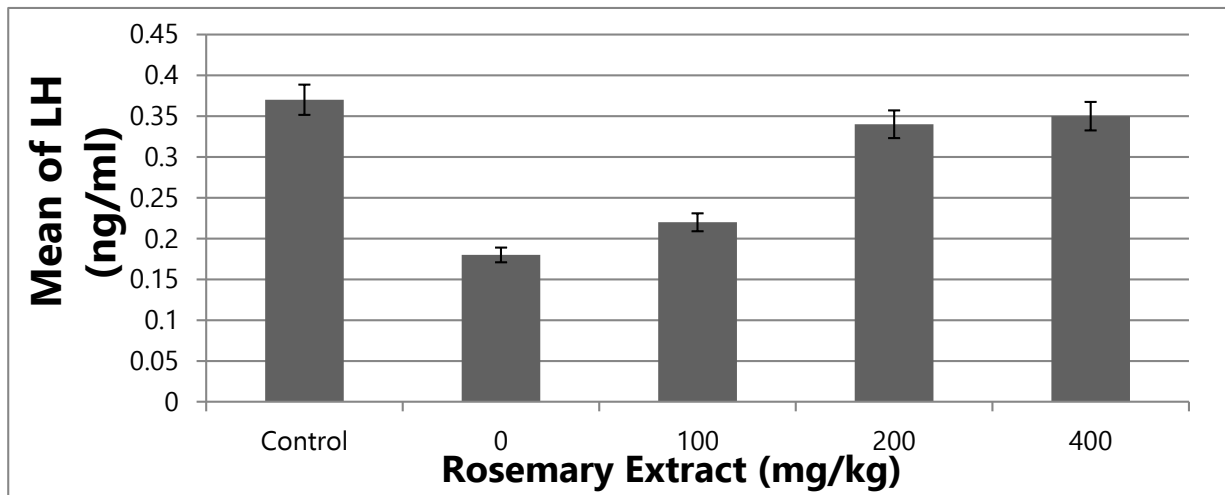


Figure 2. The mean of LH concentration of various treatment groups

Testosterone amount was decreased significantly in zero and 100 mg/kg but increased in 200 and 400 mg/kg doses [$p < 0.05$] [Figure 3].

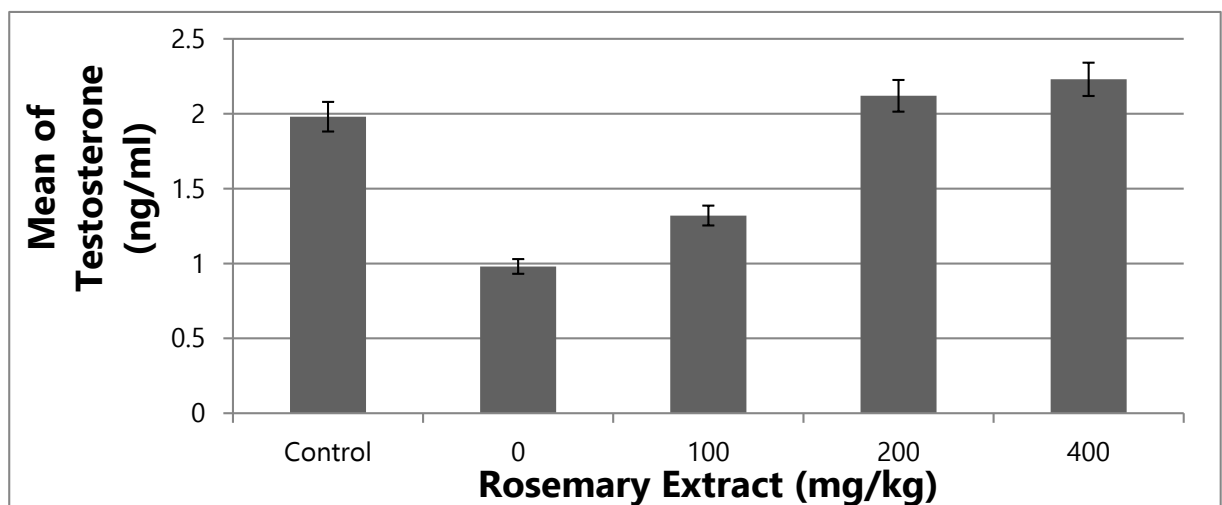


Figure 3. The mean of testosterone concentration of various treatment groups

Average primary spermatocyte number was decreased in zero group significantly whereas in 100, 200, and 400 mg/kg doses increased significantly and reached to control group [$p < 0.01$] [Figure 4].

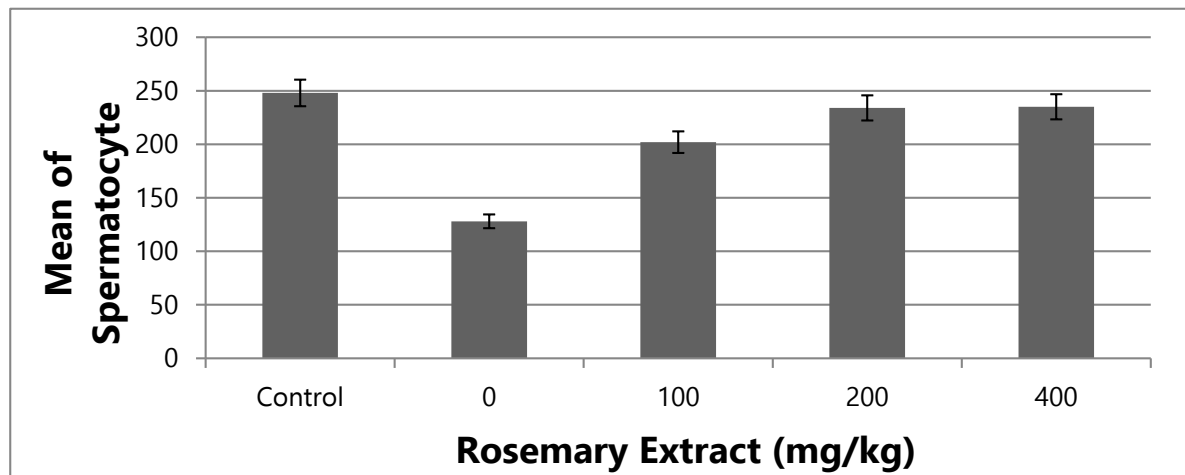


Figure 4. The mean of spermatocyte of various treatment groups

Discussion

Previous experiences have shown that heat stress have various effects on sexual hormone system. The most sensitive parts are sexual cells [7]. The main route of controlling sexual acts is hypothalamic-pituitary-gonadal axis [HPG]. This axis is under feedback control and regulates sexual acts of human and other mammals. In male sex this axis acts precisely for secreting testosterone hormone and spermatogenesis. Hypothalamus stimulates front pituitary by secreting gonadotropins release hormones [GnRH] and causes gonadotropin secretion [FSH and LH].

In this study, heat stress in lack of rosemary extract [zero dose] reduced sexual hormones significantly but LH and testosterone amounts were increased in 200 and 400 mg/kg doses significantly [$p < 0.05$]. However, FSH amount was increased in 100, 200, and 400 mg/kg groups significantly [$p < 0.05$].

LH hormone increase androgen secretion [testosterone] by affecting testis lydig cells. By increasing androgen, this axis regulates and controls testosterone secretion via feedback increase. Increase in testosterone amount affects hypothalamus and controls production of lutein releasing hormones and follicle stimulating hormone via negative feedback.

Low blood testosterone also cancels controlling effect on hypothalamus and causes testosterone secretion to normal situation [1].

Increase in serum testosterone concentration by LH increment is expected. Therefore, rosemary extract increased testosterone by preventing damages to interstitial tissue cells and destroying germinal epithelium of seminiferous tubules. Since alpha tocoferoles of rosemary extract which are a family from anti-oxidants can prohibit oxidative stress in testis of laboratory mice [8], rosemary extract [by strengthening anti-oxidant defense system] not only decreases oxidative stresses but also increases the activity of lydig cells, testosterone secretion and spermatogenesis. Anti-oxidant activity of rosemary is due to its sulfuric compounds which are really high in extract. This activity removes free radicals and also prevents active metabolites of cyclophosphamide drug [acrolein] and removes them.

CA is a lipophilic antioxidant that removes the most free radicals. Free radicals atoms and molecules that have an unpaired electron, including superoxide radical anion, hydroxyl radicals and lipid peroxy radicals may produce oxidative damage directly to critical biological molecules. Free radicals are generally the by-products of various endogenous processes that can have a noxious effect on cell macromolecules, such as proteins, lipoproteins, carbohydrates, DNA and RNA [9].

Researches showed that CA protects cortical neurons by activating the Keap1/Nrf2 pathway [10]. On the other hand, Satoh et al. suggested that the neuroprotective effects of CA critically require both free carboxylic acid and catechol hydroxyl moieties. Thus, neuroprotective effects of CA may be due to its hydrophilicity [11].

High free radicals have non desirable effects on reproduction activity and fertility of sperms and if these radicals are not controlled continuously, normal activity of cells will be destroyed [12].

Results of this study showed that FSH secretion was reduced significantly under heat stress [dose zero] whereas 100, 200, and 400 mg/mg doses increased it which shows positive effect of rosemary extract. FSH stimulates adenylate cyclase and increases cAMP by binding to Sertoli cells and causes secretion of ABP protein [Androgen binding protein]. This protein binds to testosterone and moves this androgen to seminiferous tubules for spermatogenesis [1].

FSH regulating feedback mechanism is not enforced only by testis steroids. Inhibin, activin and follistatin play roles also in FSH regulation by control effect on GnRH production. Significant FSH increase may be because of controlling effects of these factors. Heat stress increases free radicals and oxygen derivatives which have many negative effects on sexual potential [1].

Current study showed that rosemary extract protects serum concentration of testosterone via direct effect on male sexual controlling hormone system [13] and thereby protects testis tissue reduces heat stress side effects.

Conclusion

Results of this study showed that heat stress can affect sexual potential of male sex by reducing concentration of sexual hormones, but adding rosemary hydro alcoholic extract in 100, 200, and 400 mg/kg can compensate heat stress negative effects dose dependently. Therefore, it can be stated that rosemary extract can stabilize sexual potential in warm regions as a stress moderator.

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