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Impact of Black Seed Oil on Male Sex Hormones and Binding Proteins in Alloxan-Induced Diabetic Wistar Rats

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Abstract:
Background: Diabetes is a metabolic disease that has a detrimental effect on human health and patients' quality of life. The number of male patients with diabetes is increasing, and so are the infertility problems in the male population. This study aims to investigate the impact of black seed oil (BSO) on male sex hormones and binding proteins in alloxan-induced diabetic Wistar rats. Methods: Forty (40) male Wistar rats weighing 200-250g were randomly allocated into eight (8) groups of five (5) animals per group. Group 1 followed the induction of hyperglycemia, group 2 received normal saline, group 3 received 200mg/kg of metformin, group 4 received 2mg/kg of glimepiride, group 5 received 2.5ml/kg of BSO, group 6 received glimepiride and BSO, group 7 received metformin and BSO, and group 8 received BSO, glimepiride, and metformin. Results: The result shows that diabetes significantly reduced serum and testicular testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels compared to the normoglycemic-controlled group ($p<0.05$). BSO co-administered with glimepiride significantly increased serum and testicular testosterone levels compared to diabetic control and monotherapy of either glimepiride or black seed oil ($p<0.05$). There is a positive correlation between glycosylated hemoglobin (HbA1c) and sex hormone binding globulin (SHBG) with $r^2 = 0.561$ while a negative correlation between glycosylated hemoglobin and Androgen binding protein (ABP) with $r^2 = 0.345$. Conclusion Diabetes mellitus exerts an adverse impact on male sex hormones and sex hormone-binding proteins, consequently leading to a detrimental effect on male fertility. However, black seed oil enhances male sex hormones, albeit without normalizing them to normoglycemic levels. Furthermore, glycosylated hemoglobin levels affect both sex hormone-binding globulin (SHBG) and androgen-binding protein (ABP).

Keywords: *Black seed oil, SHBP, ABP, FSH, LH, intratesticular Testosterone*

1. Introduction

Diabetes mellitus is a metabolic disease that seriously threatens human health and patient's quality of life. The prevalence of diabetes mellitus (DM) is notably high. The age of onset has been decreasing globally (Liu et al., 2020). The latest data from the International Diabetes Federation (IDF) showed that the number of adults with diabetes is up to 463 million worldwide and is estimated to increase to 578 million by 2030(Liu et al., 2020). The prevalence of diabetes mellitus among male patients is steadily rising, concurrent with an increase in fertility issues within the male population (Liu et al., 2020). Hence, male reproduction is receiving increasing attention. The regulations in male reproductive function encompass a complex array of endocrine, paracrine, and metabolic interactions involving Sertoli, Leydig, peritubular, and germ cells, which maintain the proliferation and differentiation of spermatogenic cells. The hypothalamic-pituitary-gonadal (HPG) axis is the most important of these regulatory pathways because it coordinates essential functions through the action of heterodimeric glycoproteins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), as well as maintaining a high intratesticular testosterone concentration(Cavallin et al., 2018). LH stimulates the production of testosterone from the Leydig cells of the testes, which is adjudged to be the cornerstone of effective spermatogenesis, secondary sexual characteristics, and functions, as well as psychological and anabolic actions. FSH enhances testosterone action by maintaining the supporting function of Sertoli cells in spermatogenesis. The crosstalk between these hormones is crucial for the dual functions of fertility and virility in the adult testis. Therefore, the management of diabetes should include agents that reduce fasting blood glucose and address the impact of diabetes on various systems. Numerous medicinal plants and their purified components have demonstrated beneficial therapeutic potential. Nigella sativa L., a member of the Ranunculaceae family (Mashayekhi-Sardoo et al., 2020), is a valuable herb with a rich historical and religious background and is among the promising medicinal plants.

2. Materials and Method

2.1. Black Seed

Black seeds were purchased from CHILAS O' International Limited in Wuse, Abuja, Nigeria. They were identified by a botanist from the Plant Science Laboratory at Benue State University and deposited in the Herbarium with index number HBI-OC-001-BSU24.

Figure 1: Black Seeds

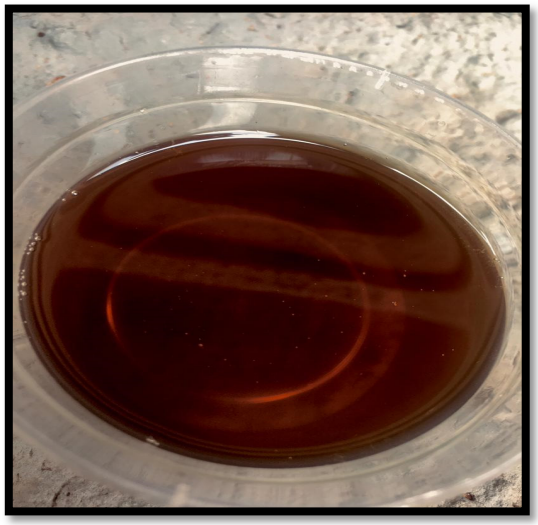

Figure 2: Black Seed Oil

2.1.1. Phytochemical Analysis of Black Seed Oil

The phytochemical analysis of black seed oil (qualitative and quantitative) was conducted in the chemistry department, Faculty of Biological Sciences, Benue State University, Makurdi, using standard protocols of Ogidi et al. (2022).

2.1.2. LD_{50} of Black Seed Oil

The LD50 values of the fixed oil of black cumin in mice and rats were examined and were found to be 28.8 ml/kg (Zaoui et al., 2002). The dose used in this study was 2.5ml/kg/day, which is considered an effective dose of black seed oil (Akhtar et al., 2022).

2.2. Preparation of Dosage

2.2.1. The Formulae

Dosage (mg/kg) x weight (kg)/stock (mg/ml).
Alloxan dose preparation in ml for a Wistar albino rat of 250g
250g= 0.25kg
0.65g of alloxan was taken using a digital weighing scale.
(Precaution: the fans were switched off. The paper was placed on the digital scale and then reset to 0.0g)
0.65g= 650mg.
10mls of normal saline was then added to 650mg of alloxan to make a stock of 650mg/10ml = 65mg/ml.
Dose to be given =65mg/kg
65mg/kg x 0.25kg/65mg/ml= 0.25ml.

An insulin 1ml syringe was used to administer 0.25ml from the 10ml containing 650mg of alloxan to a Wistar albino rat weighing 250g.

The same was done for various rats depending on their weight and agent dose.

2.3. Animal Selection and Grouping

Forty (40) Male adult Wistar albino rats were purchased from the disease-free stock of the animal house of the College of Health Sciences, Benue State University, Makurdi. They were maintained at standard laboratory conditions of temperature 28^{o}C relative humidity (with a 12-hour light-dark cycle) and adequate ventilation. The animals were fed with a commercial diet (Vital Feed Nig. Ltd.) and water *ad libitum*. Food was withheld at night before the experiments, but they had free access to water. Permission to use animals and animal houses was obtained from the Animal Ethics Committee of Benue State University Makurdi before experimentation.

2.4. Induction of Diabetes

Diabetes was induced in various groups except in group 1 by intraperitoneal injection of alloxan monohydrate (65mg/kg) in 0.9% normal saline (Al-Qudah et al., n.d.). 50% glucose solution was administered to prevent initial hypoglycemia caused by alloxan. Diabetes was confirmed three days later in alloxan-induced animals, showing a Random Blood Glucose (RBG) level ≥ 200 mg/dl by using a glucometer to monitor the blood sample from the tail vein.

2.4.1. Animal Grouping and Experimental Design

The animals underwent a 14-day acclimatization period before being randomly assigned to eight groups, with 5 animals in each group.

- Group 1: Normoglycaemic control
- Group 2: Alloxan diabetic control
- Group 3: Metformin 200mg/kg/day (Psimadas et al., 2012)
- Group 4: Glimepiride 2mg/kg/day (Dubey et al., 2020)
- Group 5: Black seed oil 2.5ml/kg/day (Seker, 2021)
- Group 6: Glimepiride 2mg/kg+ Black seed oil 2.5ml/kg/day
- Group 7: Metformin 200mg/kg +Black seed oil 2.5ml/kg/day
- Group 8: Metformin 200mg/kg+ glimepiride 2mg/kg+ blackseed oil 2.5ml/kg/day

2.5. Measurement of Sex Hormone-Binding Proteins

2.5.1. Testicular ABP

2.5.1.1. Testicular Androgen Binding Protein and Testosterone Assay

The right testes were minced and homogenized with phosphate buffer saline (PBS). The homogenates were centrifuged at 5,000 ×*g* (4°C) for 5 minutes. The supernatant obtained was used to assay testicular testosterone and androgen-binding globulin levels using appropriate ELISA kits.

2.5.1.2. Sex Hormone Binding Globulin

Serum levels of SHBG were measured by enzyme-linked immunosorbent assay (ELISA). The results were obtained in nmol / l for SHBG.

2.5.1.2.1. Determination of Plasma Concentrations of the Reproductive Hormones

The serum concentrations of testosterone, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) were determined by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits (GUANGZHOU WONDFO BIOTECH CO.LTD).

2.5.1.2.1.1. Statistical Analysis

Results are presented as mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test for multiple comparisons to assess differences among means using Statistical Package for Social Sciences software, version 22.0.

3. Results

Constituents	Qualitative	Quantitative% ±SEM
Alkaloid	++	2.45±0.01
Phenol	++++	36.33±0.03
Saponin	+	15.31±0.01
Flavanoid	++++	76.81±0.02
Tannins	++	5.21±0.01
Terpenoid	++++	19.31±0.01
Glycoside	++++	31.37±0.02

Table 1: Phytochemical Properties of Black Seed Oil

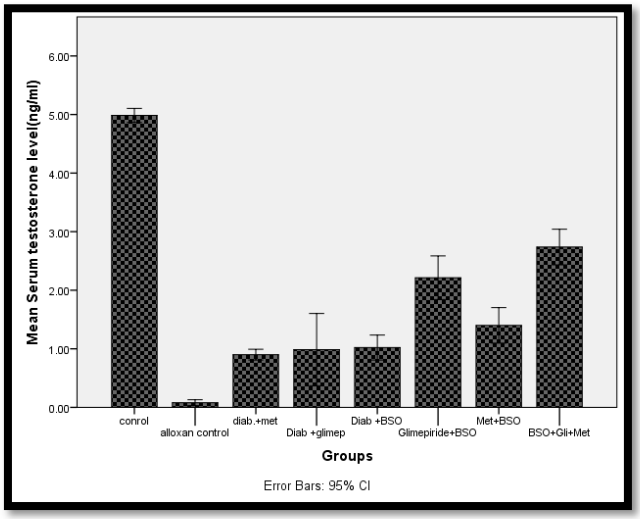

Figure 3: Impact of Black Seed Oil and Conventional Drugs on Serum Testosterone Levels in Alloxan-Induced Diabetic Rats

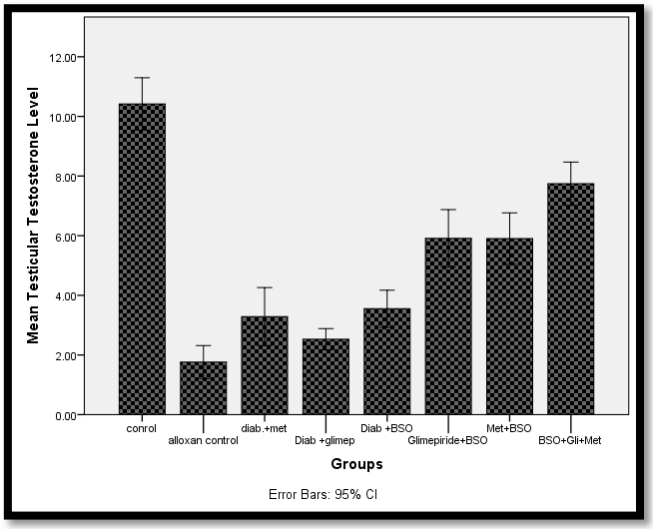

Figure 4: Effect of Black Seed Oil and Conventional Drugs on Testicular Testosterone Levels in Alloxan-Induced Diabetic Rats

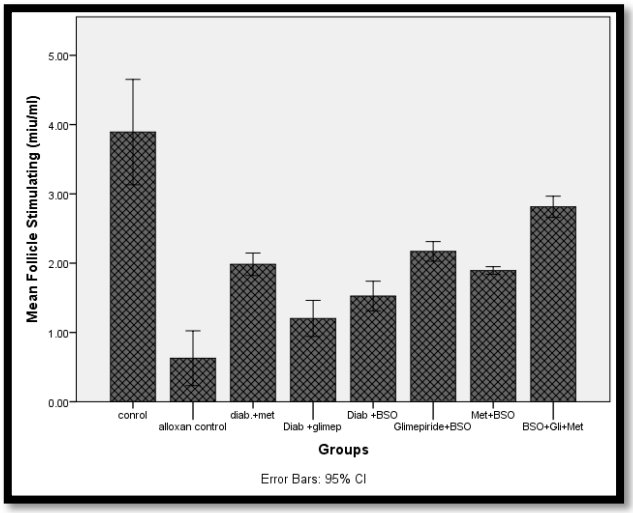

Figure 5: Impact of Black Seed Oil and Conventional Drugs on Follicle-Stimulating Hormone Levels in Alloxan-Induced Diabetic Rats

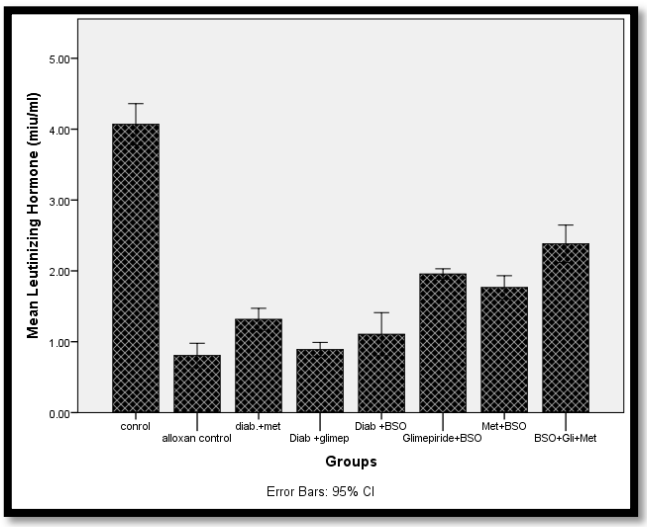

Figure 6: Impact of Black Seed Oil and Conventional Drugs on Serum Luteinizing Hormone Levels in Alloxan-Induced Diabetic Rats

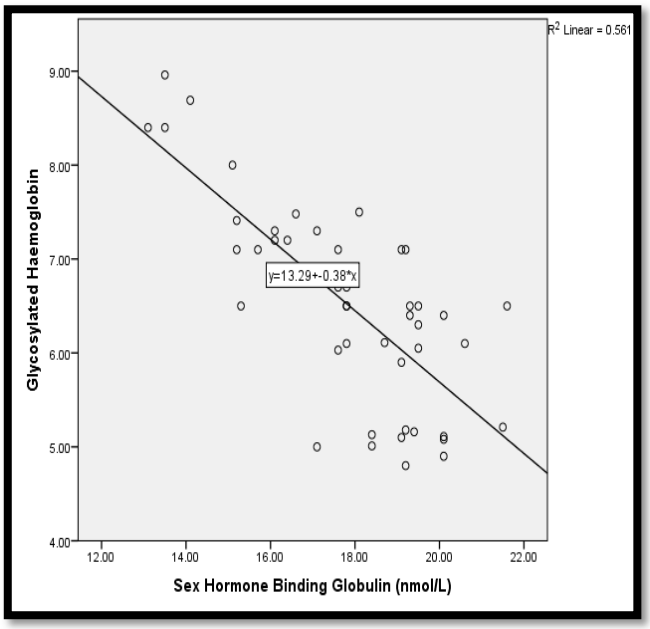

Figure 7: Scattergram of Correlation between Glycosylated Hemoglobin and Sex Hormone Binding Globulin

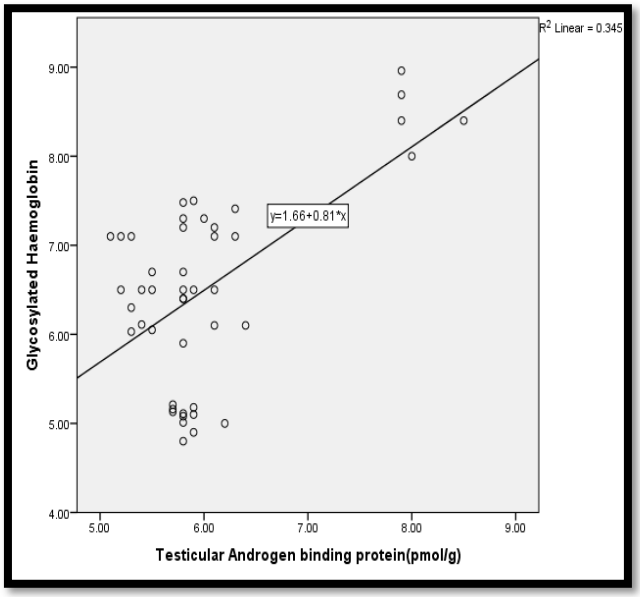

Figure 8: Scattergram of the Correlation between Glycosylated Hemoglobin and Testicular Androgen Binding Protein

The qualitative and quantitative phytochemical analysis of black seed oil (BSO) reveals a high percentage of flavonoids, phenols, glycosides, and saponins but low levels of alkaloids and tannins, as shown in table 1. The effect of BSO on serum and intratesticular testosterone levels is shown in figures 3 and 4, respectively. The result indicates that diabetes significantly reduced serum and testicular testosterone levels compared to the normoglycemic-controlled group ($p<0.05$). BSO co-administered with glimepiride significantly increased both serum and testicular testosterone levels compared to diabetic control and monotherapy of either glimepiride or black seed oil ($p<0.05$). Diabetes also significantly reduced serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels compared to the normoglycemic control group ($P<0.05$, as shown in figures 5 and 6, respectively. BSO significantly increased both hormones but not to normoglycemic level. Figure 7 shows a positive correlation between glycosylated hemoglobin and sex hormone-binding globulin with $r^2 = 0.561$, while figure 8 shows a negative correlation between glycosylated hemoglobin and androgen-binding protein with $r^2 = 0.345$.

4. Discussion

4.1. The Impact of Black Seed Oil on Male Sex Hormones

Diabetes mellitus is a global health challenge and has been identified as a clinical syndrome. The impact of diabetes mellitus on male reproductive function remains a global key focus to overcome the problem of infertility in males with diabetes.

The result of this study shows that diabetes mellitus significantly reduced both serum and testicular testosterone levels ($p<0.05$). This agrees with the work of Barsiah et al (Barsiah et al., 2019). The reduction in testosterone levels in a diabetic state could be a result of reduced insulin effect on the Leydig cells(Wagner et al., 2021) and also by hyperglycemia-induced oxidative stress on Leydig cells (Salimnejad et al., 2017).

There was no significant difference in serum testosterone level in the metformin-treated group, glimepiride group, black seed oil-treated group, and metformin plus black seed oil-treated group ($p>0.05$). Still, these groups were significantly different from the alloxan control group ($p<0.05$). Black seed oil co-administered with glimepiride had a serum testosterone level significantly higher than either black seed oil alone or glimepiride alone group ($p<0.05$).

Despite the significant difference in serum testosterone levels between the treated groups and the normoglycemic control group ($P < 0.05$), none of the treated groups could restore serum testosterone levels to the normoglycemic state.
Diabetes mellitus also significantly reduced testicular testosterone levels ($p<0.05$). This agrees with (Alsenosy et al., 2019). There was a significant difference in testicular testosterone levels in the black seed oil-treated group, the glimepiride-treated group, and the group co-administered with black seed oil and glimepiride ($p<0.05$).

Serum follicle-stimulating hormone (FSH) and luteinizing hormones were significantly reduced in the diabetic control group compared to the normoglycemic controlled group ($p<0.05$). There was no significant difference in serum FSH level in the glimepiride-treated, metformin-treated, and black seed oil groups ($p>0.05$). When black seed oil was co-administered with glimepiride in diabetic rats, it reversed FSH and luteinizing hormone levels. Consequently, there was no significant difference in serum FSH and LH levels between the group treated with black seed oil in addition to glimepiride and the normoglycemic group ($p>0.05$).

The phytochemical properties of black seed oil showed that it contains unsaturated long-chain fatty acids (LCFAs), particularly linoleic acid and oleic acid (Ahmad et al., 2021). Previous studies show that LCFAs, such as linoleic acid, enhance LHβ mRNA in rats (Tiwari & Sahu, 2017). This indicates that through its constituents, black seed oil has a direct effect on the hypothalamus, stimulating the secretion of gonadotropin-releasing hormone (GnRH), which in turn stimulates gonadotropin of the adenohypophysis (Nagy et al., 2024)

5. Summary of Findings

- Black seed oil increased both serum and testicular testosterone levels in alloxan-induced diabetes but normoglycemic levels were not achieved within the period of administration.
- The levels of follicle-stimulating hormone and luteinizing hormone were significantly elevated by the use of black seed oil. Long-chain fatty acid, particularly linoleic acid constituents of black seed oil, was responsible for the increase in the luteinizing hormone.
- There was a negative correlation between sex hormone-binding globulin and glycosylated hemoglobin
- There was a positive correlation between testicular androgen binding protein and glycosylated hemoglobin.

6. Conclusion

Diabetes mellitus exerts an adverse impact on male sex hormones and sex hormone-binding proteins, consequently leading to a detrimental effect on male fertility. However, black seed oil enhances male sex hormones, albeit without normalizing them to normoglycemic levels. Furthermore, glycosylated hemoglobin levels affect sex hormone-binding globulin (SHBG) and androgen-binding protein (ABP).

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