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Role of Mentha Extraction on Productive Hormones of Male Rats

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ABSTRACT: Mentha are persistent herbs and its therapeutic-potential is largely attributed to its bioactive mixtures particularly menthol, menthone and essential-oil which show range of pharmacological-properties. This study aims to investigates the effects of *Mentha* extract on hormone levels, specifically testosterone and follicle-stimulating hormone (FSH) in a rat model. *Mentha* leaves were procured dried and ground into powder followed by extraction with distilled water at 70°C for 24 hours. The extract was administered to rats daily as supplement for one month. The control group and treated group were compared to assess in hormones changes. Results of this study revealed a significantly increasing in testosterone value in the treated group 5.612 ± 0.3517 ng/mL related to the control-group 4.464 ± 0.3747 ng/mL with *p*-value equal to 0.0302 which suggesting a potential influence of *Mentha* extract on testosterone-regulation. In addition, there was none significantly-differencing in FSH hormone-levels among the groups, as indicated by a non-significant *p*-value of 0.7949. The results of this study demonstrated significantly elevating in testosterone levels in the treated group compared to the control group, suggesting that *Mentha* extract may have a positive influence on testosterone level and regulation.

INTRODUCTION

Mentha also named as "mint" and represents a genus of aromatic-herbs within the "*Lamiaceae* family". Encompassing approximately 25 to 30 species [1]. These genuses thrives in a variety of temperate-regions around the world [2]. Renowned for its distinctive fragrance, diverse culinary applications and therapeutic-qualities. *Mentha* show significant ecological and economic importance. Mint plants or *Mentha* are perennial herbs, easily identified by their square-stems, opposite leaf arrangement and small flowers that range in color from white to purple or lavender [3].

Mint herbs has recognized for its significant role in traditional and modern medicine. The herbs therapeutic-potential is mainly attributed to its bioactive complex compounds mostly "menthol, menthone, and essential oil" which exhibit a range of pharmacological-properties [4]. Last studies have showing mint potential in the management of some chronic disease such as diabetes, cardiovascular diseases and cancer [5]. The antioxidant characteristics of *Mentha* compounds may play role in reducing oxidative-stress which is related to these circumstances [6]. In addition mint-derived products are being studied for their efficacy as natural-insect-repellents and their role in decreasing "antibiotic resistance" [7]. Beyond its immediate therapeutic uses, *Mentha* also plays important role in mental health, offering calming and stress-reducing effects through aromatherapy [8]. Additionally, its anti-inflammatory and antioxidant-properties contribute to the inhibition of chronic-diseases including "cardiovascular diseases and diabetes" [9]. With ongoing research uncovering new potential, Mint remains a cornerstone of natural medicine and indispensable asset for keeping human health and well-being.

Hormones play essential role in regulating the reproductive-system and regulatory for sexual development, gamete production and various processes essential for reproduction [10]. These hormones are produced by the gonads "testes and ovaries" and hypothalamus and the pituitary gland. Any difference in these hormones can lead to disorders such as infertility (men or

women), menstrual irregularities or delayed puberty [11]. This study aims to evaluate role of Mentha as medical herbs in improving the sexual hormone secretion in male rats by assay of testosterone and FSH hormones.

MATERIALS AND METHODS

Preparation of *Mentha* Leaves

Fresh *Mentha* leaves were obtained from a local-market and thoroughly cleaned by washing three times with running tap water followed by a final rinse with distilled-water to remove any impurities. The cleaned-leaves were air-dried in the dark in room temperature for several days to preserve their phytochemical integrity. Once dried, the leaves were finely ground into powder using a high-speed blender. The powdered Mentha was stored in an airtight container at room temperature in a dark environment to prevent "moisture absorption and degradation of active compounds".

Preparation of *Mentha* Extract

For Mint extract preparation, 200 g of the dried *Mentha* leaf powder was placed in a 500 mL flask containing distilled water. The mixture was heated and stirring in 70°C for 24 hours n to ensure efficient extraction of "bioactive compounds". After heating, the supernatants was filtered using "Buchner funnel" with Whatman-No.1 filter-paper. The supernatant was collected and transferred into an amber-colored container to prevent "light-induced degradation", and stored at 4°C [12].

Animal Study Design

The experiment utilized 50 "healthy male Wistar rats" each weighing between 350 and 500 g. Animals rats were randomly separated into two groups (n=25 per group):

Control Group: Received a standard pellet diet without supplementation.

Treatment Group: Received the standard pellet diet supplemented with *Mentha* extraction for one month.

All animals were housed in individually ventilated cages under controlled environmental circumstances ("22 ± 2°C, 12-hour light/12-hour dark cycle", and 50–"60% relative humidity") with ad libitum access to food and water [13]. The study was conducted in accordance with "ethical guidelines for the care and use of laboratory animals", and all procedures were approved by "the Institutional Animal Ethics Committee (IAEC)".

Blood Collection

At the end of the experimental-period, blood samples were collected from the animal rats via cardiac-puncture under anesthesia to minimize stress. Approximately 1 mL of blood per rat was transferred into gel separator tubes. The blood was centrifugation at 1600 rpm for 20 minutes to getting serum, which was then aliquoted into sterile microtubes and stored at −20°C for biochemical assay [14].

Hormones assay

Testosterone and follicle-stimulating hormone (FSH) in rat serum were measured using the Cobas e411 analyzer. The principle involves the specific binding of the hormone to a labeled antibody, followed by detection of electrochemiluminescent emission, which is proportional to the concentration of the hormone in the sample [15].

Statistical Analysis

All data were analyzed using "GraphPad Prism" (Version 9) software. The "independent t-test" method was used to compare the differences between the two groups. The results were considered statistically significant if the p-value was ≤ 0.05. All tables and figures were created using the same software (GraphPad Prism), and additional data organization and graphing were performed using "Microsoft-Excel 2020".

RESULTS

The extraction of *Mentha* (mint) provides a wide range of "health benefits" due to its bioactive-compounds mainly essential oils like menthol and menthone.

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Table 1: Result of testosterone distribution in Groups, p value ≤ 0.05

Groups	N	Mean ± SEM testosterone in ng/ml	Range	P value summary	95% confidence interval
Control	25	4.464 ± 0.3747	2 - 8	S*(0.0302)	0.1147 to 2.181
Treated	25	5.612 ± 0.3517	2 – 7.5		

Result in Table 1 reveal to significant-difference in testosterone hormone value in groups when the mean value of first group (control group) was 4.464 ± 0.3747 ng/mL and standard group was 5.612 ± 0.3517 ng/mL with p value 0.0302 as show in Figure 1.

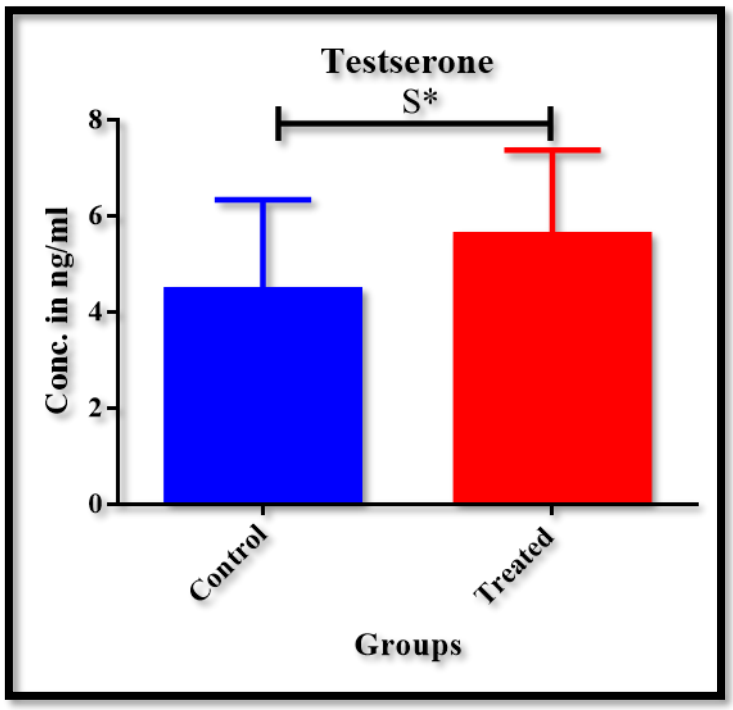

Figure 1: Testosterone value in Control and treated groups, p value ≤ 0.05

The increasing in testosterone levels observed in the experimental group suggests that the intervention (*Mentha*-extract) has a positive influence on testosterone production or secretion in the rat-animals. These results are consistent with previous studies that have suggested that "certain natural extracts including those from Mentha may influence endocrine-function and hormone regulation" [16]. The *p*-value of 0.0302 confirms that the observed difference is statistically significant at the commonly accepted when p value less of 0.05. This suggests that the main extraction has a meaningful-effect on testosterone levels, reducing the likelihood that the observed differences are due to random chance.

In contrast, the control group did not show any significant-change, supporting the reliability of results of the experimental-groups and supporting the hypothesis that the mint-extract may contribute to endocrine-modulation [17]. The results also suggest that Mint-extract could potentially be used as an adjunct-treatment for conditions related to low testosterone levels or other "hormonal-imbalances" though further studies are needed to confirm the long-term effects and mechanisms involved.

Table 2: Result of FSH distribution in Groups, p value ≤ 0.05

Groups	N	Mean ± SEM of FSH in ng/ml	Range	P value summary	95% confidence interval
Control	25	1.064 ± 0.1666	0.1 – 2.9	NS (0.7949)	-0.4550 to 0.5910
Treated	25	1.132 ± 0.1997	0.1 – 3.1		

As in Table 2, follicle stimulating hormone (FSH) show non-significant difference between groups when the mean value of control 1.064 ± 0.1666 ng/ml whereas the second group (Treated group) showed mean value 1.132 ± 0.1997 ng/ml and the p value was non-significant 0.7949 as showed in Figure 2.

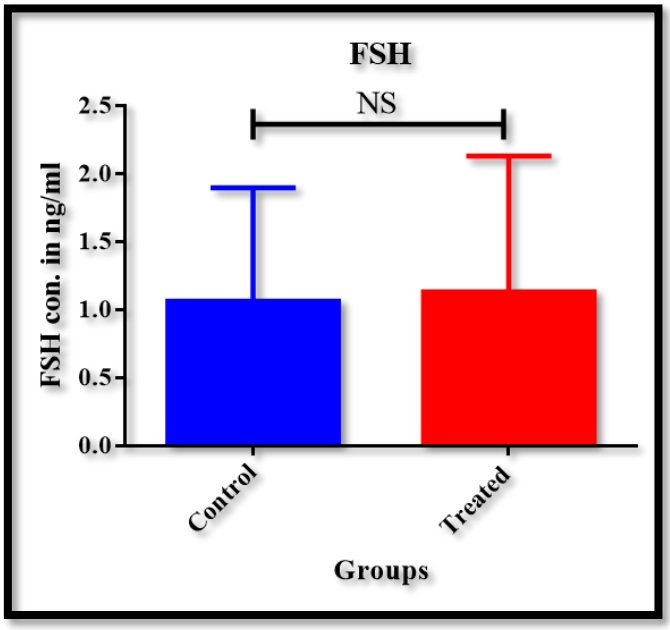

Figure 1: FSH value in Control and treated groups, p value ≤ 0.05

These results indicate that the *Mentha* –extract as administered in this study, did not exert a noticeable effect on "FSH secretion" or regulation in the rat-animals. Despite the slight increase in the mean FSH level in the treated group, the statistical analysis indicates that this difference is not large enough to be considered meaningful or to suggest any physiological-impact.

The non-significant p-value of 0.7949 is well above the conventional less than 0.05 which supports the conclusion that the observed difference is likely due to random variation rather than any effect of the treatment. Therefore, the Mentha extract does not appear to influence FSH levels in a significant manner in this study and further research may be essential to explore whether different dosages or treatment durations might have an effect on FSH level [18].

This finding contrasts with the observed significant increase in testosterone levels suggesting that the impact of Mentha extract might be specific to certain hormones, such as testosterone, but not others, like FSH [19].

DISCUSSION

The present study demonstrated that Mentha aqueous extract significantly increased serum testosterone levels in male rats without significantly altering FSH levels. These findings suggest a selective modulatory effect of Mentha on components of the hypothalamic–pituitary–gonadal axis.

Mentha species are rich in phenolic compounds and essential oils such as menthol and menthone, which are responsible for their diverse biological activities [1,3]. These bioactive constituents exhibit strong antioxidant and anti-inflammatory properties that may positively influence endocrine function [9]. Oxidative stress is known to impair Leydig cell steroidogenesis; therefore, antioxidant-rich plant extracts such as Mentha may enhance testosterone biosynthesis by reducing reactive oxygen species and protecting testicular tissue integrity [6].

The significant elevation in testosterone observed in the treated group is consistent with previous findings indicating that medicinal herbs can modulate endocrine activity and reproductive hormones [16]. Herbal preparations have been reported to influence gonadal steroid production either through direct stimulation of steroidogenic enzymes or through improvement of the testicular microenvironment [17]. The increase observed in the present study may therefore reflect enhanced steroidogenic efficiency rather than central stimulation of gonadotropins.

Testosterone production is primarily regulated by luteinizing hormone, whereas FSH mainly regulates Sertoli cell function and spermatogenesis [10]. The absence of a significant difference in FSH levels between groups suggests that Mentha extract did not significantly affect anterior pituitary secretion. This indicates that the extract's effect may occur at the testicular level rather than through hypothalamic or pituitary modulation. The selective hormonal effect observed here aligns with previous reports on herbal therapies used in reproductive disorders, where certain botanicals improved steroid hormone profiles without uniformly affecting all gonadotropins [19]. Such selective endocrine modulation may explain why testosterone levels increased while FSH remained unchanged in this experiment.

Mentha has been traditionally used in reproductive and gynecological conditions, and evidence-based evaluations have documented its hormonal regulatory potential [16]. Clinical research investigating Mentha preparations in menstrual and reproductive disorders also suggests hormonal modulation effects, although mechanisms vary depending on species, dosage, and preparation method [18]. The aqueous extraction used in this study may preferentially extract hydrophilic compounds capable of influencing steroidogenesis. The antioxidant properties of Mentha are particularly relevant to male reproductive physiology. Reactive oxygen species can disrupt mitochondrial function in Leydig cells and impair cholesterol conversion into testosterone. Mentha species have demonstrated potent antioxidant capabilities [9], and studies have shown their role in mitigating oxidative stress-related cellular damage [6]. This protective mechanism could underlie the significant increase in testosterone observed in the treated group. In addition to antioxidant effects, Mentha exhibits anti-inflammatory properties [4]. Chronic low-grade inflammation has been associated with suppression of reproductive hormone synthesis. By reducing inflammatory mediators, Mentha extract may indirectly support normal testosterone production. This anti-inflammatory mechanism may further contribute to the hormonal enhancement observed in this study. The non-significant change in FSH levels is also clinically relevant. Disturbances in gonadotropins can lead to endocrine disorders and infertility [11]. The absence of significant FSH alteration suggests that Mentha supplementation, at the administered dose and duration, does not disrupt central endocrine regulation, supporting its potential safety in hormonal modulation. It is also possible that the one-month duration of treatment was sufficient to affect peripheral steroidogenesis but insufficient to produce measurable changes in pituitary hormone secretion. Hormonal feedback mechanisms are complex, and longer exposure periods may yield different results.

Mentha has been widely studied for its nutritional and therapeutic attributes, including cardiovascular and metabolic benefits [5]. Increasing attention is now directed toward its systemic physiological effects, including endocrine modulation [2]. The findings of the present study add to this growing body of evidence by demonstrating a measurable influence on male reproductive hormones. Although these findings are promising, translation to human application requires careful consideration. Herbal extracts can vary in composition depending on species, cultivation conditions, and extraction techniques [3]. Therefore, standardization and dose optimization are necessary before considering clinical application. Limitations of this study include the absence of luteinizing hormone measurement, lack of histopathological examination of testicular tissue, and evaluation of only one dosage and duration. Future studies should explore dose–response relationships, longer treatment periods, and molecular pathways involved in steroidogenic regulation. Further investigation is also needed to identify the specific active compounds responsible for the testosterone-enhancing effect. Mentha contains diverse phytochemicals [1,3], and isolating these compounds may clarify the mechanism of action. Overall, the present findings demonstrate that Mentha extract exerts a selective endocrine effect by significantly increasing testosterone levels without altering FSH. This supports the hypothesis that Mentha may enhance testicular steroidogenesis through antioxidant and anti-inflammatory mechanisms while maintaining central hormonal balance.

CONCLUSION

The results confirmed a significant increase in testosterone hormone levels in the treated-group compared to the control-group which reveal that mint -extract may have a positive influence on testosterone secretion or regulation. Nevertheless, no significantly-difference was observed in the levels of follicle-stimulating hormone (FSH), indicating that the extract did not affect this particular hormone.

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