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Neem Seed Oil Ameliorates Key Biochemical/Clinical Dysfunctions in Streptozotocin-Induced Diabetic Wistar Rats

***M.D.Ezema[a,b*], C.A. Onodugo[a], O.S. Adedeji[a],I.M. Odoh[c], O.Y. Adetuyi[d], B.C. Obi[,e], O.H. Oyeniran[a], O.R.Nwagwe[a]**

[a]Functional Foods and Phytomedicine Research Group, Department of Biochemistry, Federal University Oye-Ekiti, PMB 373, Oye-Ekiti, Ekiti State, Nigeria

[b,] Food Science, Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada

[c]University Medical Centre, Federal University Oye-Ekiti, PMB 373, Oye-Ekiti, Ekiti State, Nigeria

[d]Department of Biochemistry, Federal University Oye-Ekiti, PMB 373, Oye-Ekiti, Ekiti State, Nigeria

[e]Department of Pharmacology and Toxicology,Faculty of Pharmacy, University of Nigeria, Nsukka

Corresponding Author: matthew.ezema@fuoye.edu.ng

Abstract

Neem oil, from the fruits and seeds of the Neem (*Azadirachtaindica*), is reported to possess many medicinal properties. The present study is to ascertain the ameliorative potentials of Neem seed oil (NSO) on some biochemical/clinical dysfunctions in streptozotocin-induced diabetic rats. Forty-eight (48) rats were distributed into eight groups of six. Standard procedures were used to evaluate the levels of haematological, liver functions, kidney functions, lipid profile indices and changes in oxidative stress. The 28 day study revealed that Neem seed oil at different dosages (0.1ml, 0.2ml and 0.5ml) significantly ($P< 0.05$) reversed some biochemical/clinical imbalances in the diabetic rats by increasing the levels of Packed cell volume, Red blood cell, Haemoglobin and white blood count and lowering the levels of liver enzymes, total protein, albumin and some lipid profile indices..Coupled with the observed reductions in levels of oxidative stress markers, the results showed that Neem seed oil at notable concentrations could potentially serve as antidote in the management of diabetes. Therefore, we recommend use of neem seed oil as alternative medication in treatment of Type 2 diabetes.

Key words: Ameliorative potentials, diabetes mellitus, biochemical dysfunctions, streptozotocin.

Introduction

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders and it is caused by a combination of two primary factors - defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin (Unai*et al*, 2020).The World Health Organisation (WHO) data indicates that diabetes was the direct cause of about 1.6 million deaths in 2021 (WHO 2024). When including deaths from kidney disease caused by diabetes and high blood glucose related cardiovascular deaths, the total increased to over 2 million deaths annually (WHO 2024).

Although there are available and popular antidiabetic medicines in the pharmaceutical market with considerable progress in the management of diabetes mellitus by these synthetic drugs, diabetes and its complications continue to be a major medical problem, hence the search for indigenous antidiabetic agents still continues.Consequently, there is increasing acceptability of the use of herbal remedies in the treatment of various ailments, including diabetes which calls for need for clinical assessment of the safety of these remedies.

Neem (*Meliaceae*), has been widely known for centuries as a source of active ingredients to develop products for health providers in remote areas and report showed that primary healthcare in some developing countries has included treatments with Neem tree or its parts (Saleem*et al*., 2018).

Neem oil, also known as margosa oil, is indigenous to the Indian sub-continent and has been introduced to many other areas in the tropics. It is reported that the oil is the most important of the commercially available products of Neem plant and it

is used for organic farming and medicines (Meeran*et al.*,2013).

In an attempt to find cure to the ravaging effect of diabetes, several animal models are continually being developed to study mechanisms and therapeutic interventions. According to African Health Science (2021), Streptozotocin (STZ) has been commonly used for the induction of diabetes in experimental models of mice and rodents because of its proven potency to exert toxic effects on pancreatic islet beta cells and induce diabetes mellitus in the animals (Raza and John, 2012). Biochemical or clinical changes in various body organs associated with diabetic or in pre-diabetic state could be investigated in experimental animals and by extension be used in studies related to clinical problems in human diabetes mellitus (Unai*et al*, 2020).

Neem seed oil has been known to exhibit antidiabetic properties (Gupta *et al.*, 2000) andthis research seeks to confirm and validate the earlier scientific reports about the antidiabetic potentials of neem seed oil. The present research was therefore undertaken to ascertain ameliorative potentials ofNeem (*Azadirachtaindica*) seed oil on key biochemical/clinical dysfunctions induced with STZ in wistar rats.

Objective of the Study

The objective of the study is to ascertain the ameliorative potentials of Neem seed oil (NSO) on some biochemical/clinical dysfunctions in streptozotocin (STZ)-induced diabetic rats.

Specific objectives of the Study

- To assess the ameliorative potentials of NSO on haematological indices in streptozotocin-induced diabetic wistar rats.
- To determine the ameliorative potentials of NSO on lipid profile parameters
- To ascertain the ameliorative potentials of NSO on liver function indices
- To examine the ameliorative potentials of neem seed oil NSO on kidney function indices
- To evaluate the ameliorative potentials of neem seed oil NSO on oxidative stress markers

Materials and Methods

Materials

Neem seed oil was purchased from Nutri-Pea Limited (Portage La Prairie, Manitoba, Canada).Streptozotocin (STZ) and glibenclamide (Daonil) was purchased from Sigma-Aldrich (St Louis, Missouri, USA). Other chemical reagents used as indicated in the methods were of analytical grade and obtained from Fisher Scientific Company (Oakville, ON, Canada).

Experimental Animals

Adult male Wistar albino rats with weights of 100–180g were maintained at the Animal House of the Department of Biochemistry, Federal University Oye-Ekiti. The rats were acclimatized under standard laboratory condition with 12-h light/dark cycle for 3 weeks prior to the commencement of the experiment. They were given regular feed (commercial Rodent Chow, Vital Feeds Nig. Ltd.) and had access to clean drinking water *ad libitum*. Treatment of the animals conformed to the guideline for the Care and Use of Laboratory Animals **(**The National Academy of Sciences, 2011).

Experimental Design

Baseline blood glucose levels were determined prior to diabetes induction in the rats. Rats were fasted overnight before the intraperitoneal injection of 60 mg/kg bwt. of streptozotocin (STZ) which was dissolved in citrate. Rats with blood glucose levels higher than 8.2 mmol/L (147.6 mg/dl) after 3 days (day 0) were considered diabetic and randomly distributed into8 groups of 6 rats each:Neem seed oil at low, medium, and high doses were administered via oral gavage for 28 days for more precise assessment of the effects of varying doses of neem seed oil on haematological and other biochemical parameters as follows:

Group 1: negative control (no induction)
Group 2: rats treated with 0.1ml neem seed oil only
Group 3: rats treated with 0.3 ml glibenclamide(daonil) only
Group 4: positive control (STZ only, no treatment)
Group 5: STZ-induced diabetic rats treated with 0.2 ml of glibenclamide
Group 6: STZ-induced diabetic rats treated with 0.1 ml neem seed oil
Group 7: STZ-induced diabetic rats treated with 0.2 ml neem seed oil
Group 8: STZ-induced diabetic rats treated with 0.5 ml neem seed oil

Administration/harvesting of organs

Treatment was done once per day and lasted for twenty-eight (28) days. The animals were then

euthanized by decapitation. Whole blood was collected from the jugular vein in polyethylene tubes with an anticoagulant (EDTA) and centrifuged at 3000*g* for 15 min at 4 °C to obtain the plasma, which was then stored at −80 °C until analysis. The thoracic and abdominal regions were opened, and the liver and kidney were harvested, rinsed with saline solution and weighed. Homogenates were prepared at a ratio of about 100 mg wet liver and kidney tissue per 1 mL of 50 mmol·L−1 phosphate buffer (pH 7.4) or 5% trichloroacetic acid (TCA) solution using a homogenizer (MA102/Mini, Marconi, Piracicaba – SP, Brazil). The homogenates were used in antioxidant enzymes and reduced glutathione content assays, respectively. The protein concentration of liver homogenates was determined by the Bradford method (Bradford, 1976).

Biochemical/Clinical Indices

Haematological indices

The complete blood count (CBC) was determined on an automated hematology analyzer using well-mixed whole blood with K2-EDTA to prevent blood clotting. A CBC differential analysis was performed according to the visual method of Dacie and Lewis (1991). The percentage of packed cell volume (PCV) was carried out using the hematocrit method, while the blood hemoglobin (Hb) concentration was measured according to the cyanomethemoglobin method of Alexander and Grifiths (1993). Mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated following the method of Dacie and Lewis (1991), while differential white blood cell counts was estimated using the method of Osim*et al.*, (2004).

Lipid profile parameters

Total cholesterol (TC) was determined using the method of Allain*et al* (1974); triacylglycerol (TAG) was carried out using the method of Albers *et a*l (1978); high-density lipoprotein-cholesterol (HDL) concentration was determined using the method of Albers et al. 1984, while low-density lipoprotein-cholesterol (LDL) concentration was determined using the method of Friedewald*et al.*, (1972), as contained in Randox commercial kits.

Liver function indices

Assays of aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT) and alkaline phosphatase activity (ALP) as well as total protein and albumin were determined using individual standard assay methods as stipulated in the Randox commercial kits.

Kidney function indices

Creatinine, urea and serum electrolytes (Na^+, K^+, Cl^-, etc) were determined using the Randox commercial kits.

Antioxidant assay

All oxidative stress biomarkers(Superoxide dismutase,SOD, Catalase (CAT), Glutathione transferase (GSTs) and Reduced Glutathione (GSH)activities were assayed using their respective commercial kits (Randox Diagnostics) while Malondialdehyde (MDA) concentration was determined by measuring spectrophotometrically the level of the lipid peroxidation productas described byWeitner*et al.*, (2016).

Statistical Analysis.

Data obtained were analyzed using (SPSS), version 25. Test of statistical significance was carried out using one-way analysis of variance (ANOVA). Results were expressed as mean ± SD of triplicate samples. Post-Hoc Dunnett's-test at 95% level of significance was used to assess significant difference between the control and treated groups. Mean values with $p < 0.05$ were considered significant.

Results

Effect of Neem seed oil on hematological indices in streptozotocin- induced diabetic rats

Results of red blood cell and white blood cell indices are presented in Tables 1and 2 respectively. In Table 1, there is significant increase in number of red blood cells in groups administered with neem seed oil when compared with both positive and negative controls and thus, a corresponding increase in haemoglobin values in the same trend, (16.53g/dl,15.82g/dl,15.8g/dl), with the highest value of Hb recorded with 0.1 NSO (16.53g/dl) (Table 1). Similar pattern is noticed with PCV (hematocrit) at different dosages (0.1ml,0.2ml,0.5ml) in the diabetic rats. In addition, there is decrease in MCV in the treated groups (96.67fl, 106.67fl,100fl) at different dosages (0.1 mL, 0.2 mL and 0.5mL) when compared with the controls (Table 1) .Meanwhile, there is no significant change in the value of MCH (36.03pg) at 0.2ml and

MCHC (33.29g/dl,33.22g/dl,33.25g/dl) at different doses (0.1ml,0.2ml,0.5ml) as shown in the table.

Table 2 shows that there is significant increase in activities of white blood count at 0.1ml and 0.2ml NSO when compared to the control; eosinophil at 0.1ml NSO with daonil(1.33) and 0.2ml NSO with daonil (1.33); monocyte at 0.1ml (7.67) and lymphocyte at 0.5ml(4.7). Further, there is no significant change in activities of monocyte at 0.2ml (7.33), lymphocyte(43.33,43.33) at 0.1ml and 0.2ml, neutrophil(46.67,48,48) at different dosages (0.1ml, 0.2ml and 0.5ml) compared to the control (Table 2).

Parameters	Treatment							
	Normal control	NSO at 0.1 ml	GBC at 0.2 ml	Diabetes group	Diabetes + GBC (0.2 ml)	Diabetes + NSO (0.1 ml)	Diabetes + NSO (0.2 ml)	Diabetes + NSO (0.5 ml)
RBC	4.71 ± 1.17[a]	5.19 ± 0.73[a]	4.68 ± 0.01[a]	3.31 ± 0.01[a]	4.68 ± 0.62[a]	5.71 ± 0.02[a]	3.87 ± 0.01[a]	4.86 ± 0.01[a]
HB	13.97 ± 0.14[a]	16.87 ± 0.01[a]	14.83 ± 0.01	13.31 ± 0.01	14.73 ± 0.01	16.53 ± 0.01	16.53 ± 0.01	16.41 ± 0.01
PCV	42.33 ± 0.01	50.33 ± 0.01	46.67 ± 0.01	37.33 ± 0.01	44.33 ± 0.01	49.67 ± 0.01	47.67 ± 0.01	47.52 ± 0.01
MCH	33.23± 0.02	34.86 ± 2.94	32.53 ± 0.01	37.43 ± 0.01	32.27 ± 0.01	32.47 ± 0.01	36.03 ± 0.01	33.40 ± 0.01
MCV	113.3 ± 0.01	96.67± 0.01	96.67 ± 0.01	110.11 ± 0.01	96.67 ± 0.01	96.67 ± 0.01	106.67 ± 0.01	100.11 ± 0.01
MCHC	33.23 ± 0.01	32.97 ± 1.49	33.27 ± 0.07	33.22 ± 0.06	33.31 ± 0.02	33.29 ± 0.05	33.22 ± 0.06	33.25 ± 0.01

Table 1: Results of Red blood cell indices

Legend: RBC - Red blood cell, Hb - haemoglobin, PCV - packed cell volume, MCV - mean cell volume, MCH - mean cell haemoglobin, MCHC - mean cell haemoglobin concentration. Values are presented as mean ± standard deviation of triplicate samples.

Table 2: Results of White blood cell indices.

Parameters	Treatment							
	Normal control	NSO at 0.1 ml	GBC at 0.2 ml	Diabetes group	Diabetes + GBC (0.2 ml)	Diabetes + NSO (0.1 ml)	Diabetes + NSO (0.2 ml)	Diabetes + NSO (0.5 ml)
WBC	9.80 ± 0.01	16.17 ± 0.05	10.08± 0.01	11.23 ± 0.25	6.60 ± 0.01	11.37 ± 0.05	13.07 ± 0.18	11.10 ± 0.05
Lymphocyte	43.10 ± 0.01	42.67 ± 0.01	43.66 ± 0.09	42.67 ± 0.05	40.33 ± 0.05	43.33 ± 0.05	43.33 ± 0.05	47.10 ± 0.05
Neutrophils	48.33 ± 0.05	48.10 ± 0.01	49.10 ± 0.01	47.67 ± 0.05	50.33 ± 0.05	46.67 ± 0.05	48.10 ± 0.01	40.20 ± 0.01

Monocytes	7.67 ± 0.05	8.33 ± 1.65	6.67 ± 0.05	8.67 ± 0.05	5.33 ± 0.23	8.67 ± 0.05	7.33 ± 0.05	6.56 ± 0.01
Eosinophils	1.20 ± 0.01	1.20 ± 0.01	0.67 ± 0.05	1.20 ± 0.01	1.20 ± 0.01	1.33 ± 0.05	1.33 ± 0.05	0.67 ± 0.01

Values are presented as mean ± standard deviation of triplicate samples.
Legend: WBC = white blood cell, NSO = Neem seed oil, GBC = Glibenclamide

Effect of Neem seed oil on liver biomarkers in streptozotocin- induced diabetic male Wistar rats.

The activities of ALP, AST and ALT as well as levels of albumin and total protein are shown in Table 3 below. It shows that there is significant increase in the levels of AST, ALT and ALP in the diabetic control (untreated) rats when compared with the normal and test groups.Although, all neem seed oil dosages resulted in a significant difference in ALP, AST and ALT activities, treatment with several dosages of neem seed oil led to decrease in the level of these liver enzymes. However, 0.2ml dosage of neem seed oil gave the highest percentage decrease in ALP and ALT activities as compared with the standard drug (glibenclamide) and other dosages.Also it was discovered that the little dosage of neem seed oil (i.e 0.1ml of NSO) has little effect on the levels of ALT in the liver whereas large dosage of neem seed oil decreased the levels of liver biomarkers in the serum. It was also noted that there is no significant difference between the neem seed oil and the standard drug i.eglibenclamide on the liver biomarkers.

From Table 3,the level of total protein increased consequent upon induction of STZ as compared to the control. However, treatment with a standard drug (daonil) produced more significant decrease in total protein level in the serum when compared with the decrease noticed in 0.2ml and 0.5ml NSO. the induction of diabetes increased the level of total protein while decreasing that of albumin as shown. However, treatment with neem seed oil (NSO) and the standard drug (glibenclamide) decreased the level of total protein while increasing albumin levels in the diabetic but treated groups, especially at 0.2ml and 0.5ml NSO for total protein and 0.1ml NSO for albumin respectively.

Parameters	Treatment							
	Normal control	NSO at 0.1 ml	GBC at 0.2 ml	Diabetes group	Diabetes + GBC (0.2 ml)	Diabetes + NSO (0.1 ml)	Diabetes + NSO (0.2 ml)	Diabetes + NSO (0.5 ml)
ALT (µmol/L)	47.05 ± 0.07	50.02 ± 0.42	38.25 ± 0.35	59.25 ± 0.71	37.00 ± 0.14	51.10 ± 0.06	37.00 ± 0.14	49.02 ± 0.01
AST (µmol/L)	130.03 ± 0.01	135.03 ± 0.72	137.45 ± 0.70	148.02 ± 0.01	132.25 ± 0.35	147.15 ± 0.07	151.00 ± 0.14	135.06 ± 0.01
ALP (µmol/L)	415.05 ± 0.95	422.24 ± 0.05	371.05 ± 0.07	688.96 ± 1.33	318.10 ± 0.28	265.07 ± 0.39	320.40 ± 0.57	305.80 ± 1.13
Total protein (g/L)	56.05 ± 1.34	68.10 ± 0.99	70.00 ± 1.48	63.25 ± 0.35	46.02 ± 1.44	71.01 ± 0.01	56.25 ± 1.48	56.75 ± 0.35
Albumin (g/L)	29.15 ± 0.04	34.42 ± 1.24	27.18 ± 0.04	25.22 ± 1.57	19.01 ± 1.43	38.01 ± 0.01	28.10 ± 0.57	20.00 ± 0.28

Table 3: Result of the activities of ALP, AST and ALT and levels of albumin and total protein. Values are presented as mean ± standard deviation of triplicate samples.

Legend: NSO- Neem seed oil, GBC- Glibenclamide, STZ-streptozotocin, ALT - Alanine transaminase, AST- Aspartate transaminase, ALP - Alkaline phosphatase.

Effect of Neem seed oil on Kidney function indices in streptozotocin-induced diabetic rats

The results of kidney function indices are presented in Figures 1a - e below: Fig. 1a shows that induction of diabetes in the rats increased the urea level as seen in STZ group (45.0±5.09) when compared with the normal control (34.2±0.00). However. administration of NSO significantly decreased the level of urea in the test groups, especially with 0.2ml NSO (32.4±2.54). 0.5ml NSO does not show an effect to the diabetic group.

In Fig.1b, the creatinine concentration level is increased upon induction with STZ (42.0±2.82) compared with the normal control (40.0± 1.41). However, upon administration of NSO in the test groups, there was significant decrease in creatinine level, especially with 0.2ml NSO combined with the standard drug(.34.0±1.41)**.** There is no significant difference in values of creatinine level in other dosages when compared with the normal, suggesting that the best efficacy is achieved when 0.2ml NSO is used with daonil.

Figures 1c - e show the concentrations of serum electrolytes (mmol/L). The results showed that induction of diabetes in the experimental animals significantly decreased the level of sodium (126.5±28.99 vs 148.0±0.00), potassium (5.3±0.85 vs 6.2±0.00) and chloride ions (96.0±11.31 vs 108.0±0.00) when compared with the normal controls. However, treatment with neem seed oil (NSO) significantly increased the levels of all the three electrolytes tested, with remarkable increase at some dosages as shown in the figures.

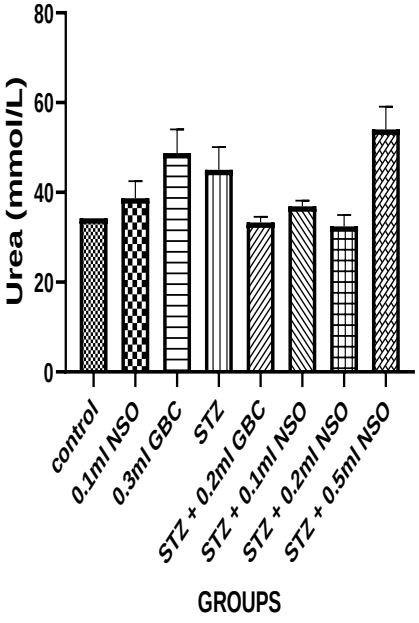


Fig.1a. Result of Urea level

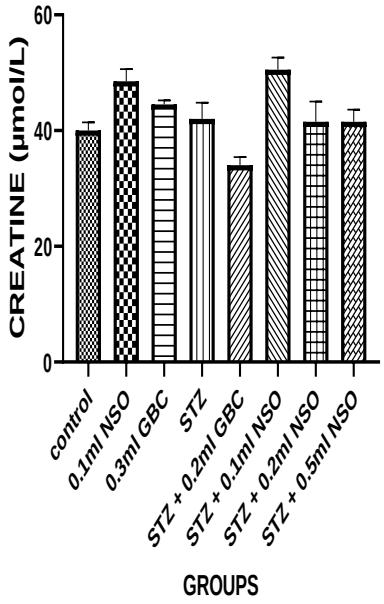
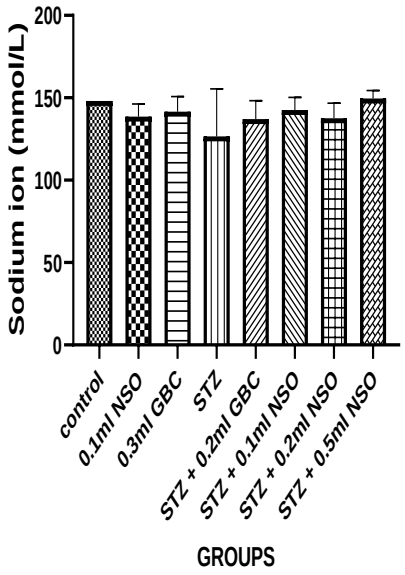


Fig. 1b.Result of Creatinine level



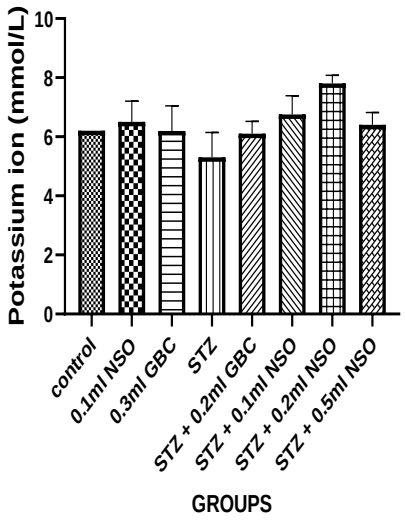


Fig.1c. Result of Sodium ion level **Fig.1d. Result of Potassium ion level**

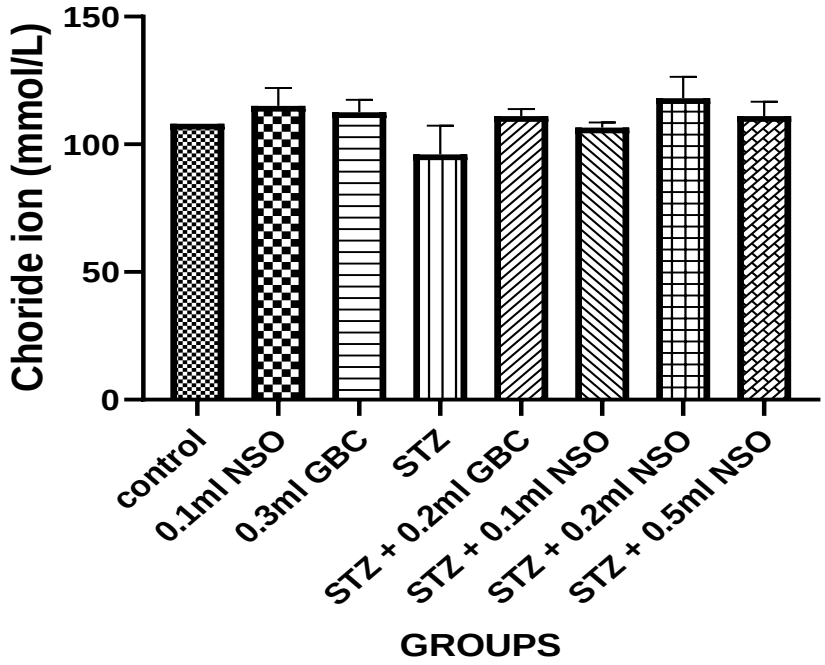


Fig.1e. Result of Chloride ion level

Figure 1 (a - e): The result of kidney function indices.

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Effect of neem seed oil on lipid profile in STZ-induced diabetic wistar rats.

Result of lipid profiles in Table 4 shows a significant increase ($p>0.05$) in the lipid profile indices as a result of induction of the rats with diabetes using STZ, except LDL. However, there was a significant decrease in groups treated with different dosages (ml) of neem seed oil, especially with 0.2ml (20.35+3.3mg/dl) and 0.5ml NSO(21.85+2.89mg/dl).

Table 4: Result of lipid profiles

Parameters	Treatment							
	Normal control	NSO at 0.1 ml	GBC at 0.2 ml	Diabetes group	Diabetes + GBC (0.2 ml)	Diabetes + NSO (0.1 ml)	Diabetes + NSO (0.2 ml)	Diabetes + NSO (0.5 ml)
HDL	24.20 ± 1.13^{a}	16.25 ± 2.48^{b}	24.45 ± 4.03^{a}	27.45 ± 3.18^{c}	18.15 ± 2.33^{b}	26.05 ± 1.20^{a}	21.98 ± 2.08^{a}	22.90 ± 1.83^{a}
LDL	17.70 ± 3.25^{a}	21.70 ± 2.68^{b}	16.65 ± 3.18^{a}	11.55 ± 3.60^{c}	23.20 ± 2.26^{b}	16.95 ± 1.06^{a}	18.00 ± 2.55^{a}	20.20 ± 3.11^{b}
TAG	18.90 ± 1.27^{a}	18.30 ± 2.97^{a}	19.75 ± 2.47^{a}	25.30 ± 0.28^{c}	20.00 ± 2.82^{b}	23.30 ± 2.40^{b}	20.50 ± 3.32^{b}	21.85 ± 2.89^{b}
T-Chol	36.85 ± 1.20^{a}	44.85 ± 2.97^{b}	47.00 ± 2.87^{b}	45.60 ± 3.39^{b}	50.50 ± 2.68^{c}	52.50 ± 3.04^{c}	48.65 ± 2.61^{b}	50.70 ± 2.97^{c}

Data are shown as mean ± S.D. Mean values with the different signs are significantly different at $P<0.05$.
Legend: STZ- Streptozotocin, NSO- neem seed oil (AzadiracthaIndica), GBC or Daonil

Effect of neem seed oil on Oxidative stress markers in the liver and kidney of STZ - induced diabetic rats.

The result of effect on oxidative stress markers is shown in Figures 2a, e below:

Fig 3a. shows that levels of MDA were significantly higher in rats with neem seed oil therapy.Fig 3b revealed that SOD activities was significantly inhibited along with an elevation ofLPO in kidney and liver of STZ-induced diabetic animals which is not reversed by different treatments. Evaluating SOD activity in hepatic and renal tissues (Fig 2b) showed a significant increase in group 4 when compared to group 1 in the liver. Groups 6,7 and 8 revealed lower levels of this enzyme activity in liver when compared to group 4. Groups treated with Neem seed oil only show a slight difference in SOD activity when compared to group 4.

Considering CAT activity in Fig 2c, the activity of this enzyme was highly expressed in the kidney compared to the liver. Group 4 and 8 show the same increase in the liver and group 7 in the kidney. No significant difference was observed between group 2, 3 and 7 in the liver.

In Fig. 2d, GST activity in the liver was slightly increased in group 4 compared to group 1 but there was no change in the activity of the enzyme in the kidney. Groups treated with Neem seed oil presented lower levels of GST Activity compared to group 1 and group 4.Group 3 treated with Glibenclamide but are not diabetic revealed a low level of the enzyme activity compared to group 4, however group 5 showed a slight increase compared to group 4.In the Kidney,there was observed decrease in enzyme activity in group 6,7 and 8.

Fig. 2e shows the effect of NSO on level of Reduced Glutathione(GSH). Induction of the animals with STZ increased the level of GSH as shown in the control. However, there was significant reduction in level of GSH in groups treated with NSO when compared with diabetic group.

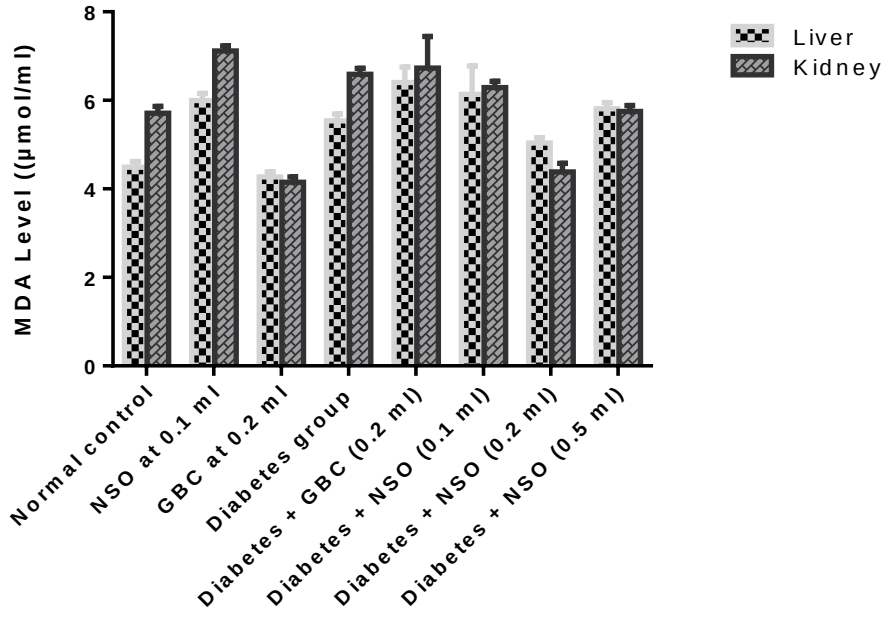

Fig 2a. Result of malondialdehyde (MDA)

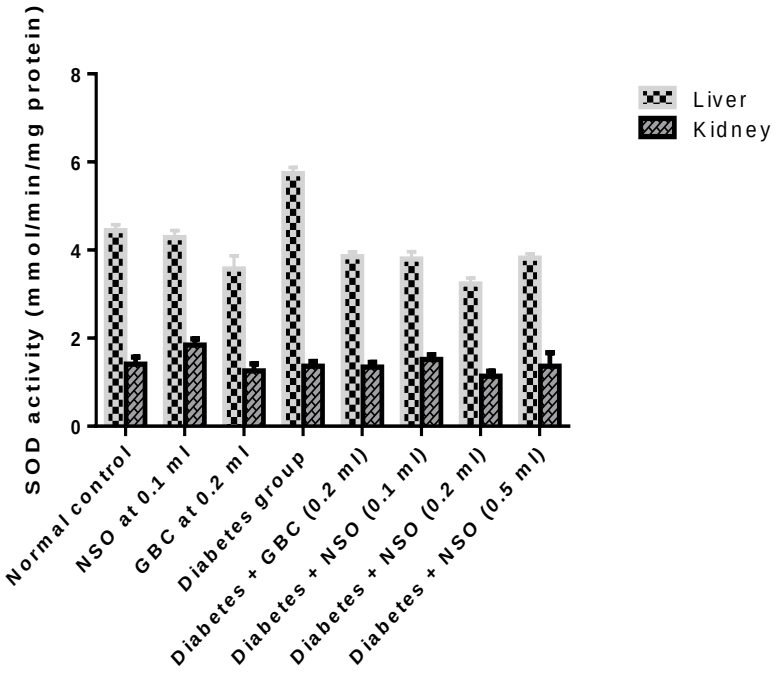

Fig.2b. Result of Superoxide Dismutase

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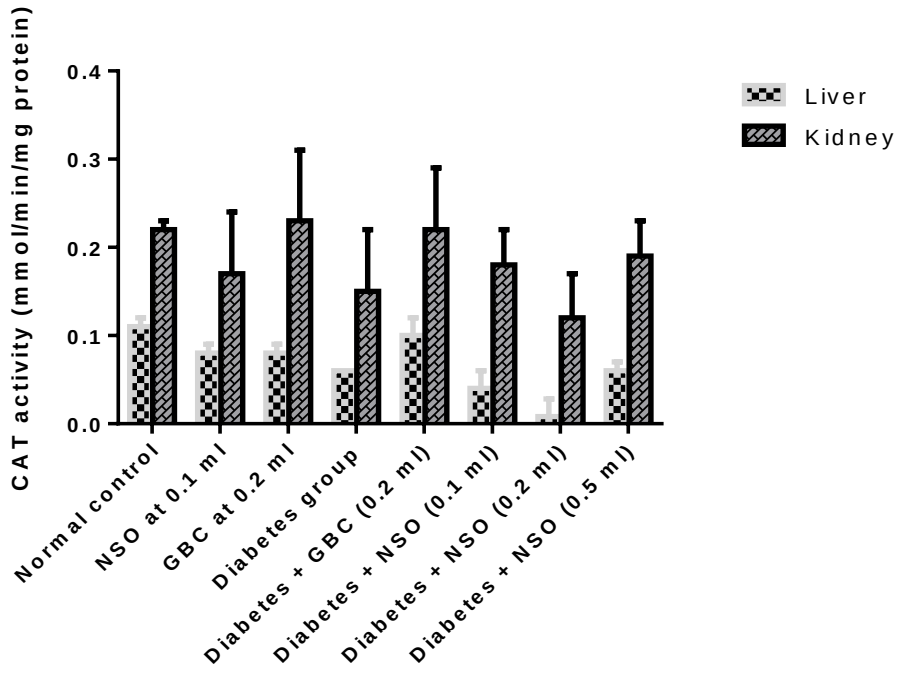

Fig.2c. Result of Catalase

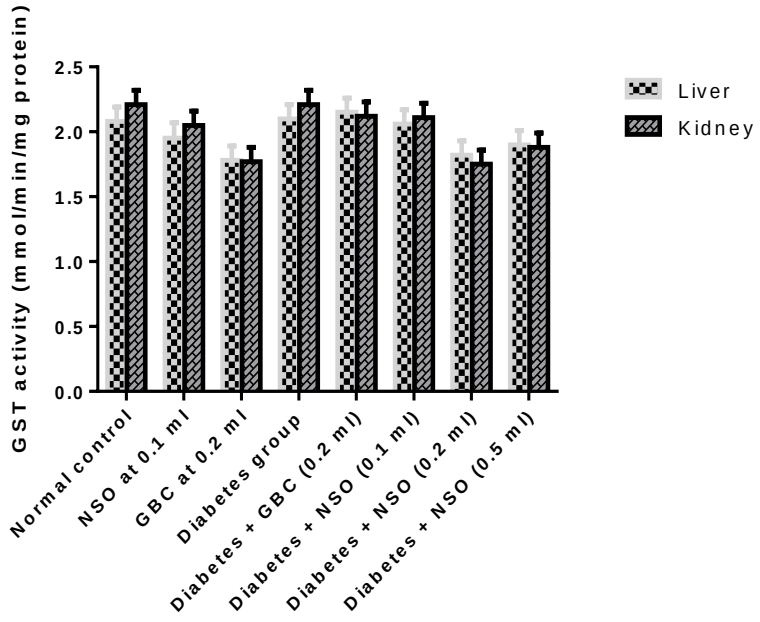

Fig.2d. Result of Glutathione- S- Transferase

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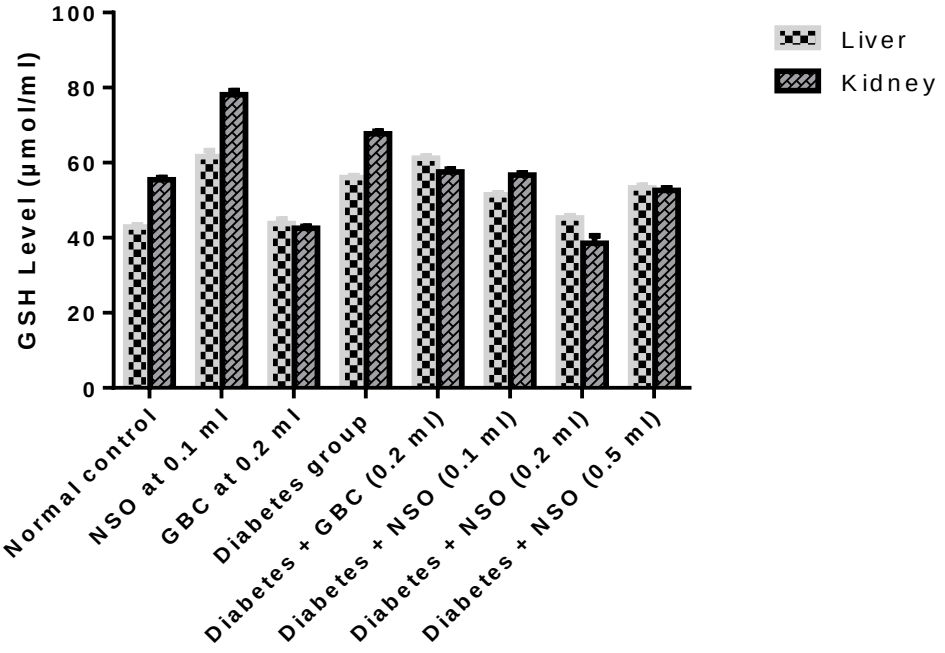

Fig.2e. Result of Reduced Glutathione (GSH).

Figs 2a - e: MDA, SOD, CAT,GST and GSH levels and activities in the liver and kidney of STZ_induced diabetic rats.
Legend: MDA (Malondialdehyde), NSO (Neem seed oil), CAT (Catalase), SOD (Superoxide Dismutase), GST (Glutathione S transferase) GSH(Reduced Glutathione).

Discussion

Haematological indices

Streptozotocin has been shown to be the most used diabetogenic chemical for creating rat models of type 1 and type 2 diabetes due to the fact that it causes both direct organ toxicity and diabetes, and *both* actions can affect the structure and function of many organs, including blood (Ghasemi and Jeddi, 2023, Watkins *et al*,.2000). Clinical analysis of changes in levels of blood in diabetic conditions is important. Specifically, evaluation of red blood cell indices is helpful in determining the cause of anemia**.**

In this study, as earlier noted, the significant increase in number of red blood cells in groups administered with neem seed oil when compared with both positive and negative controls and a corresponding increase in haemoglobin and PCV values in the same trend (Table 1) is a subsequent ameliorative effect of NSO on STZ damaged red cell indices.

Furthermore, the decrease in MCV in the treated groups at different dosages (0.1ml, 0.2ml and 0.5ml)) when compared with the controls (Table 1) can be attributed to microcytosis (iron deficiency anemia). This can lead to polycythemiathis, dehydration, polycythemia or lung disease. Generally, reduction of adequate oxygen supply (Hypoxia) can lead to anaemia (Bain ,2015, Pittman, 2011)**.** The cause of anemia (iron deficiency or thalassemia (hemoglobin disorder) could be because of blood osmoregulation defect and abnormal synthesis of hemoglobin (Stookey et al., 2007) resulting in decreasing levels of MCH (32.43pg, 33.4pg) at 0.1ml and 0.5ml in the diabetic rats.

White blood cells is known to defend the body against infections and are also involved in the inflammatory response (Pagana*et al.,* 2014). A high value of white blood cell count (leukocytosis) often occurs in infections, inflammation, and states of physiologic stress. It can also be as a result of diseases that involve abnormal production of blood cells, such as myeloproliferative and lymphoproliferative disorders **(**Walls *et al.,* 2017).

In this study, the significant increase in activities of white blood count, eosinophil and monocyte especially at specified doses (0.1ml and 0.2ml NSO) when compared to the control (Table 2) may be attributed to physiological stress associated with diabetes.A decreased white blood cell count, termed leukopenia, can lead to an increased risk of acquiring infections (Territo, 2020), which result in decrease in the activities of monocyte at 0.5ml (Table 2).

In this study, treatment of the diabetic rats with extracts of neem seed oil and standard drug (glibenclamide) significantly improved the alterations in hematological indices, especially at 0.2ml and 0.5ml dosages

Liver function indices

According to reports, the earliest and most important indicators in assessing liver injury are levels of plasma alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) (Crawford *et al.*, 2009; Longo *et al.*,2011; Nannipieri*et al.,* 2005). An increase in these enzyme activities is a clear indication of liver damage (Elisa *et al.*, 2009). Cell damage to the liver causes these cytosolic enzymes to spill into the sinusoids and finally into the blood stream. In this study there is significant increase in levels of the liver enzymes (ALP, ALT and AST)in diabetic group compared with the normal control (Table 3). However, treatment of the diabetic rats with extracts of neem seed oil and standard drug (glibenclamide) significantly decreased the concentrations of these liver enzymes in serum when compared with the diabetic control rats.This result agrees with Ohaeri (2001), who reported that induction of diabetes with STZ resulted in necrosis of the liver of rats. Hence, the increase observed in the activities of AST and ALT may result from the leakage of these aminotransferase enzymes from the cytosol of the liver into the blood, which therefore indicates the hepatotoxic impact of STZ (Zafar *et al.*, 2009). The result also agrees with the reports of other researchers who observed similar elevations in activities of liver enzymes following STZ induction (Zafar *et al.*, 2009; Najla*et al.*, 2012; Soliman, 2013; Omonkhua*et al.*, 2014). Chronic and untreated diabetes tends to induce liver injury and damage thereby affecting the liver's overall metabolic functions in diabetic patients.. . The high percentage reduction in these liver enzyme activities may not be far-fetched as several authors have reported on the hepato-protective effects of extracts of neem seed oil.

Although there are reports of decrease in serum total proteins in STZ diabetic rats (Najla*et al.*, 2012), in this study, the diabetic state or its treatment with neem seed oil and glibenclamide significantly altered the level of serum total protein concentrations.

. Albumin is one of the major proteins synthesized in the liver and it is important to note that energy supply is a very important determinant for the normal physiology of albumin production .Reduced serum albumin levels are observed in medical conditions associated with malnutrition (Levitt and Levitt, 2016; Seiler 2001). Although high serum albumin levels have been reported to be associated with metabolic syndrome, an indicator of obesity and over nutrition, both the antioxidant and anti-inflammatory properties of albumin in the atherogenetic process have been suggested as possible mechanisms for this inverse association ((Kadono*et al.*, 2010; Ishizaka *et al.*, 2007; Djousse*et al.*, 2002; Zhang *et al.*,2002).

It should be noted that the levels of total protein in body are expected to be low while albumin level are expected to be high but the induction of diabetes increased the level of total protein while decreasing that of albumin as shown in Table 3.

However, treatment with neem seed oil (NSO) and the standard drug (glibenclamide) decreased the level of total protein while increasing albumin levels in the treated groups, especially at 0.2ml and 0.5ml NSO for total protein and 0.1ml NSO for albumin respectively due to the possible ameliorative effect of NSO.

Kidney function indices

The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum because urea is a major nitrogenous end product of protein and amino acid catabolism. It is useful in differential diagnosis of acute renal failure and pre-renal condition where blood urea nitrogen–creatinine ratio is increased (Mitchell and Kline, 2006). It is reported that Increased blood urea nitrogen (BUN) is associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract.

In this study, treatment of the diabetic rats with neem seed oil and standard drug (daonil) significantly improved the alterations in serum kidney function indices - urea, creatinine and serum electrolytes (Na^+.K^+ and Cl^- (Fig. 1a, e). The increase in urea and creatinine level in STZ group and its subsequent reduction upon treatment with NSO is a testament to its therapeutic potential.

Creatinine is also commonly used as measure of kidney function and report indicates that . normal creatinine clearance test value is 110-150ml/min in male and 100-130ml/min in female (Corbett, 2008)**.**The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules (Miller et al, 2005). Creatinine values may alter as its generation may not be simply a product of muscle mass but influenced by muscle function, muscle composition, activity, diet and health status.

Figures 1c - e showed that induction of diabetes in the experimental animals significantly decreased the level of sodium, potassium and chloride ions when compared with the normal controls. However, treatment with neem seed oil (NSO) significantly increased the levels of all the three electrolytes tested, with remarkable increase at some dosages as shown in figures 1c, d and e above. Treatment with standard drug also followed same pattern with NSO, confirming the possible ameliorative effect of NSO in kidney function challenges.

Lipid function indices

Diabetes as a metabolic disorder, disturbance and malfunction characterized by hyperglycemia generating from defects in insulin production, insulin action, or both. is also connected with hyperlipidemia and hyperaminoacidemia. The typical and glaring symptoms of hyperglycemia in diabetes mellitus are polyuria, polydipsia, polyphagia and weight loss(Rajesh and Sreekala, 2020).

Triglycerides (TGs) are the most abundant lipid in human adipose tissue. It is generated when there is excess consumption of calories. In a diabetic state, insulin is dysfunctional and it is unavailable or restricted from converting the excess calorie into triglycerides. This condition is confirmed by the significant increase in the level of triglycerides in STZ group. Treatment with neem seed oil, however ameliorated this condition based on the concentrations administered which agrees with the research conducted by (Adekunle*et al.*,2016).

Table 4 shows a significant increase ($p>0.05$) in the lipid profile indices as a result of induction of the rats with diabetes using STZ, except LDL. However there was a significant decrease in groups treated with different dosages (ml) of neem seed oil, especially with 0.2ml and 0.5ml NSO**.** This decrease is in conformity with the standard drug which also showed significant decrease at 0.2ml (18.150+2.33mg/dl). This result agrees with research conducted by Yarmohammadi*et al., (*2021). HDL is antithrombogenic because it reverses LDL-induced inhibition of fibrinolysis, inhibits tissue factors (factors Va, VIIIa and X) and increases the activity of protein S and activated protein C(Ertek, 2017).

Clinically, LDL transports cholesterol and its esters in the bloodstream and consequently plays a central role in the development of cardiovascular diseases, in particular atherosclerosis (Teemu*et al.,* 2011). In the initial stages of atherogenesis, LDL-derived lipids accumulate in the arterial intima, and high LDL levels have been shown to increase the risk of atherosclerosis and when considered with formation of plaque on arterial walls, can lead to the narrowing of arteries, rupture, clotting, and potential death (Teemu*et al.*, 2011).

In addition, it can be noted that apart from the increase in LDL level both in the negative control group upon induction with STZ**,** it was also discovered that LDL increased when different dosages of the neem seed oil and daonil were administered when compared with the controls**.** This result is not in agreement with some researchers and the reason could be due to low dosage of neem seed oil (Table 4).

In the same table, the induction of diabetes with STZ in the rats also produced a significant increase in cholesterol level in the positive control (45.60+3.39) compared to the negative control (36.850+1.202). Although there was a significant increase in cholesterol level in virtually all the groups induced with STZ in spite of treatment with NSO, it should be noted that use of 0.1ml NSO without induction showed a slight decrease in cholesterol level thereby confirming the research conducted by (Dholi*et al.*, 2011).

Report shows that about 50% of the excreted cholesterol is reabsorbed by the small intestine back into the bloodstream and according to the lipid hypothesis, elevated levels of cholesterol in the blood lead to atherosclerosis which may increase the risk of heart attack, stroke, and peripheral artery disease (Cohn *et al.*, 2010). Therefore, since higher blood LDL–especially higher LDL concentrations and smaller LDL particle size contribute to this process more than the cholesterol content of the HDL particles, (Brunzell*et al.*,2008),LDL particles are often termed "bad cholesterol". High concentrations of functional HDL, which can remove cholesterol from cells and

atheromas, offer protection and are commonly referred to as "good cholesterol"(Dholi*et al.*, 2011).

Oxidative Stress indices

Oxidative stress and enhanced production of free radicals are the main events involved in the pathogenesis of diabetes and its complications (Rahimi et al., 2005). Use of antioxidants reduce oxidative stress and alleviates diabetic complications. This study investigated the effects of Neem seed oil on oxidative stress parameters in liver and kidneys of diabetic rats. Compared to other studies, neem has been said to have an effective antioxidants and anti-diabetic effect (Omóbòwálé et al.,2018)

In this study, administration of Neem seed oil to healthy (non-diabetic) rats did not cause any structural change in liver or in kidney, which can be considered beneficial because it shows that this oil per se does not cause damage to the organs studied.

In order to explore the effect of Neem seed oil on liver and kidney of the diabetic rats, lipid peroxidation, antioxidant defense system capabilities, and protein oxidation were evaluated according to appropriate methods reported in Materials and Methods. Fig 3a. shows that levels of MDA (the end product of lipid peroxidation and a marker of free radical generation in the hepatic and renal tissues) were significantly higher in rats with neem seed oil therapy. This might be as a result of environmental conditions though it was lower in the kidney of group 7 rats, whereas diabetic rats (group 4) showed enhanced levels of lipid peroxidation. Meanwhile there was no increase in hepatic and renal lipid peroxidation among rats in group 3 that are not diabetic but are subjected to Glibenclamide treatment.

Fig 3b revealed that SOD activities was significantly inhibited along with an elevation of lipid peroxidation (LPO) in kidney and liver of STZ-induced diabetic animals which is not reversed by different treatments. The results are in agreement with earlier data (Bryzewska et al, 1995; Parthiban et al, 1995).

Evaluating SOD activity in hepatic and renal tissues (Fig 3b) showed a significant increase in group 4 when compared to group 1 in the liver. Groups 6,7 and 8 revealed lower levels of this enzyme activity in liver when compared to group 4 and same in the kidney. Generally, since SOD activity was low in the study, we can conclude that it did not present an adequate ROS scavenging and the excess of free radicals led to lipid peroxidation, supporting the results found in the study.

Considering CAT activity in Fig 2c, the activity of this enzyme was highly expressed in the kidney compared to the liver.The treatment with Neem seed oil prevented the decrease in the enzyme activity in renal and hepatic tissue. The enhancement in CAT activity might be a primary defense response, acting as a scavenger under the oxidative insult provoked by diabetes (Caballero et al.,2000). There is higher ROS scavenging ability of it in the liver as well as high synthesis in hepatic tissues compared to the kidney. The liver's ability to scavenge reactive oxygen species (ROS) is crucial for maintaining redox balance and preventing oxidative stress. In hepatic tissues, ROS synthesis is high due to the liver's critical role in detoxication and metabolism regulation. The liver's antioxidant defense system is essential for managing ROS levels and any imbalance can lead to oxidative stress, resulting in liver damage (Ansari et al, 2025).

In Fig. 2d, GST activity in the liver was slightly increased in group 4 compared to group 1 but there was no change in the activity of the enzyme in the kidney. This is because the liver serves as the primary site for xenobiotic detoxification and possesses a higher metabolic capacity to adapt to toxic stress than kidney. The liver's higher adaptability is driven by its central role in metabolizing toxins, high basal GST content, and efficient signaling pathways that induce GST expression in response to oxidative stress (Thier et al, 1998)

Groups treated with Neem seed oil presented lower levels of GST activity compared to group 1 and group 4. In the Kidney,there was observed decrease in enzyme activity in group 6,7 and 8 but increased activity at 0.1ml dosage in both the liver and kidney of the rats.

The level of GSH in this study was significantly low which corroborates with the research of Ahmed (2004) in which reduced levels of GSH in diabetic rat livers have been associated with decreased GST, GPX and glutathione reductase activity and with the accumulation of oxidative stress products, such as advanced glycation end-products (AGEs), protein oxidation products (POPs) and lipid peroxidation (LPO) (Ahmed, 2004).

Conclusion

In this empirical study, attempt was made to leverage the biochemical and clinical impacts of induction of diabetes in wistar rats using streptozotocin. The clinical dysfunctions and the associated consequences obviously led to oxidative stress, increased lipid profiles and haemological indices, etc .which in long run can cause significant.dysfunctions and disruption of protein, lipid and carbohydrate metabolism that could

result in many secondary complications such as dyslipidemia, coronary artery disease, renal stroke, neuropathy, retinopathy and blindness. Conversely, use of neem seed oil reversed most of these dysfunctions in appreciable statistical levelat some doses, triggering significant positive changes in biochemical indices associated with pathogenesis of diabetes at some of the dosages tested thereby suggesting a relief in possible use of Neem seed oil in the management of Type 2 Diabetes mellitus.However, in the study, the use of Neem seed oil could not protect the hepatic and renal tissues against lipid peroxidation, and also, could not prevent the decrease in the enzymatic antioxidant activity in these organs but could only exert some of its effect at certain dosages. Generally, neem tends to show a weak inhibitory effect (Riyanti et al.,2016).

Recommendation

Based on this study, there is need for effective enlightenment strategy on the health benefits of neem seed oil, especially its use in the management of Type 2 diabetes mellitus.

Secondly, more integrated researches should be conducted to ascertain both the effective therapeutic doses of neem seed oil for a unit-dose dispensing as well as repackaging the products for market supply chain.

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