IAA Journal of Biological Sciences 8(1):164-177,2022. ISSN: 2636-7254

Evaluation of the effect of Black Seed (*Nigella sativa*) on Oxidative Stress Markers of Alloxan–Induced Diabetic Wistar Rats

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ABSTRACT

The effect of Black Seed (*Nigella sativa*) on Oxidative Stress markers of Alloxan–Induced Diabetic Wistar Rats was evaluated. 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 through oral gavage. Diabetes Mellitus (Hyperglycemia) was induced intraperitoneally by a single dose 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 by fasting blood glucose concentration of 200mg/dL and above for three consecutive days. In vitro Antioxidant Potential assay were done using standard procedures. The 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. The result also revealed a significant increase in MDA level in-group B compared to A, groups C, D, and E had significant decrease compared to group B. The CAT result demonstrated a non-significant decrease in-group B compared to group A, groups C, D, and E had a non-significant increase compared to group B. The SOD result showed a non-significant decrease in-group B compared to A, groups C, D, and E had significant increase compared to group B. The GPx result revealed a significant decrease in-group B compared to A, groups C, D, and E had significant increase compared to group B. In conclusion, This study revealed that *Nigella sativa* had hypoglycemic effect in alloxan diabetic rats. However, it has antioxidant activities, which can reduce the lipid peroxidation caused by ROS formation. It is rich in Vitamins, minerals, free scavenging activities, and high antioxidants effect.

Keywords: Black Seed, *Nigella sativa*, Oxidative Stress, Alloxan–Induced , Diabetic Wistar Rats

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of discrete aetiology characterized by prolonged hyperglycemia with turbulence of carbohydrate, protein and lipid metabolism ensuing from defects in insulin secretion, insulin action, or both [1,2,3,4]. Type II diabetes mellitus (T2DM), the loss of a direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver causes increased hepatic glucose product [5,6]. Global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), which rose to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.

However, impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045 [7,8,9]. 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. However, the treatments that involve the use of medicinal plants are recommended [10,11] in the management of diabetes and its related complications. *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. 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 [12,13]. Malondialdehyde is an expedient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid [14]. Malondialdehyde (MDA) is a stable product of lipid peroxidation and therefore can be used as an indirect measure of the cumulative lipid peroxidation [15]. The superoxide dismutase (SOD), catalase, and glutathione peroxidase, are the first line of antioxidant defense grid that plays an indispensable in the entire defense strategy of antioxidants [16]. The SOD is the first detoxification enzyme and most powerful antioxidant in the cell, which has the potency to extend life span [17,18,19]. Catalase (CAT) is a key enzyme in the metabolism of H_2O_2 and reactive nitrogen species, and its expression and localization is markedly altered in tumors [20]. CAT is known for its neutralization through decomposition of hydrogen peroxide, thus maintaining an optimum level of the molecule in the cell, which is also essential for cellular signaling processes [21,22]. Lipid peroxidation is formed by generation of reactive oxygen species (ROS) and causes substantial changes in the cell membrane, with a link to the pathogenesis of many degenerative diseases such as atherosclerosis and carcinogenesis besides diabetes mellitus and aging [23,24]. Diabetes mellitus been a diverse group of metabolic disorders is often associated with a high disease burden in developing countries such as Nigeria [25,26]. 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 [25,26]. Medicinal herbs have gained wide attention and are considered a beneficial adjuvant agent to oral antidiabetic drugs because of their integrated effects [27,28,29,30]. However there is need to investigate the effect of *Nigella sativa* on oxidative stress markers on alloxan–induced diabetic Wistar rats.

Aim of the study

The aim of this study was to evaluate the effect of Black Seed (*Nigella sativa*) on Oxidative Stress Markers of Alloxan–Induced Diabetic Wistar Rats

Fig 1 diagram of *Nigella sativa* [31].

MATERIALS AND METHODS

Materials

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 [9,17].

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]. 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 [32].

Determination of In vitro Antioxidant Potential

The total antioxidant capacity of the samples was determined using the following methods: (DPPH) radical scavenging activity assay, ferric reducing antioxidant potential assay (FRAP) and hydroxyl radical scavenging assay (HRSA) and total antioxidant capacity (TAC) method.
The DPPH radical scavenging activity, the FRAP assay,. Hydroxyl radicals scavenging ability and the Total antioxidant capacity (TAC) were determined by the method of [24].

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 as described by Parasuraman et al. (2010). 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 a micropipette. The retrieved serum was used to assay for oxidative stress markers (MDA) and antioxidants (CAT, SOD, GSH, &

GPx), Vitamin A, C, and E, and blood glucose level using enzymatic method.

Estimation of Superoxide Dismutase Activities

The serum obtained was used to assay for SOD activity by the method described by [24].

Principle: This method will employ the reaction of xanthine and xanthine oxidase to generate superoxide radicals, which will react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetrazolium chloride) to form a red formazan dye. SOD activity was measured by the degree of inhibition of this reaction.

Estimation of Serum Catalase Activities

The activity of catalase was determined according to the standard method described [24] by using a UV-VIS spectrophotometer (Model 752N, China).

Principle

Catalase catalyzes the following reaction:

$2H_2O_2$ catalase $2H_20 + 0_2$

Catalase activity was assessed by incubating the enzyme sample in substrate hydrogen peroxide in the sodium-potassium phosphate buffer, (pH 7.4) at 37 ^{o}C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide were measured at 374 nm against the blank. This is equivalent to the catalase activities in the serum.

Estimation of serum glutathione peroxidase (GPx) activity

The activity of glutathione peroxidase was determined by using a UV-VIS spectrophotometer (Model 752G, China) according to the method of [24].

Principle

Glutathione peroxidase in the presence of hydrogen peroxide (H_2O_2) oxidizes reduced glutathione (GSH) to form H_2O. The amount of GSH consumed is directly proportional to the activity of GPx, and it is expressed as U/ml (µmol of GSH consumed/minute). The GSH remains after the reaction is allowed to react with 5'-5' dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412 nm.

$2GSH + H_2O_2 \longrightarrow$ R-O-H + GSSG + $2H_2O$

GPx **ermination of Malondialdehyde (MDA) Activity**

Tissue MDA was assayed using the method described by Aguilar Diaz De Leon and Borges, (2020). The testes will be stored in a phosphate-buffered solution, which was centrifuged, and the homogenate will be used. The standard stock solution of MDA (1 mM) will be prepared using glacial acetic acid. MDA (31.35 mg) was accurately weighed and dissolved in 100 mL solvent. From the stock solution, different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM will be prepared. The calibration curve were constructed in the concentration range of 0.1 to 1.0 mM.

Preparation of TBA (Thiobarbituric acid) Reagent

The standard solution of 4.0 mM of TBA was prepared in glacial acetic acid, by dissolving 57.66 mg of TBA in 100 mL of glacial acetic acid.

Principle

The standard MDA solution (1 mL) was taken in a 10 mL test tube and mixed with TBA (1 mL). The mixture was heated in a boiling water bath at 95°C for 60 minutes. The test tubes will be cooled at room temperature and absorbance was measured at 532 nm using UV-visible spectrophotometer model PharmaSpec 1700 (Shimadzu, Japan). Each standard for the calibration will be repeated (n=3) according to the above procedure. A blank sample was repeated (n=5) replacing standard or sample by acetic acid or water.

The extract of each sample (1 mL) was mixed with 1 mL TBA reagent, and the above procedure was repeated five times (n=5). The TBARS will be calculated using the formula as M/g of the sample:

TBARS (µM/g) = (Ac x V) / W

Where 'Ac' is the amount determined from the calibration curve, and 'W' is the weight of the sample taken while 'V' is the

volume in mL or dilution factor of the total extract prepared.

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

The results of Antioxidants Minerals 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 1 Antioxidants Minerals of EEBS

MINERALS(PPM)	BLACKSEED
IRON(Fe)	0.73 ± 0.01^{b}
COPPER(Cu)	0.21 ± 0.01^{b}
MANGANESE(Mn)	0.53 ± 0.01^{a}
ZINC(Zn)	0.00 ± 0.01^{a}
SELENIUM(Se)	0.32 ± 0.01^{a}

The result of the Study of EEBS on DPPH Radical Scavenging Assay has been summarized and presented below in table 2. Values are mean ± standard deviation of triplicate determination. Values within the same row bearing he same superscript letters are not statistically significant at P<0.05.

Table 2 Study of EEBS on DPPH Radical Scavenging Assay

CONCENTRATIONS (µg/ml)	% INHIBITION BLACKSEED	STANDARD ASCORBIC ACID (mg/ml)
62.50	35.19 ± 0.01^{c}	59.22 ± 0.01^{b}
125.00	49.10 ± 0.01^{c}	69.64 ± 0.02^{b}
250.00	66.95 ± 0.02^{c}	75.34 ± 0.01^{b}
500.00	74.74 ± 0.02^{c}	7.21 ± 0.02^{b}
Ec_{50}	2.09 ± 0.01^{a}	1.25 ± 0.01^{c}

The result of the Study of EEBS on Hydroxyl Radical Scavenging Assay has been summarized and presented below in Table 3. Values are mean ± standard deviation of triplicate determination. Values within the same row bearing he same superscript letters are not statistically significant at P<0.05.

Table 3 Study of EEBS on Hydroxyl Radical Scavenging Assay

CONCENTRATIONS (µg/ml)	STANDARD BLACKSEED	ASCORBIC ACID (mg/ml)
62.50	9.34±0.01[b]	56.00±0.01[c]
125.00	22.39±0.01[a]	68.21±0.01[c]
250.00	44.23±0.01[a]	68.34±0.01[c]
500.00	67.58±0.01[a]	70.42±0.01[c]
Ec50	2.45±0.01[b]	1.14±0.01[c]

The results of Study of Black seeds on TAC Assay has been summarized and presented below in Table 4. 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 4 Study of Black seeds on TAC Assay

CONCENTRATIONS (µg/ml)	% INHIBITION BLACKSEED	STANDARD ASCORBIC ACID (mg/ml)
62.50	0.34±0.01[a]	0.002±0.00[c]
125.00	0.38±0.01[a]	0.12±0.01[c]
250.00	0.61±0.01[a]	0.13±0.01[c]
500.00	0.77±0.01[a]	0.22±0.01[c]

The result of the Study of EEBS on FRAP Assayhas been summarized and presented below in Table 5. 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 5 Study of EEBS on FRAP Assay

CONCENTRATIONS (µg/ml)	BLACKSEED	STANDARD GALLIC ACID (mg/ml)
500.00	0.84±0.01[b]	0.01±000[c]
250.00	0.56±0.01[b]	0.02±0.01[c]
125.00	0.47±0.01[b]	0.10±0.00[c]
62.50	0.37±0.01[b]	0.13±0.01[c]

The result of the effect of *Nigella sativa* on blood glucose level following alloxan diabetic rat has been summarized and presented below in Table 6

Table 6 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 6 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.

The result of effect of *Nigella sativa* on body weight following alloxan diabetic rat has been summarized and presented below in Table 6

Table 7 effect of *Nigella sativa* on body weight following alloxan diabetic rat

		Mean	±SEM	P-value
Bodyweight (g)	Group A (Normal control)	169.47	±2.59	0.001
	Group B (Diabetic untreated)	140.87	±6.85	
	Group C (DM + Gluformin 100mg/kg)	109.80	±5.39	0.001
	Group D (DM + 20% pulverized NS)	126.45	±3.25	0.107
	Group E (DM + 40% pulverized NS)	112.55	±3.96	0.001

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

Table 7 result revealed a significant decrease in the body weight in-group B compared to A, groups C and E had significant decrease, while group D had an insignificant decrease compared to group B.

Table 8 effect of *Nigella sativa* on MDA, CAT, GPx and SOD level following alloxan diabetic rat

		Mean	±SEM	P-value
Malondialdehyde (umol/L)	Group A (Normal control)	1.02	±0.02	0.000
	Group B (Diabetic untreated)	3.94	±0.33	
	Group C (DM + Gluformin 100mg/kg)	1.85	±0.48	0.001
	Group D (DM + 20% pulverized NS)	1.94	±0.40	0.002
	Group E (DM + 40% pulverized NS)	1.58	±0.43	0.000
Catalase (umol/L)	Group A (Normal control)	24.46	±4.56	0.661
	Group B (Diabetic untreated)	20.94	±3.05	
	Group C (DM + Gluformin 100mg/kg)	34.08	±7.54	0.116
	Group D (DM + 20% pulverized NS)	29.66	±7.60	0.286
	Group E (DM + 40% pulverized NS)	29.13	±3.20	0.315
Glutathione Peroxidase (umol/L)	Group A (Normal control)	170.62	±31.52	0.060
	Group B (Diabetic untreated)	104.93	±23.54	
	Group C (DM + Gluformin 100mg/kg)	174.36	±17.32	0.048
	Group D (DM + 20% pulverized NS)	184.60	±18.35	0.026
	Group E (DM + 40% pulverized NS)	228.05	±20.43	0.002
Superoxide Dismutase (umol/L)	Group A (Normal control)	1.76	±0.03	0.001
	Group B (Diabetic untreated)	0.85	±0.19	
	Group C (DM + Gluformin 100mg/kg)	1.71	±0.08	0.001
	Group D (DM + 20% pulverized NS)	1.35	±0.21	0.038
	Group E (DM + 40% pulverized NS)	1.51	±0.17	0.009

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

Table 8 result revealed a significant increase in MDA level in-group B compared to A, groups C, D, and E had significant decrease compared to group B. The CAT result demonstrated a non-significant decrease in-group B compared to group A, groups C, D, and E had a non-significant increase compared to group B. The SOD result showed a non-significant decrease in-group B compared to A, groups C, D, and E had significant increase compared to group B. The GPx result revealed a significant decrease in-group B compared to A, groups C, D, and E had significant increase 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 [7]. 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. 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 [33]. 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-cell [8], which leads to the destruction of Beta- to hyperglycemia. The findings of [1,3,5,7,8,9] 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 [10,14,17,19] had similarities to the study findings revealing a decrease in FBG levels in diabetic rats by *Nigella sativa* treatments. The study's outcome showed a significant decrease in the body weight in-group B compared to A; however, groups C and E had a significant decrease, while group D had an insignificant decrease compared to group B. The mechanism of action for the weight loss could be attributed to reactive oxygen species (ROS) causing loss of appetite and dehydration resulting from renal failure [8]. Further, the report of [11] aligned with the study findings revealing significant weight loss following diabetes mellitus induction with alloxan. Also, [12,13,15,19,20] reported a significant reduction in the bodyweight following the alloxan-induced diabetic model, which has similarities with the study findings. [20] reported a non-significant weight decrease in the bodyweight following alloxan monohydrate, contradicting the study report. Further, the groups administered with *N. sativa* had a significant decrease in-group E. Its precise mechanism was not fully understood but suggested inhibition of satiety centres by the saponin contents. However, [26] report revealed that *N. sativa* had a significant decrease in body weight, which corresponds to the study findings. Furthermore [11] report showed weight loss following MET and *Nigella* sativa, which is in agreement with the study's findings. [20] reported a significant increase in body weight following *N. Sativa* administration in a diabetic model, which contradicts the study result. The result obtained in the study revealed a significant upsurge in MDA level in-group B compared to A, groups C, D, and E had a significant decrease compared to group B. However, the rise in MDA levels in the diabetic model could be attributed to ROS formation. Also, ROS activation by alloxan toxicity is linked to either increased production or decreased destruction of these formed free radicals by the enzymatic and non-enzymatic antioxidants [33]. The report

of [34,35,36] revealed a significant higher MDA following the alloxan diabetic model, which has similarities to the study report. However, the *Nigella sativa* and Gluformin showed a significant decline in the diabetic treated groups, attributed to the presence of saponin and flavonoids from the plant extract. The report of [37,38] had similarities to the study findings, revealing a significant decline in MDA activity. However, antioxidants are enzymes and non-enzymatic involved in reducing redox activities, which lead to the ameliorative effect of plant extracts or drugs to known toxicity that does result in a decline in their function [39]. The CAT result demonstrated a non-significant decrease in-group B compared to group A; groups C, D, and E had a non-significant increase compared to group B; the physiology behind the increase in the treated groups are not fully elucidated. However, the report of [40,41,42,43,44] showed an attenuated increase compared to diabetic control, which has correspondence to the study findings. Further, the SOD activity showed a non-significant decrease in group B compared to A; groups C, D, and E had higher significance than group B, which is linked with the saponin and flavonoid contents in the *N.* Sativa. It revealed that these phytochemicals play a significant role in the ROS reduction activity, through which the various systems affected by the diabetes gain restoration. The report of [11,14,17] have similarities to the current study findings. The GPx result revealed a significant decrease in-group B compared to A; groups C, D, and E had a significant increase compared to group B. GPx is a critical antioxidant that is involved in the combating of ROS production during alloxan toxicity, which helps reduce its formation and deleterious effect on body systems [19]. The study showed that GPx activity significantly declined in the diabetic control, which resulted from increased ROS formation [25,29] and has an agreement with the study results. However, the study demonstrated significantly higher levels of GPx activities in the treated groups with *Nigella sativa* and Gluformin, which brings about a decline the ROS activity. The report of [13,14,15,17] which has a similar report to the study findings, revealing a significant increase in the GPx activity in a diabetic model. The result showed that the vitamin E antioxidant level in EEBS (24.29), vitamin C, and A were lower in EEBS (303.68mg/100g) and (180.66mg/100g) in EEBS, respectively. The result showed a free scavenging effect of ROS elimination through DPPH, Hydroxyl Radical, TAC, and FRAP. It indicates that EBBS has a high antioxidant effect on diabetic activity, as shown by the reduction in FBG, SOD and GPx levels and reducing MDA activities. The report of [15] had similar findings to the study result.

CONCLUSION

This study revealed that *Nigella sativa* had hypoglycemic effect in alloxan diabetic rats. However, it has antioxidant activities, which can reduce the lipid peroxidation caused by ROS formation. It is rich in Vitamins, minerals, free scavenging activities, and high antioxidants effect.

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