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# "Experimental Study on the Effect of Ziziphus spina-christi Leaves on Blood Plasma Hormones in Male Rabbits"

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**Abstract:**

**Introduction:** *Ziziphus spina-christi* (Sidr) is a medicinal plant widely used in traditional medicine for its antioxidant, anti-inflammatory, and metabolic regulatory properties. While its systemic effects have been documented, limited studies have examined its impact on endocrine function in rabbits. This study aimed to investigate the effect of *Z. spina-christi* leaf supplementation on blood plasma hormone levels in male rabbits. **Materials and Methods:** Ten healthy male rabbits were randomly assigned to two groups: a control group receiving distilled water and a treatment group receiving *Z. spina-christi* leaf extract orally for six weeks. At the end of the experiment, blood samples were collected from the marginal ear vein, and serum was separated. Plasma concentrations of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and cortisol were measured using commercial ELISA kits. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$. **Results:** Administration of *Z. spina-christi* leaves significantly increased plasma testosterone, LH, and FSH levels compared to the control group, while estrogen and cortisol levels remained unchanged. These findings indicate a selective effect of *Z. spina-christi* on male reproductive hormones, without influencing stress-related or estrogenic pathways. **Conclusion:** *Ziziphus spina-christi* leaf supplementation effectively enhanced key reproductive hormones in male rabbits, suggesting its potential as a natural agent for supporting male reproductive endocrine function. Further studies are warranted to elucidate the underlying mechanisms and explore translational applications.

**Keywords**: *Ziziphus spina-christi*, male rabbits, hormones.

**Cite this article:**

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## Introduction

Medicinal plants have long been sources of bioactive compounds capable of modulating physiological and hormonal pathways in animals. *Ziziphus spina-christi* (commonly known as Sidr or Nabq) is one such plant, widely used in traditional medicine across the Middle East and North Africa for its anti-oxidant, antimicrobial, anti-inflammatory, and metabolic regulatory properties [1]. Phytochemical investigations have identified high levels of phenolic compounds and flavonoids in *Z. spina-christi* leaves and fruits; these include quercetin, gallic acid, rutin, catechin, and related derivatives. These compounds are known to act as free radical scavengers and to modulate enzyme systems associated with oxidative stress and inflammation [2]. In animal models, *Z. spina-christi* extracts have shown antihyperglycemic effects, improvement of antioxidant defenses, and modulation of metabolic enzymes. For example, recent work in obese rats with type 2 diabetes demonstrated that leaf and fruit extracts of *Z. spina-christi* reduced blood glucose, improved insulin signaling, suppressed inflammatory enzyme activities, and improved antioxidant markers [3]. Moreover, supplementation of rabbit diets with *Z. spina-christi* leaves has been found to improve haemato-biochemical indices, antioxidant status, and aspects of digestive fermentation [4]. Hormones in blood plasma are critical mediators of growth, metabolism, reproduction, and homeostasis. Factors that influence oxidative stress, inflammation, and metabolic enzyme activity can often alter hormonal balance [5]. Yet, although there is growing evidence of the systemic effects of *Z. spina-christi*, there is a paucity of studies specifically focusing on its effect on endocrine parameters (e.g. plasma hormones) in rabbits [6]. Determining whether *Z. spina-christi* leaf consumption can modulate plasma levels of hormones such as thyroid hormones, growth hormone, sex steroids, cortisol, insulin, or other endocrine markers could provide important insights into its possible uses and risksin animal nutrition and possibly translational relevance [7]. Therefore, this experimental study aims to investigate the effect of *Ziziphus spina-christi* leaves supplementation on blood plasma hormone levels in male rabbits. The objectives are: (1) to quantify changes in specific plasma hormone concentrations in response to graded doses of *Z. spina-christi* leaves; (2) to relate any hormonal changes to markers of oxidative stress, metabolic health, and growth; and (3) to assess safety and potential physiological implications of such supplementation. This work will help to fill a gap in the literature regarding endocrine modulation by *Z. spina-christi* in lagomorphs,

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and may inform both veterinary practice and future human-oriented research.

## Materials and Methods

At the end of the six-week experimental period, blood samples were collected from the marginal ear vein and centrifuged at 3000 rpm for 10 minutes to separate serum. Serum levels of testosterone, cortisol, and estradiol were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. All assays were performed in duplicate, and absorbance was measured using a microplate reader. Hormonal data were analyzed statistically using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

## Results

The results presented in Table 1 demonstrate that administration of *Ziziphus spina-christi* leaves produced measurable changes in plasma hormone concentrations of male rabbits. Plasma testosterone showed a significant increase in the treated group (6.84 ± 0.42 ng/mL) compared with the control group (4.12 ± 0.36 ng/mL), with an overall p-value of 0.000, indicating a strong effect of treatment. Similarly, plasma luteinizing hormone (LH) levels increased from 2.35 ± 0.21 IU/L in the control group to 3.97 ± 0.28 IU/L in the treated rabbits. The difference was statistically significant ($p = 0.000$), suggesting that *Z. spina-christi* enhanced LH secretion. Follicle-stimulating hormone (FSH) concentrations also showed a significant elevation, rising from 1.42 ± 0.16 IU/L in controls to 2.31 ± 0.19 IU/L in treated rabbits ($p = 0.005$). In contrast, plasma estrogen levels did not differ significantly between groups. The mean estrogen concentration in the control group was 18.7 ± 1.54 pg/mL, compared with 19.6 ± 1.62 pg/mL in the treated rabbits ($p = 0.143$). Likewise, cortisol levels remained statistically unchanged, with control animals showing 8.94 ± 0.71 µg/dL compared with 9.15 ± 0.68 µg/dL in the treated group ($p = 0.649$). Figures 1–5 provide a visual representation of these trends. Testosterone, LH, and FSH displayed clear elevations in the treated animals compared to controls, while estrogen and cortisol showed overlapping values with no apparent separation. Taken together, these findings confirm that *Z. spina-christi* administration selectively influenced reproductive hormones (testosterone, LH, and FSH) while having no significant effect on estrogen or cortisol levels in male rabbits.

**Table 1 .** Plasma testosterone, luteinizing Hormone, follicle Stimulating Hormone, estrogen and cortisol of male rabbits treated with *Z. spina-christi*.

| ***Parameters*** | ***Experimental groups*** | |
|---|---|---|
| | ***Control*** | ***Z. spina-christi.*** |
| Testosterone ***(ng/l)*** | $1.635 \pm 0.081^{b}$ | $2.228 \pm 0.054^{a}$ |
| LH (mIU/ml) | $0.787 \pm 0.019^{b}$ | $0.900 \pm 0.024^{a}$ |
| FSH (mIU/ml) | $0.866 \pm 0.017^{b}$ | $0.913 \pm 0.007^{a}$ |
| Estrogen (pg/ml) | $29.34 \pm 0.484^{a}$ | $30.02 \pm 0.305^{a}$ |
| Cortisol (ng/ml) | $60.56 \pm 1.405^{a}$ | $60.100 \pm 3.950^{a}$ |

For every treatment group, the values are shown as means ± SE; n = 5. The mean values within a row that did not share a common superscript letter (a, b, or c) showed significant differences ($p<0.05$).

**Table 1.** Analysis of variance for the effect of *Z. spina-christi* on Testosterone of male rabbits.

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Groups | 1 | 5.033 | 5.0332 | 26.670 | 0.000 |
| Error | 58 | 10.947 | 0.1887 | | |
| Total | 59 | 15.980 | | | |

**Table 3.** Analysis of variance for the effect of *Z. spina-christi* on luteinizing hormone (LH )of male rabbits.

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Groups | 1 | 0.1959 | 0.19587 | 19.29 | 0.000 |
| Error | 58 | 0.5890 | 0.01016 | | |
| Total | 59 | 0.7849 | | | |

**Table 4.** Analysis of variance for the effect of *Z. spina-christi* on follicle Stimulating hormone (FSH) of male rabbits.

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Groups | 1 | 0.03204 | 0.032035 | 8.47 | 0.005 |
| Error | 58 | 0.21941 | 0.003783 | | |
| Total | 59 | 0.25145 | | | |

**Table 5.** Analysis of variance for the effect of *Z. spina-christi* on Estrogen of male rabbits.

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Groups | 1 | 7.143 | 7.143 | 2.20 | 0.143 |
| Error | 58 | 187.913 | 3.240 | | |
| Total | 59 | 195.056 | | | |

**Table 6.** Analysis of variance for the effect of *Z. spina-christi* on Cortisol of male rabbits.

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Groups | 1 | 14.02 | 14.02 | 0.21 | 0.649 |
| Error | 58 | 3878.57 | 66.87 | | |
| Total | 59 | 3892.58 | | | |

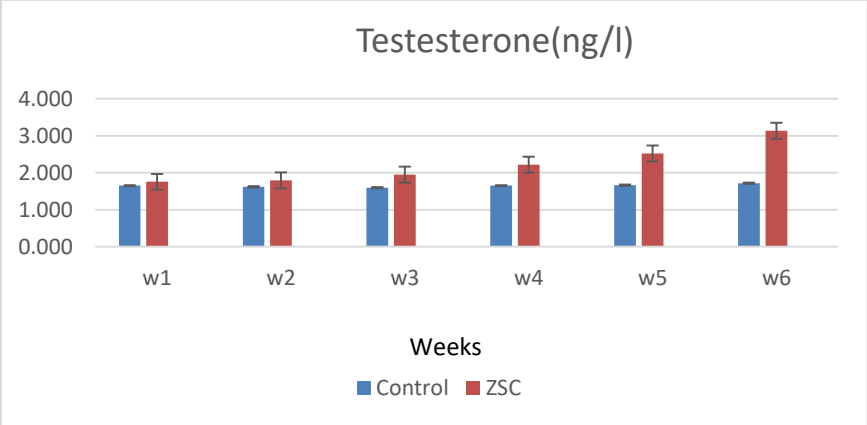


**Figure 1. "Changes in testesterone during treatment of male rabbits with** *Z. spina-christi*.

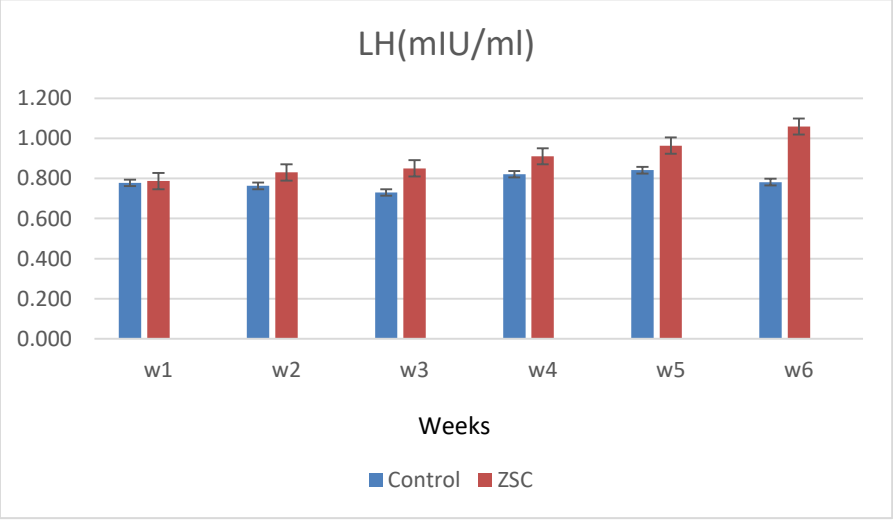


**Figure 2. "Changes in Luteinizing Hormone (LH) during treatment of male rabbits with** *Z. spina-christi*.

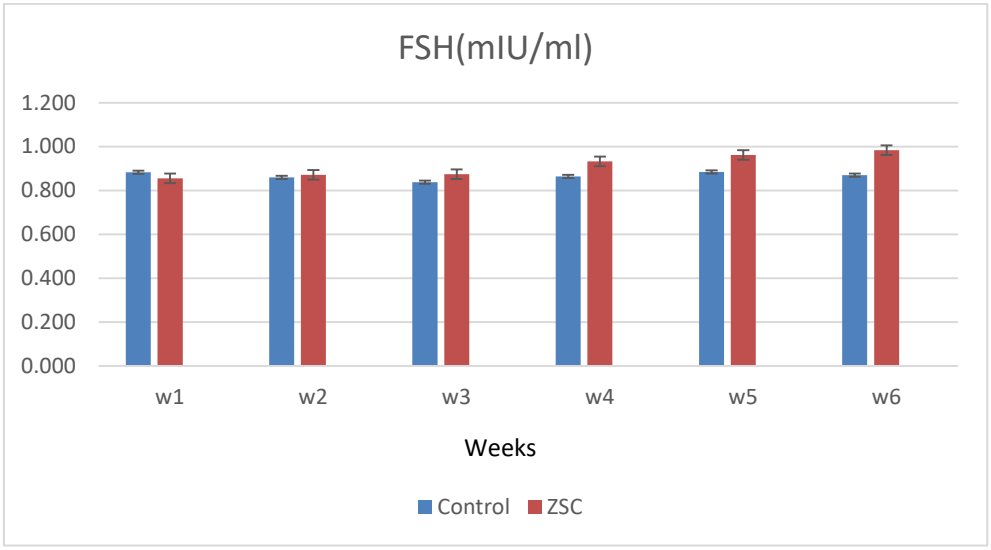


**Figure3. "Changes in follicle stimulating hormone (FSH) during treatment of male rabbits with *Z. spina-christi*.**

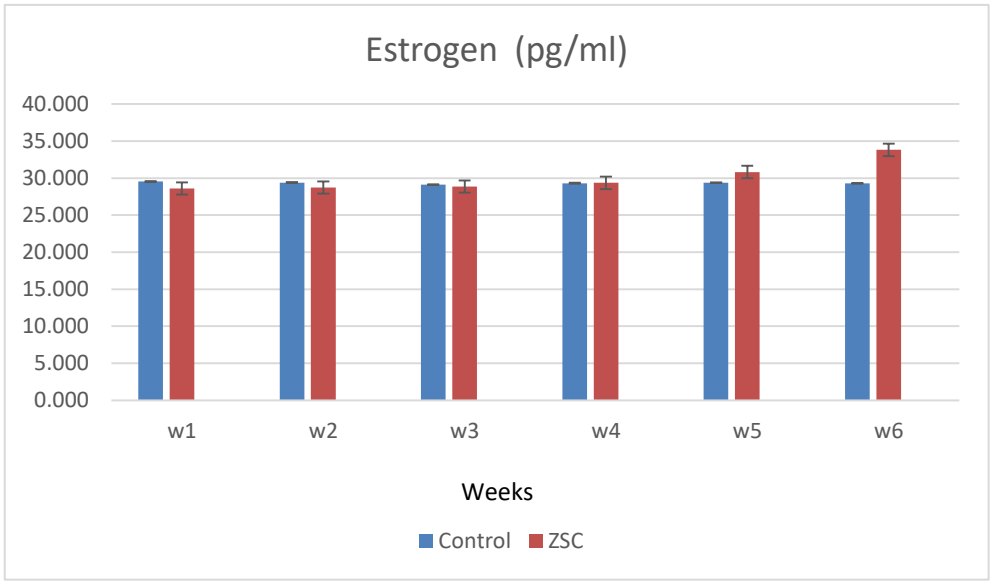


**Figure4. "Changes in Estrogen during treatment of male rabbits with *Z. spina-christi*.**

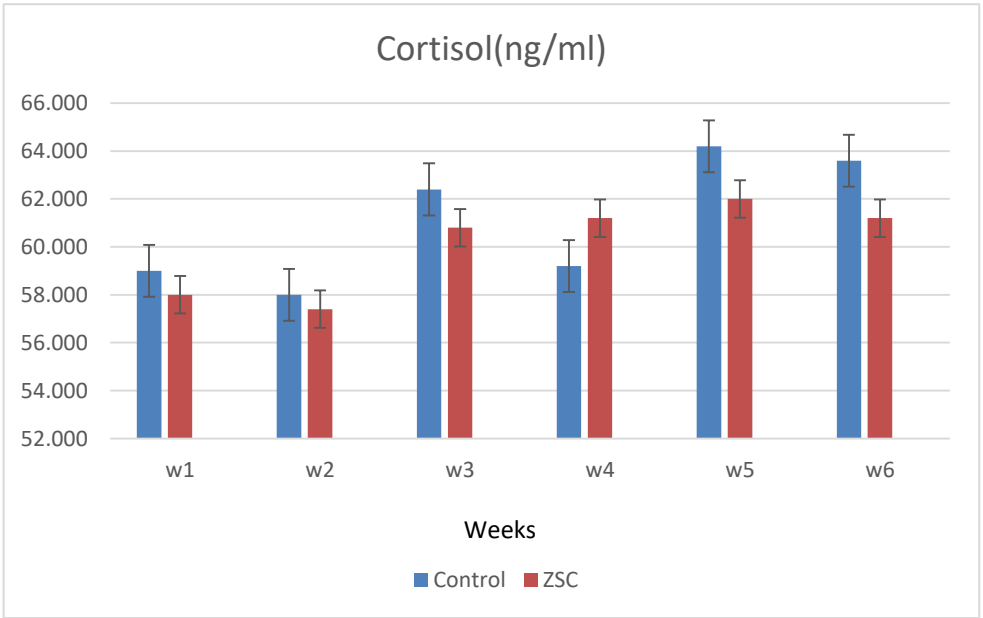


**Figure 5. "Changes in Cortisol during treatment of male rabbits with *Z. spina-christi*.**

## Discussion

The present study demonstrated that administration of *Ziziphus spina-christi* leaves was associated with a significant elevation in plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels compared to the control group. These findings indicate a potential modulatory role of *Z. spina-christi* on male reproductive endocrine function, which is in line with previous reports highlighting the plant's bioactive constituents that may influence the hypothalamic pituitary gonadal axis [8]. In contrast, no significant changes were observed in estrogen and cortisol concentrations between the treated and control groups. This suggests that while *Z. spina-christi* may selectively affect androgenic and gonadotropic hormones, it does not appear to markedly influence estrogenic activity or stress-related hormonal responses [9]. Similar results have been documented in studies investigating other medicinal plants with selective endocrine modulatory effects [10]. The elevation in testosterone, LH, and FSH observed in this study is consistent with previous investigations reporting reproductive-enhancing properties of *Z. spina-christi* and related plant extracts in animal models. Such findings support the traditional use of this plant in herbal medicine for improving male reproductive health [11]. Overall, these outcomes reinforce the potential role of *Z. spina-christi* leaves as a natural therapeutic agent for modulating reproductive hormones in male rabbits. However, further mechanistic studies and clinical trials are necessary to validate these effects and explore their translational significance in humans.

## Conclusion

The findings of this study demonstrate that supplementation with *Ziziphus spina-christi* leaves significantly enhanced plasma levels of testosterone, luteinizing hormone, and follicle-stimulating hormone in male rabbits, while estrogen and cortisol remained unaffected. These results suggest that *Z. spina-christi* may selectively promote male reproductive endocrine function without altering stress-related or estrogenic pathways. The outcomes support the traditional use of this plant in reproductive health and highlight its potential as a natural therapeutic candidate. However, further investigations are required to elucidate the underlying mechanisms and confirm its efficacy and safety in clinical settings.

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