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Comparative Endocrine and Metabolic Effects of Walnut Oil, Saussurea costus, and L-Carnitine in Domperidone-Induced Hyperprolactinemia: An Experimental Rabbit Model

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I. ABSTRACT

Background:

A frequent endocrine condition that impacts both metabolic and reproductive processes is hyperprolactinemia. Hormonal imbalance is frequently linked to disruptions in dopaminergic control. Natural substances have recently drawn interest because of their possible regulatory and antioxidant properties.

Objective:

The purpose of this study was to compare the preventive effects of L-carnitine, walnut oil, and Saussurea costus oil on endocrine and metabolic changes in a male rabbit model of hyperprolactinemia caused by domperidone.

Methods:

The goal of the current study was to assess the potential protective effects of L-carnitine, walnut oil, and Saussurea costus oil against the hormonal and metabolic changes caused by domperidone in male rabbits. Twenty-five adult male rabbits were split into five groups at random for this purpose: a control group, a hyperprolactinemic group, and three treatment groups that received costus oil, walnut oil, or L-carnitine in addition to domperidone over a 30-day period.

Results:

The treatment of domperidone considerably increased serum prolactin, cortisol and fasting blood glucose levels and significantly decreased testosterone concentrations compared with the negative control group ($P < 0.05$). Saussurea costus oil and Walnut oil treatments significantly decreased the prolactin level. Walnut oil showed the strongest prolactin reduction impact. Costus oil treatment showed the biggest improvement in testosterone levels in all treated groups. Costus oil and walnut oil also lowered the fasting blood glucose compared to the hyperprolactinemic group. L-Carnitine exhibited limited endocrine benefit.

Analysis of lipid profile measures revealed that triglyceride levels were increased in all treated groups as compared to control. L-carnitine therapy showed a significant increase in HDL-C and relatively low LDL-C levels, indicating a better influence on lipid metabolism. On the other hand, VLDL-C was increased in the costus oil and walnut oil groups. Overall, walnut oil and costus oil showed more significant protective benefits against endocrine disorders, but L-carnitine showed greater lipid-modulating action.

Conclusion:
Overall, the results point to a potential function for walnut oil and Saussurea costus in mitigating some of the endocrine disorders linked to hyperprolactinemia. However, their effect on metabolic markers seems to be less significant, suggesting that more research is necessary to fully comprehend their methods of action and their therapeutic uses.

***Keywords** :Hyperprolactinemia, Domperidone, Walnut oil , Saussurea costus , L-carnitine, Prolactin, Testosterone, Lipid profile, Endocrine regulation*

II. INTRODUCTION

Elevated levels of prolactin, a peptide hormone released by lactotroph cells of the anterior pituitary gland, are the hallmark of hyperprolactinemia, a frequent endocrine condition [1]. Prolactin and growth hormone are structurally similar, but prolactin is transcribed by a different gene and has many molecular forms, the most common of which has 199 amino acids and a molecular weight of about 23 kDa [2]. In physiological settings, prolactin is crucial for reproductive function, particularly during lactation, although there is rising evidence for involvement in both immune responses and metabolic management.

Many physiological, pharmacological and pathological conditions may cause elevation of the prolactin level. Diseases like hypothyroidism, renal insufficiency, hepatic dysfunction and pituitary tumors are known to cause elevation of prolactin secretion. Some medicines, notably those that affect dopaminergic pathways are also known to cause this [3]. Hyperprolactinemia is one of the most prevalent endocrine issues. It is clinically described as a prolonged elevation of prolactin levels above the normal physiological limits, often exceeding 15 mg/L [4].

Dopamine is the main regulator of prolactin release and is a powerful inhibitory regulator through the tuberoinfundibular pathway, linking the hypothalamus to the pituitary gland [8]. Disruption of this regulatory system (particularly with dopamine receptor antagonists, such as domperidone) leads to increased prolactin

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release. Domperidone has been widely utilized in experimental and clinical settings as a reliable model to induce hyperprolactinemia due to its ability to antagonize dopamine receptors and increase prolactin production [9,10].

Hyperprolactinemia has been associated to alterations in metabolic processes and hormonal balance, including endocrine diseases. Some of the changes include increased release of cortisol, impaired glucose metabolism and reduced synthesis of gonadal hormones, particularly testosterone in men. These effects result from the complicated interaction between prolactin and other hormonal axes, including the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes.

Natural products have received increased attention as possible therapeutic agents due to their broad array of biological functions and very low side effects. Traditional medicinal plants have been used for millennia and it is estimated that a large part of the world population depends on herbal treatments for health care [5]. Saussurea costus (Indian costus) is among them that has been popularly used in traditional medicine and reported to contain antioxidant, anti-inflammatory, anti-microbial and immunomodulatory effects [6].

Likewise, walnuts provide a good source of essential nutrients and bioactive compounds, especially unsaturated fatty acids, that are beneficial to metabolic and cardiovascular health. Previous studies have demonstrated that walnut consumption is associated with improved lipid metabolism and decreased risk of coronary heart disease [7]. These properties suggest a potential role for walnut oil in the treatment of metabolic and endocrine diseases.

L-carnitine is a naturally occurring compound that has been examined for its ability to enhance metabolic function and decrease oxidative stress. It has a role in the transfer of fatty acids into mitochondria. Its antioxidant properties and role in energy metabolism may help to reduce the effects of hormonal imbalance.

Individual trials of these medicines are accessible, but few comparative studies evaluating their preventative efficacy against drug-induced hyperprolactinemia are currently available. The purpose of the present study was to analyze and compare the effects of L-carnitine, walnut oil and Saussurea costus oil on endocrine and metabolic alterations induced by domperidone in male rabbits.

III. MATERIALS AND METHODS

Experimental Animals

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The present experiment was carried out on twenty five healthy adult male local rabbits, weighing between 1000-1900 g and aged between 4 and 7 months. Animals were obtained from local markets and were acclimatized to the laboratory settings for three weeks before they were employed for the tests. The rabbits were housed in wooden cages (125 × 60 × 50 cm) with a sawdust bed, which was changed every 48 hours to guarantee hygienic conditions. The habitat was standardized by a 12 h light/dark cycle and free access to water and balanced nutrition was given to the animals, with feed components that were widely available in the area.

Induction of Hyperprolactinemia

Experimental hyperprolactinemia was induced by subcutaneous administration of domperidone (4mg/kg body weight) in normal saline with Tween-80 as dispersion agent. The therapy was performed daily for 30 days according to the previously published experimental protocols [11].

Experimental Design

The animals were randomly divided into five equal groups (n = 5 per group) as follows:

- **Negative control group (C−ve):** Received normal saline, Tween-80, and standard diet without any treatment.
- **Positive control group (C+ve)**: Received domperidone (4 mg/kg) to induce hyperprolactinemia.
- **Group G1 (Costus oil):** Received domperidone (4 mg/kg) along with oral administration of *Saussurea costus* oil (0.5 ml/kg).
- **Group G2 (Walnut oil):** Received domperidone (4 mg/kg) with oral administration of walnut oil (0.5 ml/kg).

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- **Group G3 (L-carnitine):** Received domperidone (4 mg/kg) along with oral L-carnitine at a dose of 300 mg/kg.

All treatments were continued for 30 consecutive days**.**

Sample Collection

Animals were fasted for 12 hours at the conclusion of the experiment before samples were taken. Cardiac punctures were used to collect blood samples under the proper handling circumstances. Ten milliliters of blood were drawn into simple tubes and left to clot at room temperature. After being separated by centrifugation at 3000 rpm for 15 minutes, the serum was kept at low temperatures until additional biochemical and hormonal studies could be carried out.

Hormonal Analysis

Following the manufacturer's instructions, serum prolactin and cortisol concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (Changhai, China). To guarantee accuracy and repeatability, all operations were performed in accordance with accepted laboratory practices.

Biochemical Analysis

Serum lipid profile parameters, including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C), were measured using commercially available kits (Biolabo, France). Low-density lipoprotein cholesterol (LDL-C) was calculated using the standard equation:

LDL-C = TC − HDL-C − VLDL-C

Very low-density lipoprotein cholesterol (VLDL-C) was estimated using the formula:

VLDL-C = TG / 5 [12]

Fasting blood glucose (FBS) levels were also measured using standard biochemical methods.

Statistical Analysis

Data are presented as mean ± standard deviation. Statistical analysis of the data was performed using GraphPad Prism version 9.0 . The Shapiro–Wilk test was used to assess normality of data distribution.Differences between

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the experimental groups were examined using one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison test. Groups in the same row with different superscripted letters are statistically different at $P < 0.05$. The % change compared to the positive control group was also determined to measure the extent of treatment response. Reporting Cohen's d impact magnitude may also be useful to increase interpretation of biological relevance.

IV. RESULTS

Prolactin, Cortisol, and Fasting Blood Glucose

As shown in Figures 1, 2, 3 Table 1 presents endocrine and metabolic changes between experimental groups. The positive control group (C+ve) revealed a substantial increase in serum prolactin levels compared to the negative control group ($P < 0.05$). This confirmed the successful induction of hyperprolactinemia by Domperidone. The treatment with Saussurea costus oil and Walnut oil showed a considerable reduction in the levels of prolactin compared to the positive control group, the walnut oil being the most effective. Also the levels of prolactin were decreased in the L-carnitine treated group although not to the same extent.

The positive control group showed a significant increase in serum cortisol levels compared to the negative control group. The greatest cortisol levels were recorded in costus oil and walnut oil groups. In contrast, the relative lower cortisol concentrations were detected in L-carnitine group compared to other treatment groups.

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Table 1. Endocrine and Glucose Parameters in Experimental Groups

Parameter	C−ve	C+ve	Costus oil	Walnut oil	L-carnitine	Main interpretation
Prolactin	0.33±0.04[b]	0.58±0.02[a]	0.34±0.01[b]	0.24±0.01[c]	0.30±0.04[ab]	Walnut oil showed the strongest prolactin reduction
Cortisol	76.09±1.02[c]	138.91±10.22[b]	198.24±15.33[a]	199.82±17.23[a]	127.10±13.34[b]	Costus and walnut increased cortisol
FBS	149.50±20.05[b]	173.40±22.20[a]	146.66±19.22[b]	128.66±18.11[c]	171.00±20.21[a]	Walnut oil showed the best glucose improvement

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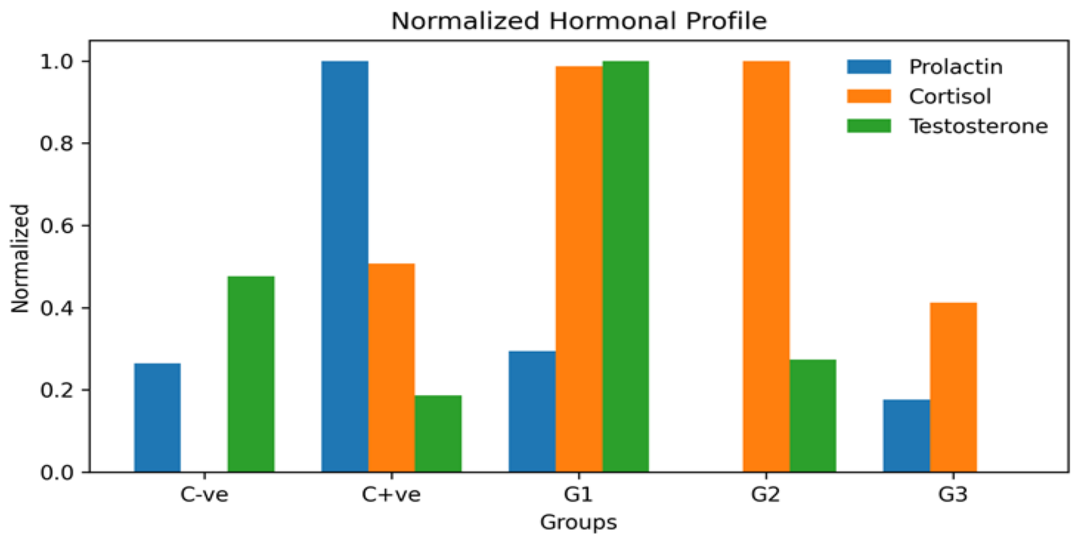

Figure 1 shows a normalized comparison of testosterone, cortisol, and prolactin levels in the serum from each experimental group. In order to compare variables with various measurement scales, data were standardized. While treatment groups showed a partial restoration of endocrine function, the hyperprolactinemic group displayed a noticeable hormonal imbalance.

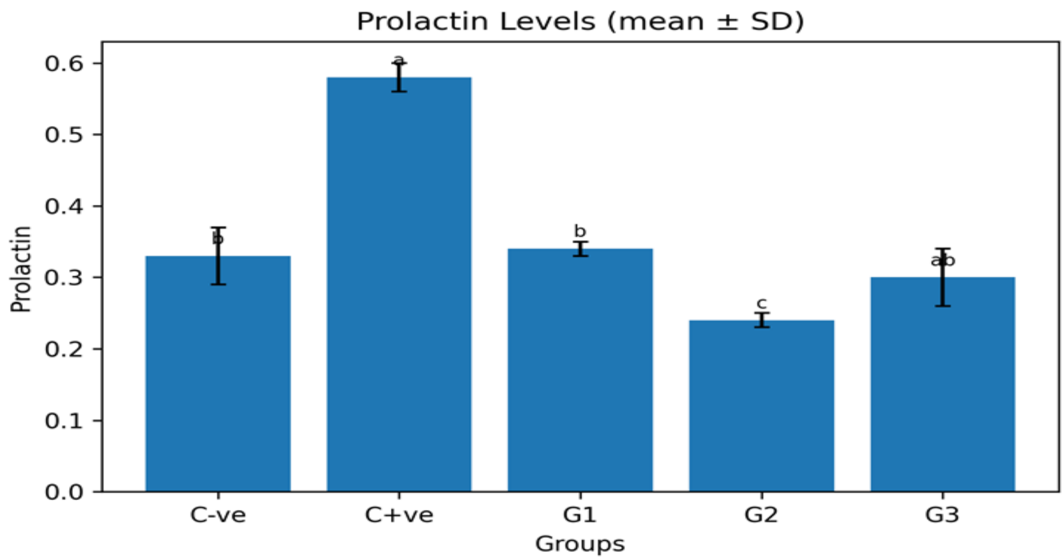

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Figure 2: Prolactin levels in the experimental groups were compared. The data are displayed as mean ± SD. Tukey's post hoc test (p < 0.05) was used after one-way ANOVA to establish statistical significance.

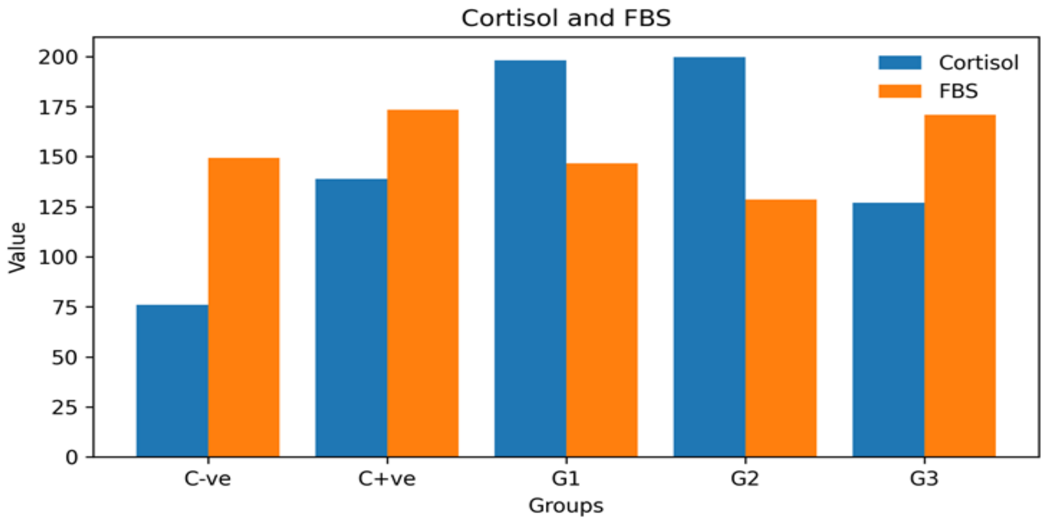

Figure 3: Comparison of Cortisol and FBS among experimental groups. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Testosterone Levels

Serum testosterone levels were considerably lower in the positive control group (C+ve) than in the negative control group (C−ve), as seen in Figure 4 and Table 2 ($p < 0.05$). Testosterone levels were significantly higher in the costus oil group (G1) than in any other group. The L-carnitine group (G3) showed lower testosterone levels than the other treated groups, but the walnut oil group (G2) showed a slight increase.

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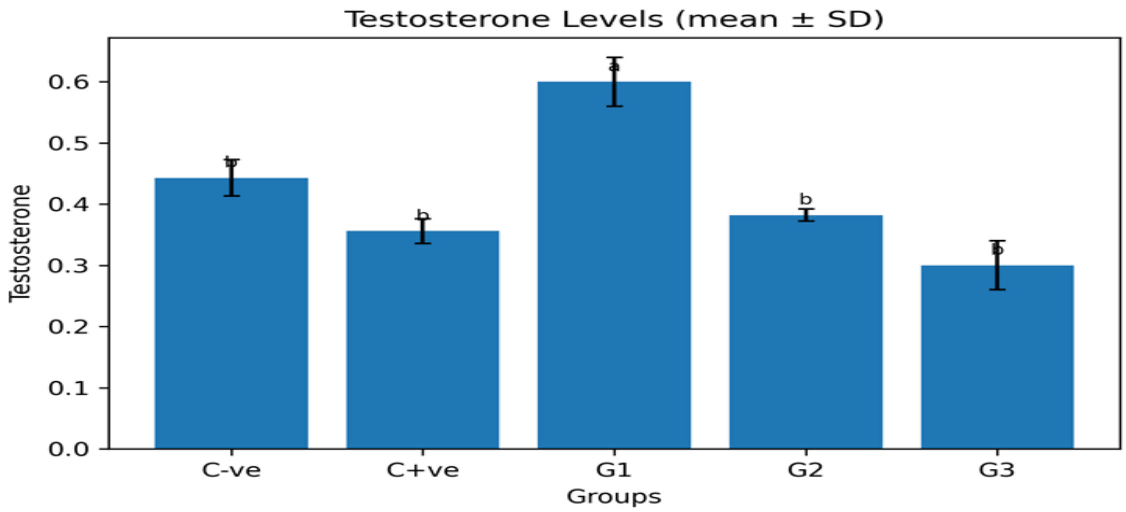

Figure 4: Comparison of Testosterone among experimental groups. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Lipid Profile Parameters

Figure 5 and Table 2 summarizes the effects of different treatments on testosterone levels and lipid profile parameters. Serum testosterone levels were significantly reduced in the positive control group compared with the negative control group, reflecting the suppressive effect of hyperprolactinemia on gonadal function. Costus oil treated group demonstrated highest concentration of testosterone across all groups with marked protective effect on reproductive hormonal balance. Walnut oil therapy induced a slight increase in testosterone levels, while L-carnitine treatment had the lowest testosterone concentration compared to the treated groups.

Total cholesterol (TC) levels were considerably higher in the costus oil and L-carnitine groups than in the control groups. Triglyceride (TG) concentrations were considerably elevated in all treated groups compared to the two control groups.

The L-carnitine group showed a significant increase in levels of high-density lipoprotein cholesterol (HDL-C), suggesting a possible positive influence on lipid metabolism. On the other hand, the positive control, costus oil and walnut oil groups exhibited substantial rise in low-density lipoprotein cholesterol (LDL-C) levels whereas L-carnitine group showed considerably lower LDL-C levels.

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Very low-density lipoprotein cholesterol (VLDL-C) contents in costus oil and walnut oil groups were considerably higher than the rest groups. Overall, the data suggest diverse metabolic responses among the treatments studied, with L-carnitine exhibiting the highest effect on HDL-C improvement and walnut oil showing superior glucose management.

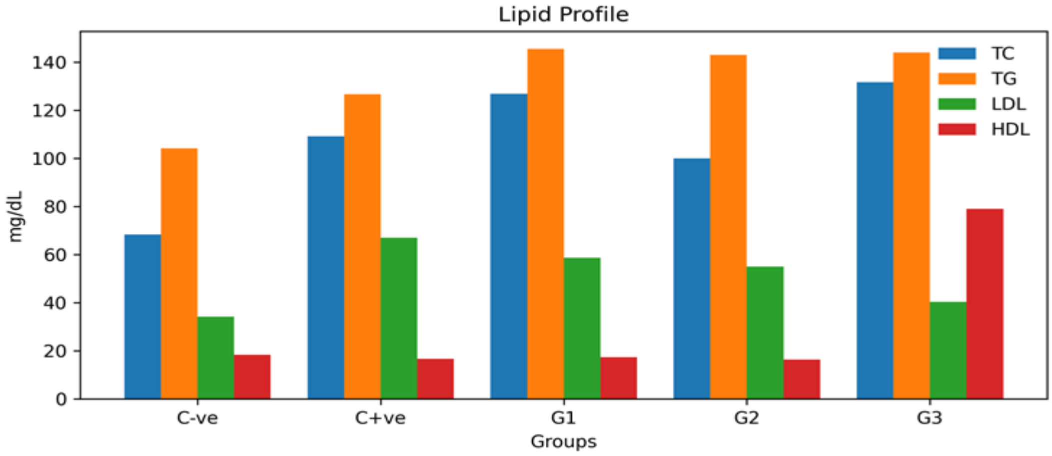

Figure 5: Comparison of Lipid Profile among experimental groups. Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Table 2. Testosterone and Lipid Profile in Experimental Groups

Parameter	C−ve	C+ve	Costus oil	Walnut oil	L-carnitine	Main interpretation
Testosterone	0.443±0.03^{b}	0.356±0.02^{b}	0.600±0.04^{a}	0.382±0.01^{b}	0.300±0.04^{b}	Costus oil gave the strongest testosterone improvement
TC	68.40±6.02^{c}	109.25±12.22^{b}	126.80±11.31^{a}	100.00±9.23^{b}	131.67±10.34^{a}	TC increased especially with costus and L-

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						carnitine
TG	104.20±7.02 ^{c}	126.75±10.11 ^{b}	145.60±13.01 ^{a}	143.00±9.10 ^{a}	144.00±11.34 ^{a}	Treatments increased TG
HDL	18.40±3.02 ^{b}	16.75±2.10 ^{b}	17.40±2.01 ^{b}	16.33±2.20 ^{b}	79.00±5.30 ^{a}	L-carnitine strongly increased HDL
LDL	34.20±4.02 ^{c}	67.00±7.11 ^{a}	58.67±8.01 ^{b}	55.00±7.10 ^{b}	40.33±8.34 ^{c}	L-carnitine showed the best LDL reduction
VLDL	15.80±3.05 ^{c}	25.25±6.20 ^{b}	29.00±5.22 ^{a}	28.67±4.11 ^{a}	25.00±3.21 ^{b}	Costus and walnut increased VLDL

Table 3 shows the % change of biochemical and hormonal indicators compared to the positive control group. Walnut oil treatment was related to the highest decrease in prolactin level and costus oil was related to the highest increase in testosterone concentration. Costus oil and walnut oil lowered fasting blood glucose levels when compared to the positive control group, with walnut oil having the most hypoglycemic effect.

The biggest rise in HDL-C level and the greatest decrease in LDL-C concentration were found for L-carnitine treatment. These results suggest a more positive effect of L-carnitine on lipid metabolism than on endocrine parameters. However, L-carnitine had little influence on prolactin levels and testosterone levels.

Table 3. Percent Change Compared with Positive Control

Marker	Costus oil	Walnut oil	L-carnitine
Prolactin	−41.4%	−58.6%	−48.3%
Testosterone	+68.5%	+7.3%	−15.7%
Cortisol	+42.7%	+43.8%	−8.5%
FBS	−15.4%	−25.8%	−1.4%

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TC	+16.1%	−8.5%	+20.5%
TG	+14.9%	+12.8%	+13.6%
HDL	+3.9%	−2.5%	+371.6%
LDL	−12.4%	−17.9%	−39.8%
VLDL	+14.9%	+13.5%	−1.0%

The total therapeutic efficacy of the treatments examined is presented in Table 4. Costus oil showed the highest endocrine protection effects through decreasing prolactin and restoring testosterone. Walnut oil showed the strongest combined effect on prolactin suppression and glycemic regulation. On the other hand, the effects of L-carnitine on the endocrine indicators were very mild, but it had the best effects on lipid profile parameters especially on increasing HDL-C and decreasing LDL-C. The results point to different endocrine and metabolic effects of each therapy under experimental hyperprolactinemic circumstances.

Table 4. Overall Therapeutic Ranking

Treatment	Strongest beneficial effects	Main limitations	Overall interpretation
Costus oil	Reduced prolactin, strongly increased testosterone	Increased cortisol, TC, TG, VLDL	Best endocrine improvement
Walnut oil	Strongest prolactin reduction, best FBS reduction	Increased cortisol, TG, VLDL	Best prolactin/glucose improvement
L-carnitine	Strong HDL increase, LDL reduction	Weak effect on prolactin and testosterone	Best lipid-specific effect

DISCUSSION

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The significant increase in prolactin levels seen in the positive control group supports the current findings that domperidone treatment effectively generated hyperprolactinemia. Given domperidone's documented function as a dopamine receptor antagonist that disrupts dopamine's inhibitory control on prolactin secretion, this result was anticipated [9,10]. The reliability of this approach has been demonstrated in previous experimental studies [11] with similar results.

The walnut oil and Saussurea costus oil groups exhibited a significant decrease of the level of prolactin. This effect could be related to the presence of bioactive compounds, specifically unsaturated fatty acids such as omega-3, which have been shown to alter dopaminergic function [12]. Dopamine is important for the inhibition of prolactin release and even a modest improvement in this pathway could explain the observed improvement. These oils also have antioxidant properties that may benefit indirectly by reducing oxidative stress, which is known to affect neuroendocrine function.

However, despite these reductions, the majority of treated groups, especially those treated with costus and walnut oils, still had elevated cortisol levels. This may be a reflection of the complex interaction between prolactin and adrenal function. Prolactin has been proposed to stimulate steroidogenesis and adrenal activity [14]. Thus the adrenal response may remain even after the prolactin levels begin to fall.

The elevated prolactin inhibits the hypothalamic-pituitary-gonadal axis, which is consistent with the drop in testosterone levels reported in the hyperprolactinemic group [19]. Interestingly, the increase in testosterone levels in G1 revealed that costus oil partially alleviated this effect. This may indicate an effect on testicular function, directly or indirectly, possibly through increased steroidogenic activity.

The effect of walnut oil on testosterone was smaller. This may be attributed to its nutritional composition, specifically the antioxidants and essential fatty acids, which have been related to improved reproductive performance in previous studies [20]. Furthermore, routes of nitric oxide [21] might maintain gonadal activity and hormone synthesis.

On the contrary, L-carnitine did not reveal a clear favorable effect on testosterone levels in the present study. Despite its well-known function in lowering oxidative stress and promoting mitochondrial function, it seems to have no effect in the current experimental setup. This may be due to the severity of the induced hormonal imbalance or the duration of treatment [23–25].

From a metabolic standpoint, oxidative stress and decreased insulin production could be linked to the hyperprolactinemic group's elevated fasting blood glucose. Free radical damage to pancreatic β-cells may be a factor in this outcome [15]. The oil-treated groups showed some improvement, but not enough to get the glucose levels back to normal.

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In a similar vein, the therapies did not completely reverse changes in lipid profile values. Increased triglyceride and cholesterol levels could be a sign of underlying oxidative stress-related disruptions in lipid metabolism [26]. Although omega-3 fatty acids are typically linked to better lipid profiles, their impact in this model seems to be restricted, perhaps as a result of the experimental setup or length of therapy [29, 30].

When considered collectively, these findings imply that natural oils like walnut oil and Saussurea costus might have some protective effects on endocrine function, notably in relation to prolactin and testosterone. However, their effect on metabolic diseases is less consistent, indicating a role for other variables.

Like prior studies, the current study found that domperidone injection significantly increased prolactin levels due to its inhibitory effect on dopamine pathways (8,9).

Prolactin levels are decreased in walnut oil and Saussurea costus treated groups. Bioactive chemicals, notably unsaturated fatty acids and antioxidants, may affect dopaminergic activity and hormone regulation.

Oxidative stress seems to be a key factor in the genesis of endocrine imbalance, especially in the case of hyperprolactinemia (31, 32). However, the effect of L-carnitine was quite small in this study. This could be because of the duration of treatment or the degree of the induced hormonal disruption, although it is known to have an antioxidant effect (33).

Conclusion

This study reveals that the domperidone-induced hyperprolactinemia leads to a significant endocrine disturbance characterized by elevated cortisol and prolactin levels, impaired glucose management and reduced testosterone production. These results show the relationship between dopaminergic control, adrenal activity and gonadal function in cases of hormonal imbalance.

Two of the studied treatments, Saussurea costus oil and walnut oil have demonstrated significant efficacy in reduction of prolactin rise and partial restoration of testosterone levels suggesting probable modulatory action on neuroendocrine pathways. In contrast, L-carnitine showed no influence on most of the parameters examined under the present experimental settings.

These endocrine improvements are not always associated with a consistent normalization of cortisol levels and lipid profile tests, suggesting that the metabolic effects of hyperprolactinemia are more complicated and may include processes other than prolactin management.

“Collectively, these findings indicate the potential for the use of selected natural oils as an adjunctive treatment for hormonal disturbances induced by hyperprolactinemia.” Further studies with larger sample sizes, longer treatment periods and more biomarkers, especially those related to oxidative stress, are needed to better elucidate their therapeutic efficacy and processes.

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