International Digital Organization for Scientific Research ISSN: 2579-0781
IDOSR JOURNAL OF EXPERIMENTAL SCIENCES 7(1) 112-125, 2022.

Evaluation of the effect of Black Seed (*Nigella sativa*) on lipid profile Markers of Alloxan-induced diabetic Wistar Rats

Amatu Chisom S., Nwaka Chinyere S. and Nwaka Andrew C.
Department of Biochemistry Chukwuemeka Odumegwu Ojukwu University Uli Campus, Anambra State, Nigeria.

ABSTRACT

The effect of Black Seed (*Nigella sativa*) on lipid profile Markers of Alloxan-induced diabetic Wistar Rats was evaluated. *Nigella sativa* seed was air-dried under ambient temperature and milled into a coarsely powdered form using a local grinder. A total of thirty five-(35) wistar albino rats were randomly divided into five (5) groups of seven rats each and used for the study. Group A: Normal control, Group B: Diabetic untreated, Group C: Diabetic+Standard drug(Gluformin 100mg/kg), Group D: Diabetic+feed formulated with 20% pulverized *Nigella sativa* and Group E: Diabetic +feed formulated with 40% pulverized *Nigella sativa.* All experimental protocols were observed under strict supervision, the experiment lasted for fifteen days and the administration was done via oral gavage. The study's findings showed a significant increase in the Total cholesterol level in-group B compared to A. However, groups C and E had a significant decrease, and D had a non-significant increase compared to group B. The triglyceride result showed a significant increase in-group B compared to A; however, groups C, D, and E had a significant decrease compared to group B. Dyslipidemia characterized by lipoprotein abnormalities linked with increased triglyceride level, decreased high-density lipoprotein-cholesterol levels and increased in small dense low-density lipoprotein (LDL) particles. However, it is ubiquitous in type-2-diabetes (T2DM), affecting around 70 % of patients and a significant risk factor for atherosclerotic cardiovascular disease. The significantly higher levels of TGs and total cholesterol activity in the diabetic treated rats are associated with increased lipid peroxidative activity. However, the study reports a non-significant decrease in HDL activity in-group B compared to A. Groups C, D, and E increased compared to B. However, it was statistically significant in Group D, while groups C and E were insignificant. The LDL result showed a non-significant increase in-group B compared to A; groups C, D, and E had a significant decrease compared to group B. The VLDL result showed a significant increase in-group B compared to A; groups C, D, and E had a significant decrease compared to group B. However, the differences seen in the levels of HDL and VLDL are not well understood, as shown by the diabetic control to positive control. However, treatment with *N. sativa* showed a significant decrease in VLDL and LDL. In conclusion, the results revealed that *Nigella sativa* had hypoglycemic and hypolipidemic effects in alloxan diabetic rats.
Keywords: Black Seed, *Nigella sativa*, lipid profile, Markers and diabetes.

INTRODUCTION

The use of medicinal plants has gained grounds in recent times in the management of several ailments associated with metabolic disorders, of which diabetes mellitus is not an exception [1,2,3,4,5]. However, the treatments that involve the use of medicinal plants are recommended in the management of diabetes and its related complications [6,7,8,9]. *Nigella sativa* L. (N. sativa), also known as black seed or black cumin, is a plant grown in Mediterranean Countries, and Southwest Asia, and is known for its content of bioactive compounds (i.e., tocopherols, vitamin A and C, and B-carotene, etc.) in the seed. Notably, the seed biological activity has been associated with its thymoquinone content [10,11,12]. However, the *N. sativa* seed contains other compounds, such as fixed oil (22-38%),volatile oil (0.40-1.5%), proteins (21-31%), carbohydrates (25-40%),minerals(3.7-7%),vitamins(1 4%),saponins(0.013%),and alkaloids (0.01%), which can all contribute to its

biological properties [13,14,15,16,17]. Dyslipidemia has being linked to diabetes mellitus, which is modifiable risk factor for cardiovascular complications in type 2 DM [18,19], however, it is characterised by hypertriglyceridemia, reduced HDL cholesterol levels, and an increased concentration of Low-Density-Lipoprotein particles [20,21,22,23]. It was reported that dyslipidemia is connected to insulin resistance and hyperglycemia, resulting in overproduction of triglyceride-rich lipoproteins from the liver, and low clearance rate of triglyceride-rich lipoproteins, and an altered postprandial lipoprotein metabolism, which is rare in some cases [24,25,26,27]. [28,29,30] reported high triglycerides activity alters the particle diameters and triglyceride content of LDLs, an effect associated with increased atherogenicity. Dyslipidemia is a global burden with an increase concern to health professionals, and results from unhealthy diets, sedentary life style, and obesity been the modest risk factors among all [31,32,33,34]. Medicinal herbs have gained wide attention and are considered a beneficial adjuvant agent to oral antidiabetic drugs because of their integrated effects [35,36,37].

Aim of the study

The aim of this study was to determine the effect of Black Seed (*Nigella Sativa*) on lipid profile Markers of Alloxan-induced Diabetic Wistar Rats

Fig 1 diagram of *Nigella sativa*

MATERIALS AND METHODS

Location of the Study

This study was carried out in the Animal House, Department of Biochemistry, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu, University, Uli Campus.

Ethical Approval

Ethical approval was obtained from the Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu, University, Uli Campus. Rats handling and treatments conform to the National Institute of Health guidelines for laboratory animal care and use.

Experimental Animals

Wistar Rats (thirty-five-males) weighing 120 to 180g were purchased from the Animal House, Department of Zoology, Faculty of Science, Nnamdi Azikiwe University, Awka. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (Grower feed) and water *ad libitium*. The animals were acclimatized for two weeks and before administering the *Nigella sativa* and induction of diabetes, and were kept in 12hours light and dark cycles.

Plant Procurement and Identification

Samples of *Nigella sativa* was purchased from Osse market Onitsha and was identified by The Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

Plant Extraction Procedure

Nigella sativa seed was air-dried under ambient temperature and milled into a coarsely powdered form using a local grinder. Two hundred and fifty grams of the dried *Nigella sativa* seed was macerated in 1000mls of 95% absolute ethanol for 48hours. It was filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator and dried further using a laboratory oven at 45^{oc} into a gel-like form. The extract was preserved in airtight container and kept in a refrigerator for further usage. The extraction method was done with modifications as described according to the method employed by [1,2,3]. However, the extract undergoes further pulverization of 20% and 40% based on the groups.

Experimental Design

A total of thirty five-(35) wistar albino rats was randomly divided into five (5) groups of seven rats each and used for the study.

Group A: Normal control

Group B: Diabetic untreated

Group C: Diabetic+Standard drug (Gluformin 100mg/kg)

Group D: Diabetic+feed formulated with 20% pulverized *Nigella sativa*

Group E: Diabetic +feed formulated with 40% pulverized *Nigella sativa*

All experimental protocols were observed under strict supervision, the experiment will last for fifteen days, and administration was done through oral gavage daily.

Induction of Diabetes

Diabetes Mellitus (Hyperglycemia) was induced in the experimental rats by a single dose of intraperitoneally injection of 120mg/kg of Alloxan Monohydrate. The animals were fasted 12-hours prior to the induction of diabetes and was administered the Alloxan Monohydrate. Hyperglycemia was confirmed when fasting blood glucose concentration was greater than 200mg/dL for 3 consecutive days. This confirmed diabetes has taken place, and was described by [35].

Phytochemical Analysis

Test for glycosides (Keller Kilianin Test)

Two milliliter of glacial acetic acid, few drops of ferric chloride ($FeCl_3$) solution and 1ml of concentrated Sulphuric acid (H_2SO_4) were added in a test tube containing 5 ml of the extract. The formation of brown ring at interface indicates the presence of glycosides.

Test for Flavonoids (Alkaline Reagent Test)

Few drops of 20% Sodium hydroxide (NaOH) solution was added into a test tube containing 2ml of the extract, an intense yellow is formed which becomes colourless upon addition of dilute hydrochloric acid (HCl), this colour change indicated the presence of flavonoids.

Test for Saponins (Foam Test)

Six milliliter of distilled water was added into a graduated cylinder containing 2ml of the extract. The mixture was shaken vigorously for few minutes and formation of persistent foam was observed which indicated the presence of saponins. The presence of saponins was then confirmed by treating 1ml of the extract with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

Test for Tannins (Braimer's Test)

One milliliter of distilled water was added into a test tube containing 0.5ml of the extract followed by addition of 5% ferric chloride solution, presence of tannins was indicated by formation of blue-green colour. The procedure was further confirmed by additionfew drops of 1% lead acetate was added into a test tube containing 2 ml of the extract. Development of yellowish precipitate indicatedthe presence of tannins.

Test for Phenol

One milliliterof the extract was added to 3ml of 10% lead acetate solution. Formation of a bulky white precipitate indicated the presence of phenolic compounds.

Sample collection and termination of the experiment

At the end of the experiment, animals in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of the ethanolic *Nigella sativa*. Blood was collected from the animals using a heparinized capillary tube through ocular puncture. Blood obtained was put in a plain bottle, allowed to cool, and centrifuged for 10-minutes at 3000rpm, after which the serum was retrieved using amicropipette.

Determination of Lipid Profile

Total cholesterol

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol [. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a colour. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows

Cholesteryl ester + H_2O ---------Cholesterol oxidase----------->cholesterol + fatty acid

Cholesterol +O2 ---Cholesteryl ester hydrolase---> cholest-4-en-3-one + H_2O_2

2H2O2 + 4-aminophenazone + phenol ----------Peroixdase----------> 4-(p-benzoquinone-monoimino)-phenazone + 4 H_2O

Triglycerides

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H2O2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm.

Triglycerides + $3H_2O$ ---Glycerokinase---> glycerol + fatty acids

Glycerol + ATP ---glycerophosphate oxidase---> glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 ----------> dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4-chlorophenol ---Peroxidase---> 4-(p-benzoquinone-monoimino)- phenazone + $2H_2O$ + HCl.

High-density lipoprotein (HDL) cholesterol

Principle

In the presence of Mn^{2+} and heparin, chylomicrons, VLDL, and LDL are precipitated, leaving only HDL in solution. The precipitated materials are sedimented by centrifugation, and the HDL containing supernatant can be removed.

Procedure

One mililiterof precipitating reagent (1.06 M $MnCl_2$ with sodium heparin added) was added to each tube and mixed well using a vortex type mixer .One mililiter of each control and sample were pipetted into labeled 10 x 75 mm test tubes.The clear supernate was then removed into labeled 10 x 75 mm test tubes with Pasteur pipets and saved it for subsequent analysis .They were then centrifuged at 2000 g for 30 minutes at 4°C..

Low-Density-Lipoprotein Cholesterol (LDL-cholesterol)

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] - [TG]/5

where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

Statistical Analysis

Data obtained from this study was analyzed using Statistical Package for Social Sciences (SPSS) version 25. Data obtained for antioxidant activity (SOD, GPx, and CAT), bodyweight, oxidative stress markers (MDA), Vitamin A, C, and E, and fasting blood glucose level, and values were considered significant at $p<0.05$.

RESULTS

Table 1 Qualitative Screening of Phytochemical Composition of EEBS

PARAMETERS	BLACKSEED
FLAVONOIDS	++
TANINS	+
PHENOLS	+
ALKALOIDS	+
SAPONINS	++
PHYTATES	+
GLYCOSIDES	+
OXALATES	+

Key: ---, not present, + present at low concentration, + + Present at moderate concentration

The results of the Quantitative Study of Phytochemical Levels of EEBS have been summarized and presented below in table 1.Values are mean ± standard deviation of triplicate determination. Values within the same row bearing the same superscript letters are not statistically significant at $P<0.05$.

Table 2;Quantitative Study of Phytochemical Levels of EEBS

PARAMETERS	BLACKSEED
FLAVONOIDS	0.06 ± 0.01^{b}
TANINS	0.12 ± 0.01^{b}
PHENOLS	0.02 ± 0.01^{a}
ALKALOIDS	13.60 ± 0.01^{b}
SAPONINS	1.60 ± 0.01^{a}
PHYTATES	0.05 ± 0.02^{a}
STEROIDS	1.40 ± 0.01^{b}
OXALATES	0.15±0.01a

The result of the effect of *Nigella sativa* on blood glucose level following alloxan diabetic rat has been summarized and presented below in Table 2.

Table 3 effect of *Nigella sativa* on blood glucose level following alloxan diabetic rat

Blood Glucose level (mg/dl)		Mean	±SEM	P-value
Day 0	Group A (Normal control)	88.00	±5.14	
	Group B (Diabetic untreated)	447.83	±22.64	0.000
	Group C (DM + Gluformin 100mg/kg)	449.71	±60.04	0.001
	Group D (DM + 20% pulverized NS)	569.00	±31.00	0.000
	Group E (DM + 40% pulverized NS)	458.14	±91.57	0.000
Day 7	Group A (Normal control)	91.42	±4.17	0.000
	Group B (Diabetic untreated)	414.40	±55.57	
	Group C (DM + Gluformin 100mg/kg)	212.83	±60.97	0.011
	Group D (DM + 20% pulverized NS)	183.80	±44.83	0.006
	Group E (DM + 40% pulverized NS)	281.40	±77.88	0.095
Day 14	Group A (Normal control)	83.57	±5.15	0.000
	Group B (Diabetic untreated)	467.40	±60.99	
	Group C (DM + Gluformin 100mg/kg)	179.33	±38.07	0.000
	Group D (DM + 20% pulverized NS)	137.40	±21.88	0.000
	Group E (DM + 40% pulverized NS)	145.20	±21.47	0.000

Data was analyzed using ANOVA following post hoc LSD multiple comparison and data was considered significant at $p<0.05$. Table 3 result showed a significant increase in the blood glucose level in groups B, C, D, and E compared to group A at day 0. At day 7, a significant increase in the blood glucose level in-group A compared to B, groups C and D had significant decrease and group E had an insignificant decrease compared to group B. At day 14, a significant increase in the blood glucose level in-group A compared to B, groups C, D, and E had significant decrease compared to group B.

Table 4 effect of *Nigella sativa* on total cholesterol and triglyceride level following alloxan diabetic rat

		MEAN	±SEM	P-value
Total cholesterol (mmol/L)	Group A (Normal control)	72.32	±8.95	0.000
	Group B (Diabetic untreated)	205.06	±29.28	
	Group C (DM + Gluformin 100mg/kg)	137.54	±10.02	0.026
	Group D (DM + 20% pulverized NS)	174.50	±20.65	0.279
	Group E (DM + 40% pulverized NS)	87.65	±19.71	0.001
Triglyceride (mmol/L)	Group A (Normal control)	71.92	±6.77	0.000
	Group B (Diabetic untreated)	349.86	±14.25	
	Group C (DM + Gluformin 100mg/kg)	157.33	±19.05	0.000
	Group D (DM + 20% pulverized NS)	112.70	±17.70	0.000
	Group E (DM + 40% pulverized NS)	133.28	±16.55	0.000

Data was analyzed using ANOVA following post hoc LSD multiple comparison and data was considered significant at $p<0.05$.

Table 4 result showed a significant increase in the Total cholesterol level in-group B compared to A, however, groups C, E had significant decrease, and D had non-significant increase compared to group B. The triglyceride result showed a significant increase in-group B compared to A, however, groups C, D, and E had significant decrease compared to group B.

Table 5 effect of *Nigella sativa* on HDL, LDL, and VLDL level following alloxan diabetic rat

		MEAN ±SEM	P-value
High-Density-Lipoprotein (mmol/L)	Group A (Normal control)	92.62 ±24.20	0.207
	Group B (Diabetic untreated)	68.47 ±6.83	
	Group C (DM + Gluformin 100mg/kg)	96.93 ±4.61	0.141
	Group D (DM + 20% pulverized NS)	130.96 ±12.11	0.004
	Group E (DM + 40% pulverized NS)	87.87 ±6.25	0.306
Low-Density-Lipoprotein (mmol/L)	Group A (Normal control)	73.05 ±20.05	0.142
	Group B (Diabetic untreated)	112.36 ±26.65	
	Group C (DM + Gluformin 100mg/kg)	19.20 ±1.16	0.002
	Group D (DM + 20% pulverized NS)	52.95 ±19.06	0.033
	Group E (DM + 40% pulverized NS)	39.23 ±11.44	0.011
Very-Low-Density-Lipoprotein (mmol/L)	Group A (Normal control)	14.24 ±1.22	0.000
	Group B (Diabetic untreated)	69.97 ±2.85	
	Group C (DM + Gluformin 100mg/kg)	31.98 ±3.64	0.000
	Group D (DM + 20% pulverized NS)	22.54 ±3.53	0.000
	Group E (DM + 40% pulverized NS)	26.65 ±3.31	0.000

Data was analyzed using ANOVA following post hoc LSD multiple comparison and data was considered significant at $p<0.05$.

Table 5 result reveal a non-significant decrease in HDL activity in group B compared to A, groups C, D, and E had increase compared to B, but was statistically significant at group D, while groups C and E are insignificant. The LDL result showed a non-significant increase in group B compared to A, groups C, D, and E had significant decrease compared to group B. The VLDL result showed a significant increase in-group B compared to A, groups C, D, and E had significant decrease compared to group B.

DISCUSSION

Diabetes mellitus (Dm) is a disorder that results from impairment of insulin action or secretion seen in chronic hyperglycemia and long-term severe vascular complications [37] Further, the use of medicinal plants in managing or treating type-2-diabetes Mellitus linked to hyperglycemia is linked to the secondary metabolite present [38,39].Medicinal plants attract growing interest in the therapeutic management of diabetes mellitus; however, *Nigella sativa* is a plant with numerous bioactive components, which has the potential of mitigative effect on DM. Further, the study revealed that *N. sativa* is rich in flavonoids and saponin more than tannins, alkaloids, phytates, glycosides, and oxalates. [40] reported a high amount of saponin, which has similarities to the study report. [41] reported that *N. sativa* of different extraction had flavonoids, saponin, tannins, and alkaloids, which have similarities to the study findings.

However, the quantitative analysis of EEBS was high in Alkaloids, which has correspondence to the report of [42].

The study showed a significant upsurge in the blood glucose levels in the treated groups (groups B-E) compared to positive control at day 0. However, the precise mechanism of action linked to significantly higher FBG levels could be the generation of ROS, which causes impaired pancreatic dysfunction resulting in insulin sensitivity [43]. However, the destruction of pancreatic beta cells, leading to the surge formation of reactive oxygen species, bringing about a tremendous increase in cytosolic calcium concentration, destroying Beta-cells [43], which leads to the destruction of Beta- to hyperglycemia. The findings of [44], has correspondence to the study's outcome, which revealed a significant increase in the FBG following alloxan toxicity. Further, the study showed that on day 7, groups C and D had a significant decrease and group E had an insignificant decrease ($p > 0.05$) compared to group B. The significant decrease seen in-group C, which was fed with gluformin, could be linked to insulin sensitization of the tissues at the cellular level. However, on day 14, groups C, D, and E significantly decreased compared to group B. The mechanism of action is attributed to the presence of flavonoids and saponins present, which has the potency of reducing FBG and ROS formation. However, the reports of [45,46,47,48,49] had similarities to the study findings revealing a decrease in FBG levels in diabetic rats by *Nigella sativa* treatments.

The study's findings showed a significant increase in the Total cholesterol level in-group B compared to A. However, groups C and E had a significant decrease, and D had a non-significant increase compared to group B. The triglyceride result showed a significant increase in-group B compared to A; however, groups C, D, and E had a significant decrease compared to group B. Dyslipidemia characterized by lipoprotein abnormalities linked with increased triglyceride level, decreased high-density lipoprotein-cholesterol levels and increased in small dense low-density lipoprotein (LDL) particles. However, it is ubiquitous in type-2-diabetes (T2DM), affecting around 70 % of patients and a significant risk factor for atherosclerotic cardiovascular disease [46]. The significantly higher levels of TGs and total cholesterol activity in the diabetic treated rats is associated with increased lipid peroxidative activity by alloxan leading to the formation of ROS, which has an agreement with the reports of [1,4,7,47]. The report of [48,49] had a similar report to the study findings revealing *N. Sativa* had a significant decline in TGs and *total* cholesterol levels following *N. Sativa*.

However, the study reports a non-significant decrease in HDL activity in-group B compared to A. Groups C, D, and E increased compared to B. However, it was statistically significant in Group D, while groups C and E were insignificant. The LDL result showed a non-significant increase in-group B compared to A; groups C, D, and E had a significant decrease compared to group B. The VLDL result showed a significant increase in-group B compared to A; groups C, D, and E had a significant decrease compared to group B. However, the differences seen in the levels of HDL and VLDL are not well understood, as shown by the diabetic control to positive control; the report contradicts the findings of [2,5,8,9]. However, treatment with *N. sativa* showed a significant decrease in VLDL and LDL, which is linked with the flavonoids and saponin effects on the lipid peroxidative activity by reducing excessive ROS formation. The report of [14,18,25] had similarities to the current study.

CONCLUSION

The study revealed that *Nigella sativa* had hypoglycemic and hypolipidemic effects in alloxan diabetic rats.

REFERENCES

1. Abbasnezhad, A., Hayatdavoudi, P., Niazmand, S., & Mahmoudabady, M. (2015). The effects of hydroalcoholic extract of Nigella sativa seed on oxidative stress in hippocampus of STZ-induced diabetic rats. *Avicenna Journal of Phytomedicine*, *5*(4), 333–340.
2. Abbasnezhad, A., Niazmand, S., Mahmoudabady, M., Rezaee, S. A., Soukhtanloo, M., Mosallanejad, R., & Hayatdavoudi, P. (2019). Nigella sativa L. seed regulated eNOS, VCAM-1 and LOX-1 genes expression and improved vasoreactivity in aorta of diabetic rat. *Journal of Ethnopharmacology*, *228*, 142–147.
3. Abd-Elkareem, M., Abd El-Rahman, M. A. M., Khalil, N. S. A., & Amer, A. S. (2021). Antioxidant and cytoprotective effects of Nigella sativa L. seeds on the testis of monosodium glutamate challenged rats. *Scientific Reports*, *11*(1), 13519–13527.
4. Adwas, A., Elsayed, A., Azab, A., & Quwaydir, F. (2019). Oxidative stress and antioxidant mechanisms in human body. *Journal of Biotechnology*, *6*, 43–47.
5. Aguilar Diaz De Leon, J., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Visualized Experiments*, *2020*(159), e61122.
6. Akhtar, M. T., Qadir, R., Bukhari, I., Azeem, R., Malik, Z., Zahoor, S., Murtaza, M. A., Naseer, S., & Shah, H. (2020). Antidiabetic potential of Nigella sativa L seed oil in alloxan-induced diabetic rabbits. *Tropical Journal of Pharmaceutical Research*, *19*(02), 283–289.
7. Al-Attar, A. M., & Abu Zeid, I. M. (2013). Effect of tea (camellia sinensis) and olive (olea europaea L.) leaves extracts on male mice exposed to diazinon. *BioMed Research International*, *2013*.
8. Alimohammadi, S., Hobbenaghi, R., Javanbakht, J., Kheradmand, D., Mortezaee, R., Tavakoli, M., Khadivar, F., & Akbari, H. (2013). Protective and antidiabetic effects of extract from Nigella sativa on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: an experimental study with histopathological evaluation. *Diagnostic Pathology*, *8*(1), 137.
9. Altobelli, G. G., Van Noorden, S., Balato, A., & Cimini, V. (2020). Copper/Zinc Superoxide Dismutase in Human Skin: Current Knowledge. *Frontiers in Medicine*, *7*, 183.
10. American Diabetes Association. (2018). 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care*, *41*(Suppl 1), S13–S27.
11. Amin, B., & Hosseinzadeh, H. (2016). Black Cumin (Nigella sativa) and Its Active Constituent, Thymoquinone: An Overview on the Analgesic and Anti-inflammatory Effects. *Planta Medica*, *82*(1–2), 8–16.
12. Disorder. *Journal of In Silico & In Vitro Pharmacology*, *03*(01).
13. Elseweidy, M. M., Amin, R. S., Atteia, H. H., & Aly, M. A. (2017). Nigella sativa Oil and Chromium Picolinate Ameliorate Fructose-Induced Hyperinsulinemia by Enhancing Insulin Signaling and Suppressing Insulin-Degrading Enzyme in Male Rats. *Biological Trace Element Research 2017 184:1*, *184*(1), 119–126
14. Fawwad, A., Sabir, R., Riaz, M., & Moin, H. (2016). Measured versus calculated LDL-cholesterol in subjects with type 2 diabetes. *Pakistan Journal of Medical Sciences*, *32*(4), 955–960.
15. Federiuk, I. F., Casey, H. M., Quinn, M. J., Wood, M. D., & Ward, W. K. (2004). Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: Route of

administration, pitfalls, and insulin treatment. In *Comparative Medicine* (Vol. 54, Issue 3, pp. 252–257).

16. Gorus, F. K., Malaisse, W. J., & Pipeleers, D. G. (1982). Selective uptake of alloxan by pancreatic B-cells. *Biochemical Journal*,*208*(2), 513–515.
17. Gounden, V., Ngu, M., Anastasopoulou, C., & Jialal, I. (2021). Fructosamine. *StatPearls. /*
18. Gülbayzar, S., Arica, V., Hatipoğlu, S., Kaya, A., Arica, S., & Karatekin, G. (2011). Malondialdehyde Level in the Cord Blood of Newborn Infants. *Iranian Journal of Pediatrics*, *21*(3), 313–319. /pmc/articles/PMC3446175/
19. Gupta, K., Kumar Gupta, P., Vinayak Lokur, A., Praveen Kumar Gupta, C., & Rithu, B. (2019). Phytochemical screening and qualitative analysis of Cymbopogon citratus. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), 3339.
20. Haile, K., & Timerga, A. (2020). Dyslipidemia and Its Associated Risk Factors Among Adult Type-2 Diabetic Patients at Jimma University Medical Center, Jimma, Southwest Ethiopia</p>. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *13*, 4589–4597.
21. Hambright, W. S., Fonseca, R. S., Chen, L., Na, R., & Ran, Q. (2017). Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration. *Redox Biology*, *12*, 8–17.
22. Hamdan, A., Idrus, R. H., & Mokhtar, M. H. (2019). Effects of Nigella Sativa on Type-2 Diabetes Mellitus: A Systematic Review. *International Journal of Environmental Research and Public Health*, *16*(24), 4911–4923.
23. Juan, C. A., de la Lastra, J. M. P., Plou, F. J., & Pérez-Lebeña, E. (2021). The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *International Journal of Molecular Sciences* , *22*(9), 4642–4653.
24. Kaatabi, H., Bamosa, A. O., Badar, A., Al-Elq, A., Abou-Hozaifa, B., Lebda, F., Al-Khadra, A., & Al-Almaie, S. (2015). Nigella sativa improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo controlled participant blinded clinical trial. *PloS One*, *10*(2), e0113486.
25. Kadiiska, M. B., Gladen, B. C., Baird, D. D., Graham, L. B., Parker, C. E., Ames, B. N., Basu, S., FitzGerald, G. A., Lawson, J. A., Marnett, L. J., Morrow, J. D., Murray, D. M., Plastaras, J., Roberts, L. J., Rokach, J., Shigenaga, M. K., Sun, J., Walter, P. B., Tomer, K. B., … Mason, R. P. (2005). Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl4 poisoning. *Free Radical Biology & Medicine*, *38*(6), 711–718.
26. Lenzen, S. (2008a). The mechanisms of alloxan- and streptozotocin-induced diabetes. In *Diabetologia* (Vol. 51, Issue 2, pp. 216–226). Springer.
27. Lenzen, S. (2008b). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216–226.
28. Lenzen, Sigurd, & Mirzaie-Petri, M. (1992). Inhibition of aconitase by alloxan and the differential modes of protection of glucose, 3-O-methylglucose, and mannoheptulose. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *346*(5), 532–536.
29. Li, L. H., Dutkiewicz, E. P., Huang, Y. C., Zhou, H. B., & Hsu, C. C. (2019). Analytical methods for

cholesterol quantification. *Journal of Food and Drug Analysis*, *27*(2), 375–386.
30. Obia, O., Ogwa, C. O., Ojeka, S. O., Ajah, A. A., & Chuemere, A. N. (2016). Effect of honey on the body weight of glibenclamide treated alloxan induced diabetic rats. *Journal of Apitherapy*, *1*(1), 33–35.
31. Ogbera, A., & Ekpebegh, C. (2014). Diabetes mellitus in Nigeria: The past, present and future. *World Journal of Diabetes*, *5*(6), 905–911.
32. Rakhit, M., Gokul, S., Agarwal, A., Plessis, S. du, & Azia, N. (2013). *Studies on women's health*.
33. Ramadan, M. F. (2016). Black Cumin (Nigella sativa) Oils. *Essential Oils in Food Preservation, Flavor and Safety*, 269–275.
34. Rohilla, A., & Ali, S. (2012). Alloxan Induced Diabetes: Mechanisms and Effects. *Int J Res Pharm Biomed Sci*, *3*.
35. Saadane, A., Lessieur, E. M., Du, Y., Liu, H., & Kern, T. S. (2020). Successful induction of diabetes in mice demonstrates no gender difference in development of early diabetic retinopathy. *PLOS ONE*, *15*(9), e0238727.
36. Sada, K. B., Sabir, A. A., Sakajiki, A. M., Umar, M. T., Abdullahi, U., & Sikiru, Y. A. (2021). Clinical profile of patients with diabetes mellitus in gusau, Northwestern, Nigeria. *Annals of African Medicine*, *20*(2), 78–83. https://doi.org/10.4103/aam.aam_16_20
37. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., Saeedi, P., Almutairi, R., Montoya, P. A., Basit, A., Phane Besanç, S., Bommer, C., … Zhang, P. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*(107843), 1–10.
38. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*(1), 44–84.
39. Van Heerden, C., Magwete, A., & Mabuza, D. (2020). Evaluating the need for free glycerol blanking for serum triglyceride measurements at Charlotte Maxeke Johannesburg Academic Hospital. *Clinical Chemistry and Laboratory Medicine*, *58*(8), 1257–1264.
40. Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders. *Antioxidants* , *10*(2), 201.
41. Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *The Journal of Cell Biology*, *217*(6), 1928.
42. Weaver, D., Barry, C., & McDaniel, M. (1979). Molecular requirements for recognition at a glucoreceptor for insulin release. *Molecular Pharmacology*, *16*(2), 361–368.
43. Woldekidan, S., Mulu, A., Ergetie, W., Teka, F., Meressa, A., Tadele, A., Abebe, A., Gemechu, W., Gemeda, N., Ashebir, R., Sileshi, M., & Tolcha, Y. (2021). Evaluation of Antihyperglycemic Effect of Extract of Moringa stenopetala (Baker f.) Aqueous Leaves on Alloxan-Induced Diabetic Rats . *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *14*, 185–192.
44. Yalcin, M., Gulesci N, Bilgin R, & Koltas, I. (2020). Superoxide dismutase, glutathione peroxidase and catalase activities in patients with viral hepatitis C. *Research*

Article Integrative Molecular Medicine, *7*, 1–3.

45. Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N., & Anwar, F. (2019). Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *2019*, 1528635.
46. Younus, H. (2018). Therapeutic potentials of superoxide dismutase. *International Journal of Health Sciences*, *12*(3), 88–93. /pmc/articles/PMC5969776/
47. Zeinvand-Lorestani, H., Nili-Ahmadabadi, A., Balak, F., Hasanzadeh, G., & Sabzevari, O. (2018). Protective role of thymoquinone against paraquat-induced hepatotoxicity in mice. *Pesticide Biochemistry and Physiology*, *148*, 16–21.
48. Zhang, X., Imperatore, G., Thomas, W., Cheng, Y., Lobelo, F., Norris, K., HM, D., MK, A., Gruss, S., Bardenheier, B., Cho, P., Garcia de Quevedo, I., Mudaliar, U., Saaddine, J., Geiss, L., & Gregg, E. (2017). Effect of lifestyle interventions on glucose regulation among adults without impaired glucose tolerance or diabetes: A systematic review and meta-analysis. *Diabetes Research and Clinical Practice*, *123*, 149–164.
49. Zhang, Y., Chen, S. Y., Hsu, T., & Santella, R. M. (2002). Immunohistochemical detection of malondialdehyde-DNA adducts in human oral mucosa cells. *Carcinogenesis*, *23*(1), 207–211.