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Original Research Article

Impact of *Nigella sativa* oil on glycemic control in alloxan-induced diabetic Wistar rats

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ABSTRACT

Background: To effectively manage diabetes, it is crucial to achieve optimal glycemic control by ensuring that A1C levels remain below 7.0%. This study aimed to investigate the impact of *Nigella sativa* oil (black seed oil, BSO) on glycemic control in alloxan-induced diabetic Wistar rats.
Methods: Forty (40) male Wistar rats weighing 200-250 gm were randomly allocated into eight (8) groups of five (5) animals per group. Group 1 received normal saline as the normoglycemic control, while groups 2 to 8 were given alloxan monohydrate to induce hyperglycemia, following the method of Osikwe et al. Following the induction of hyperglycemia, group 2 received normal saline, group 3 received 200 mg/kg of metformin, group 4 received 2 mg/kg of glimepiride, group 5 received 2.5 ml/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 results showed that BSO significantly reduced fast blood glucose levels compared to the diabetic control group ($p<0.05$), lowered glycosylated hemoglobin to <7%, and improved pancreatic beta cell function.
Conclusions: Black seed oil reduces fasting blood glucose, exhibits synergism with glimepiride, and improves pancreatic beta-cell function in alloxan-induced diabetic Wistar rats.

Keywords: Black seed oil, Glycosylated hemoglobin insulin

INTRODUCTION

Diabetes mellitus is due to the absolute or relative deficiency of insulin and insulin resistance, which leads to a decrease in the insulin utilization rate and metabolic disorder.[1] Effective management of diabetes requires achieving optimal glycemic control. Glycated hemoglobin (A1C) levels >7.0% are associated with an increased risk of microvascular and cardiovascular complications, regardless of treatment modality. Effective diabetes management requires continuous monitoring of blood sugar levels to minimize the risks of diabetic complications.

The incidence rate of DM is very high, and the age of onset has been decreasing worldwide.[2] 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.[3]

Numerous medicinal plants and their purified components have demonstrated beneficial therapeutic potential. *Nigella sativa L.*, a member of the Ranunculaceae family, is a valuable herb with a rich historical and religious background and is among the promising medicinal plants.[4]

METHODS

Black seed

Black seeds were purchased from CHILAS O' International Limited, Wuse, Abuja, Nigeria. They were identified by a botanist from the plant science laboratory of the department of biological sciences, Benue State University. Samples were deposited in the herbarium with herbarium index number HBI-OC-001-BSU24.

Figure 1: Black seed.

Black seed oil extraction

Black seeds were pressed at room temperature using a cold press (ScrewPress Model 85 mm) at 10 MPa for 10 min according to the method described by Willems, Kuipers, and De Haan, where the temperature was kept below 40°C.[5]

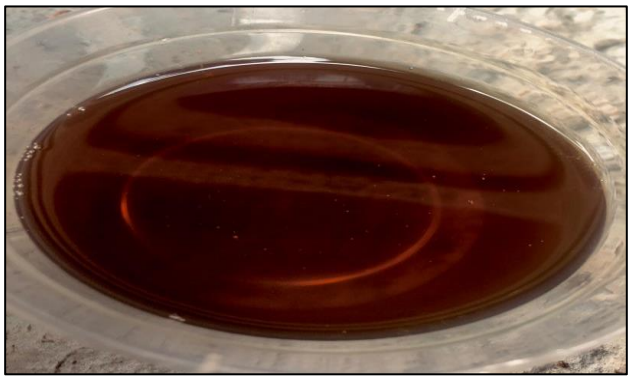

Figure 2: Black seed oil.

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 described by Ogidi et al.[6]

LD_{50} of black seed oil

The toxicity of the fixed oil of black cumin in mice and rats was also examined, and the LD_{50} values were found to be 28.8 ml/kg.[7] The dose (2.5 ml/kg/day) used in this study was said to be the effect dose of black seed oil.[8]

Preparation of dosage

The formulae used was:

Dosage (mg/kg) x weight (kg)/stock (mg/ml).

Alloxan dose preparation in ml for a Wistar albino rat of 250 gm

250 gm = 0.25 kg

0.65 gm of alloxan was taken using a digital weighing scale (precaution: the fans were switched off. Paper was placed on the digital scale and then reset to 0.0 gm).

0.65 gm = 650 mg.

10 ml of normal saline was then added to 650 mg of alloxan to make a stock of 650 mg/10 ml = 65 mg/ml.

Dose to be given = 65 mg/kg

65 mg/kg x 0.25 kg/65 mg/ml = 0.25 ml.

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

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

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°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.

Induction of diabetes

Diabetes was induced in various groups except in group 1 by intraperitoneal injection of alloxan monohydrate (65 mg/kg) in 0.9% normal saline.[9] 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.

Serum insulin assay

Serum insulin concentrations were determined using radioimmunoassay (Diagnostic Product, Co., Ltd, LA, USA).

Experimental design

The animals were allowed a 14-day acclimatization period, after which they were randomly allocated into nine groups (n=5). Group 1 was the normoglycemic control; group 2 alloxan diabetic control; group 3 metformin 200 mg/kg/day; group 4 glimepiride 2mg/kg/day; group 5 black seed oil 2.5 ml/kg/day; group 6 glimepiride 2 mg/kg+ black seed oil 2.5 ml/kg/day; group 7 metformin 200 mg/kg + black seed oil 2.5 ml/kg/day and group 8 metformin 200 mg/kg + glimepiride 2 mg/kg + blackseed oil 2.5 ml/kg/day.[8,10,11]

Statistical analysis.

Results are presented as mean ± standard error of 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 (SPSS) software, version 22.0.

RESULTS

The qualitative and quantitative phytochemical analysis of *Nigella sativa* oil (BSO) shows a high percentage of flavonoids, phenol, glycoside, and Saponin but low in alkaloids and tannins as shown in Table 1.

Table 1: Phytochemical properties of black seed oil.

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

The effect of BSO on fasting blood glucose is represented in Figure 3. Alloxan significantly increases fasting blood glucose compared to the normoglycemic control group ($p<0.05$). After 8 weeks of administration, the fasting blood glucose of the alloxan group increased showing that diabetes worsened without appropriate intervention. The percentage change in the fasting blood glucose for metformin, glimepiride, BSO, glimepiride + BSO, metformin + BSO, and BSO + metformin + glimepiride groups were -34.7%, -25.2%, -31.5%, -45.9%, -37.5% and -58.2% respectively as shown in Figure 4.

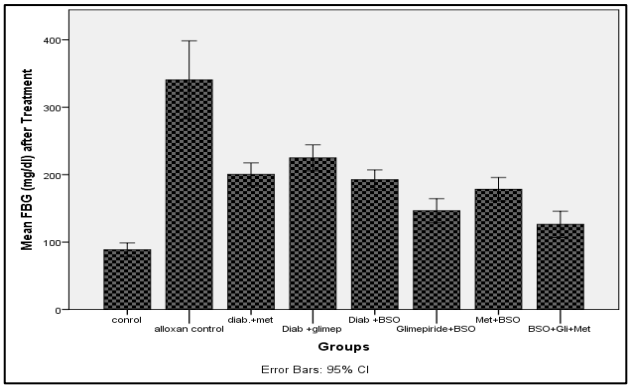

Figure 3: Effect of black seed oil on fasting blood glucose in alloxan-induced diabetic rats.

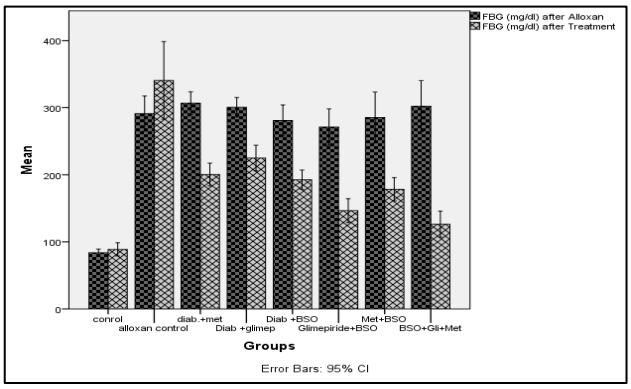

Figure 4: Effect of black seed oil and conventional drugs on fasting blood glucose level after induction with alloxan versus after 8 weeks of treatment.

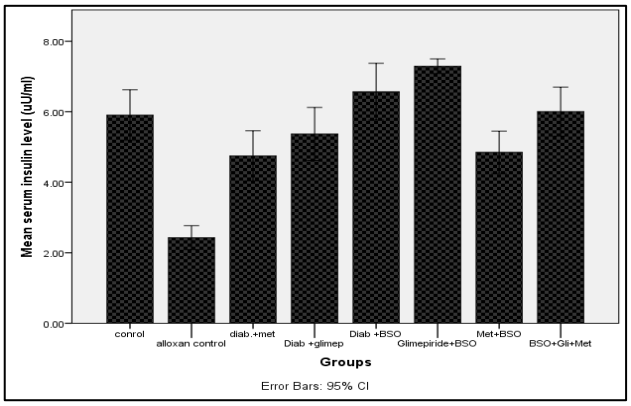

Figure 5: Effect of black seed oil, metformin, and glimepiride on serum insulin level in alloxan-induced Wistar rats.

Figure 5 shows that insulin is significantly reduced in the diabetic state. BSO and BSO co-administered with glimepiride increased serum insulin levels compared to the alloxan control group. The glycosylated hemoglobin in the diabetic control group was significantly higher than the physiological range of 4-6%. The glycosylated hemoglobin for the alloxan control, metformin, glimepiride, BSO, glimepiride +BSO, metformin +BSO,

and BSO + metformin + glimepiride groups were 8.5%, 7.2%, 7.0%, 6.9% 6.3%, 6.5% and 6.1% respectively.

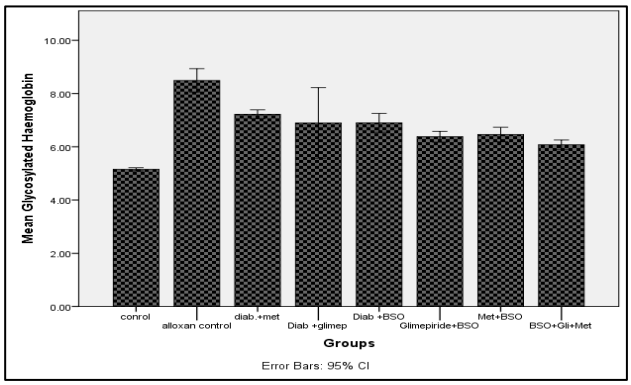

Figure 6: Effect of black seed oil, metformin, and glimepiride on glycosylated hemoglobin in alloxan-induced Wistar rats.

DISCUSSION

The results from this study show that blackseed oil administered to diabetic Wistar rats significantly reduced fasting blood glucose levels compared to the diabetic-controlled group ($p<0.05$). This agrees with the study.[12-14] There was no significant difference in fasting blood glucose between the group treated with black seed oil and the metformin-treated group ($p>0.05$).

Blackseed oil co-administered with glimepiride significantly reduced fasting blood glucose levels compared to either blackseed oil or glimepiride alone. This indicates synergism between black seed oil and glimepiride.

After 8 weeks of treatment, the diabetic-controlled group's glycosylated hemoglobin level was significantly higher than the normoglycemic control group ($p<0.05$). However, blackseed oil significantly reduced glycosylated hemoglobin levels compared to the diabetic-controlled group ($p<0.05$). However, it could not reverse it to the prediabetic state but achieved a glycemic goal of less than 7%.[15] This agrees with the work.[16]

After 8 weeks of treatment, the diabetic group treated with black seed oil showed a significant increase in serum insulin levels compared to the alloxan control group ($p<0.05$). This demonstrates that black seed oil promotes pancreatic beta cell integrity and proliferation, thus improving insulin secretion. This agrees with Abdelrazek et al who demonstrated that thymoquinone a constituent of black seed oil improved insulin secretion in streptozotocin-induced diabetic Wistar rats.[17]

Black seed oil regulates blood glucose levels through both pancreatic and extra-pancreatic pathways. The extrapancreatic pathway involves reducing glucose absorption by inhibiting the sodium-glucose co-transporter.[18] The polyphenol constituents of black seed oil have an inhibitory effect on alpha-glycosidase enzyme.[19] Thymoquinone one of the major constituents of black seed oil has been shown to decrease the expression of glucogenic enzymes (glucose-6-phosphatase and fructose 1,6-bisphosphatase) thus decreasing hepatic gluconeogenesis.[17]

The pancreatic mechanism involves the insulinotropic action of thymoquinone constituents of black seed oil which causes partial regeneration of pancreatic beta cells, thus leading to improvement in insulin production.[17,20]

Summary of findings

Black seed oil improves glycemic control by reducing both fasting blood glucose and glycosylated hemoglobin. Black seed oil and glimepiride exhibit synergism in glycemic control. Black seed oil improves pancreatic beta-cell function after diabetic induction with alloxan.

There are few limitations of the study. Due to financial constraints, gene expression was not carried out on the beta cells of the pancreas and insulin. Only serum insulin levels were measured after 8 weeks of administration.

CONCLUSION

Black seed oil reduces fasting blood glucose and improves pancreatic beta-cell function in alloxan-induced diabetic Wistar rats.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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