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Original article

Effects of Libyan *Balanites aegyptiaca* Extract on Steroid Hormone Levels in Male Rabbits

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Abstract
Balanites aegyptiaca, commonly known as the desert date or Heglig, is a multifunctional tree native to arid and semi-arid regions of Africa and the Middle East, including Libya. Traditionally, it has been used to treat diabetes, liver disorders, skin infections, and reproductive problems due to its rich content of bioactive compounds such as flavonoids, saponins, alkaloids, and phenolic compounds. Phytochemical studies indicate that steroidal saponins in *B. aegyptiaca* are structurally similar to compounds involved in steroid hormone biosynthesis, potentially influencing male reproductive functions. Despite its ethnomedicinal relevance, limited research has investigated the effects of Libyan *B. aegyptiaca* on steroid hormone modulation in male rabbits. This study aimed to evaluate the effects of Libyan *B. aegyptiaca* extract on steroid hormone levels, including testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and cortisol in male rabbits. Ripe fruits of *B. aegyptiaca* were collected from the Sebha region of southwest Libya, authenticated by a botany expert, and processed into a fine powder. Twenty healthy male rabbits were randomly assigned to four groups (n=5). The treatment group received 100 mg/kg body weight of *B. aegyptiaca* extract orally every other day for six weeks, while the control group received distilled water. Serum hormone levels were measured using ELISA kits. Statistical analysis was performed using ANOVA followed by Tukey's multiple comparison test, with significance set at $P < 0.05$. Administration of *B. aegyptiaca* extract significantly increased plasma testosterone (2.158 ± 0.037 ng/L vs. 1.627 ± 0.077 ng/L) and LH levels (0.856 ± 0.005 mIU/ml vs. 0.780 ± 0.017 mIU/ml) compared to controls ($p < 0.05$). FSH showed a non-significant upward trend. Estrogen (27.415 ± 0.111 pg/ml vs. 28.691 ± 0.361 pg/ml) and cortisol (49.70 ± 3.487 ng/ml vs. 59.92 ± 1.244 ng/ml) levels were significantly reduced in treated rabbits ($p < 0.05$), suggesting adaptogenic and anti-stress effects. These hormonal changes indicate that *B. aegyptiaca* may positively modulate the hypothalamic-pituitary-gonadal (HPG) axis and stress-related endocrine pathways.
Keywords. *Balanites aegyptiaca*, Steroid Hormones, Rabbits, Reproductive Health.

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Introduction

The multifunctional tree Balanites aegyptiaca, also called the desert date, is indigenous to dry and semi-arid parts of Africa and the Middle East, including Libya [1]. In folk medicine, it has long been used to cure a variety of conditions, including diabetes, liver problems, skin infections, and reproductive problems [2]. The bioactive elements of B. aegyptiaca, including flavonoids, saponins, alkaloids, and phenolic compounds, which support its pharmacological actions, have drawn attention recently [3]. However, little research has been done on Libyan B. aegyptiaca's physicochemical properties, particularly its phytochemical profile and chemical stability, which calls for more investigation into the plant's safety and potential for treatment [4]. Its various parts—particularly the fruit, seeds, and leaves—have been utilized in traditional medicine for their purported therapeutic properties, including antidiabetic, antioxidant, and anti-inflammatory effects [5]. Phytochemical analyses have identified several bioactive compounds in B. aegyptiaca, notably steroidal saponins, which are structurally similar to compounds involved in steroid hormone biosynthesis. These compounds have been shown to influence reproductive functions in male rodents. For instance, an aqueous extract of B. aegyptiaca demonstrated protective effects against reproductive toxicity induced by aluminum chloride in male rats, suggesting its potential to modulate steroid hormone levels [6]. In the context of animal husbandry, particularly rabbit farming, understanding the impact of phytochemical-rich plant extracts on reproductive health is crucial. While studies have explored the effects of various plants on rabbit reproduction, there is a paucity of research focusing on the specific impact of B. aegyptiaca on steroid hormone levels in male rabbits [7]. This study aims to fill this gap by investigating the effects of Libyan B. aegyptiaca extract on steroid hormone levels in male rabbits. The findings could provide valuable insights into the potential applications of B. aegyptiaca in enhancing reproductive health and productivity in rabbit farming.

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Methods

This study was carried out in July 2024 at the Chemistry Department of the Faculty of Science at Omar Al-Muktar University. Balanites aegyptiaca (L.) Delile, often referred to as "Heglig" or desert date in Libya, was the plant material used in this investigation. During the summer of 2024, the ripe fruits were harvested from trees that grew naturally in the Sebha area of southwest Libya. The native spread of B. aegyptiaca is supported by the sandy soil and dry climate of this region. A botany expert from Sebha University's Faculty of Science verified the plant's taxonomy, and a voucher specimen was placed in the university's herbarium for future use. Following collecting, the fruits were carefully cleaned to get rid of dust and debris before being allowed to air dry for two weeks at room temperature in a well-ventilated, shady location. The fruit pulp was manually removed from the seeds when it had completely dried, and an electric grinder was used to crush it into a fine powder.

Until additional extraction and analysis were completed, the powdered material was kept at 4°C in sealed, amber-colored glass containers to shield it from light and moisture. We purchased twenty healthy, robust male rabbits from reputable local farms. The room in which these rabbits were kept was suitable for the trial period and was equipped in compliance with the US-EPA 2004. The principles and standards of the Libyan Ministry of Agriculture, as well as the US-EPA 2004 for animal care, were followed in the care of the rabbits. Every rabbit was housed in an appropriate steel cage that had a 12-hour light cycle, a temperature between 22 and 26°C, and a humidity level between 40 and 70%. A suitable diet comprising clean water and balanced feed has been provided for the duration of the study. The animals received the following treatment after being randomly assigned to four groups, each consisting of five rabbits: Group 1: For six weeks, each rabbit received 100 mg/kg body weight of Balanites aegyptiaca (L.) orally every other day [8]. Group 2: was given eight milliliters of distilled water orally for six weeks as a control. ELISA kits were used to measure the levels of serum testosterone, cortisol, and estradiol in accordance with the manufacturer's instructions.

When necessary, statistical analysis was performed using GraphPad Prism 8 or Minitab software (version 17). After determining that the data had a normal distribution, ANOVA analysis was conducted using the Tukey multiple comparison test in order to achieve a significance level of $P < 0.05$.

Results

Male rabbits' hormonal profiles changed significantly after receiving Balanites aegyptiaca extract, indicating that it may modulate the hypothalamic-pituitary-gonadal (HPG) axis and stress-related endocrine pathways (Table 1; Figures 1–5). The treated group's testosterone levels were significantly higher (2.158 ± 0.037 ng/L) than the control group's (1.627 ± 0.077 ng/L, $p<0.05$), suggesting that Balanites aegyptiaca may promote Leydig cell activity or androgen production.

Additionally, the treated animals' levels of luteinizing hormone (LH) were considerably higher (0.856 ± 0.005 mIU/ml) than those of the controls (0.780 ± 0.017 mIU/ml, $p<0.05$). This might be due to increased pituitary production after hypothalamic activation by gonadotropin-releasing hormone (GnRH). On the other hand, the treated group's follicle-stimulating hormone (FSH) levels showed a non-significant upward trend (0.970 ± 0.015 mIU/ml vs. 0.844 ± 0.016 mIU/ml), indicating that although Balanites aegyptiaca may have a minor impact on Sertoli cell function, its effect on hormonal control related to spermatogenesis may be less noticeable. The treated group's estrogen levels were substantially lower (27.415 ± 0.111 pg/ml) than the controls' (28.691 ± 0.361 pg/ml, $p<0.05$). Furthermore, the treated rabbits' cortisol levels were significantly lower (49.70 ± 3.487 ng/ml) than the controls' (59.92 ± 1.244 ng/ml, $p<0.05$). The reduction in cortisol, a major stress hormone, suggests that Balanites aegyptiaca may have an adaptogenic or anti-stress impact. This might be due to its anti-inflammatory and antioxidant qualities, which reduce the activation of the hypothalamic-pituitary-adrenal (HPA) axis**.** All things considered, these results point to the possibility that Balanites aegyptiaca may be beneficial for both reproduction and stress reduction, making it a viable natural treatment option for illnesses linked to stress and reproductive failure.

Table 1. Plasma testosterone, luteinizing Hormone, follicle-stimulating hormone, estrogen, and cortisol of male rabbits treated with Balanites aegyptiaca

Parameters	***Experimental groups***	
	Control	**Balanites aegyptiaca**
Testosterone *(ng/l)*	1.627±0.077[a]	2.158±0.037[b]
LH (mIU/ml)	0.780±0.017[b]	0.856±0.005[a]
FSH (mIU/ml)	0.844±0.016[a]	0.970±0.015 [a]
Estrogen (pg/ml)	28.691±0.361[a]	27.415±0.111[b]
Cortisol (ng/ml)	59.92±1.244[a]	49.70±3.487[b]

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For each treatment group (n = 5), the means ± SE are given. Significant differences ($p<0.05$) were noted when mean values in a row did not share a common superscript letter (a, b, or c).

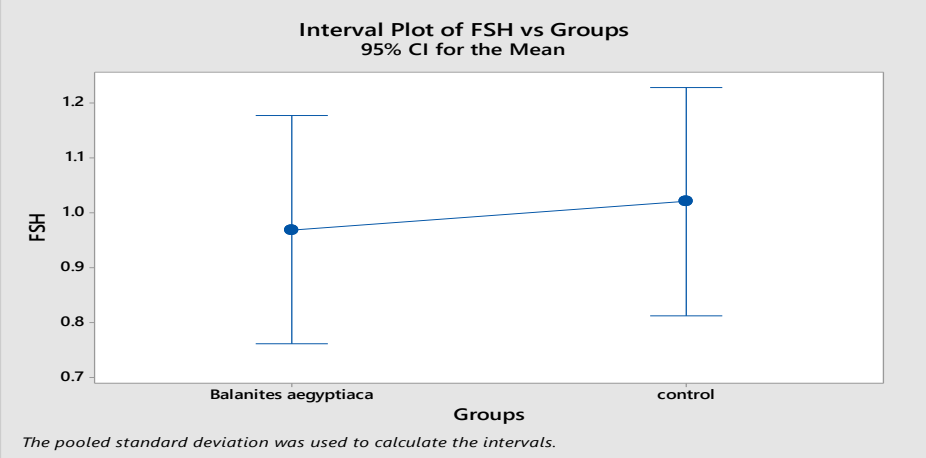

Figure 1. Alteration in Follicle Stimulating Hormone during Balanites aegyptiaca therapy in male rabbits

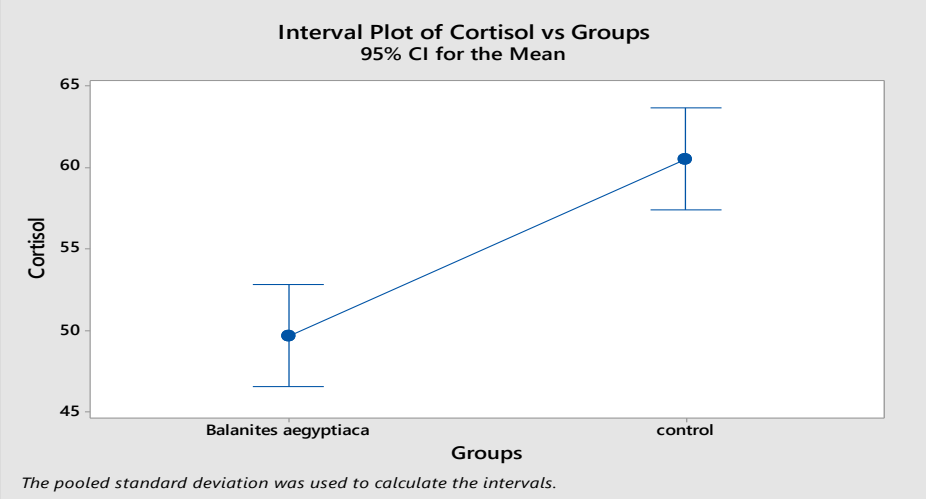

Figure 2. Cortisol changes in male rabbits treated with Balanites aegyptiaca.

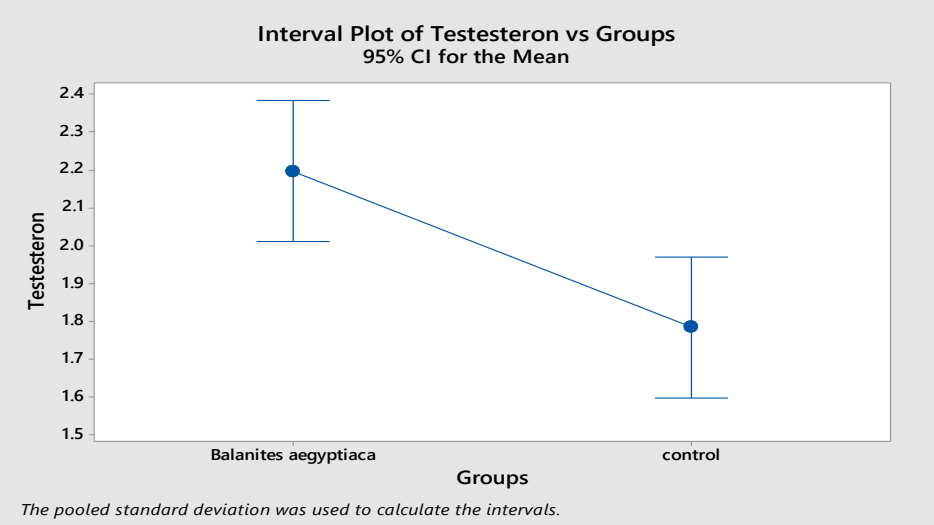

Figure 3. Testosterone changes in male rabbits treated with Balanites aegyptiaca

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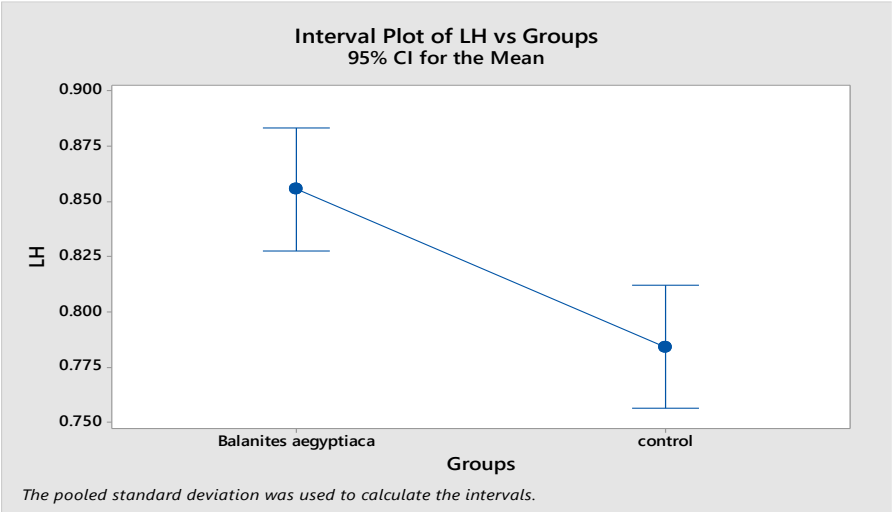

Figure 4. Modification of luteinizing hormones in male rabbits treated with balanites aegyptiaca

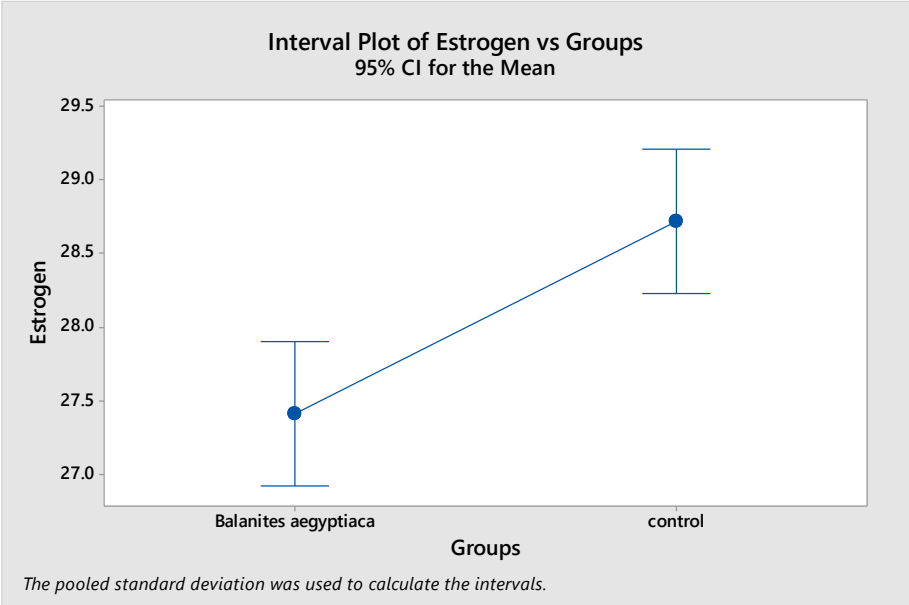

Figure 5. Alteration of estrogen hormone in male rabbits treated with Balanites aegyptiaca

Discussion

The extract may stimulate androgen synthesis, as evidenced by the considerable rise in plasma testosterone levels in the group treated with Balanites aegyptiaca as compared to the control group ($p < 0.05$). Phytochemicals like saponins and flavonoids, which are known to increase Leydig cell activity, promote testosterone production, and shield testicular tissue from oxidative damage, may be responsible for this improvement. Saponin-rich plant extracts have been shown in several studies to improve male animal steroidogenesis and reproductive function [1]. Additionally, luteinizing hormone (LH) levels in the treated group were significantly higher than in the control group ($p < 0.05$), suggesting that the testes' Leydig cells were more stimulated. The rise of LH, which is essential for controlling testosterone production, indicates that Balanites aegyptiaca may increase the activity of the hypothalamic-pituitary-gonadal (HPG) axis. Studies showing that antioxidants derived from plants can enhance the endocrine control of male reproductive hormones and modify pituitary function lend credence to this study [9]**.** Additionally, compared to controls, follicle-stimulating hormone (FSH) rose in the treated rabbits; however, this difference was not statistically significant ($p > 0.05$). FSH stimulates Sertoli cell activity, which is essential for spermatogenesis. Improved testicular function and spermatogenic potential might be the cause of the higher trend in FSH levels.

According to earlier studies, phytochemicals can increase the release of FSH by protecting the gonads from oxidative stress and regulating the release of gonadotropin-releasing hormone (GnRH) [10]. When compared to controls, the group treated with Balanites aegyptiaca had marginally but considerably lower

estrogen levels ($p < 0.05$). A change in steroidogenesis that prioritizes androgen synthesis over aromatization to estrogen might be the cause of this decline. In male reproductive physiology, where high estrogen can inhibit gonadotropin release and affect testicular function, such hormonal regulation is advantageous. In male models, plant antioxidants have been demonstrated to decrease estrogen production and inhibit aromatase activity [11]. An index of physiological stress, cortisol levels, were considerably lower in the treated group than in the control group ($p < 0.05$). This decrease implies that Balanites aegyptiaca has an adaptogenic or anti-stress impact. Prolonged stress raises cortisol levels, which inhibit immunological and reproductive hormones. By altering the hypothalamic-pituitary-adrenal (HPA) axis and strengthening antioxidant defenses, the plant's phenolic and flavonoid components most likely helped lower cortisol levels [12]. The numerical results are corroborated by the graphical representations (Figures 24 to 28), which clearly show hormonal changes that promote stress reduction and reproductive enhancement. According to Abd [13, 14], these findings support the possible use of Balanites aegyptiaca as a natural medicinal agent to enhance male fertility and hormonal balance.

Conclusion

Balanites aegyptiaca extract demonstrates the potential to enhance male reproductive hormone levels and reduce physiological stress in rabbits. Its bioactive phytochemicals, particularly steroidal saponins and flavonoids, likely contribute to increased testosterone and LH production while decreasing estrogen and cortisol levels. These findings support the potential use of *B. aegyptiaca* as a natural therapeutic agent for improving male reproductive health and managing stress-related hormonal imbalances.

Conflict of interest. Nil

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