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Effect of Crude Oil Exposure on Diabetic Rats Treated with Metformin and *Allium sativum*

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ABSTRACT

Diabetes mellitus is rapidly increasing in prevalence and petroleum hydrocarbons contribute to its pathogenesis. This study investigates the effect of crude oil exposure on management of diabetes mellitus. A total of 54 adult male albino rats were used. Diabetes was induced by a single intra-peritoneal injection of 65 mg/kg streptozotocin dissolved in citrate buffer using insulin syringe and confirmed after 7 days. Rats were exposed to crude oil by preparing 5% v/v and 1% v/v and treated with extract and metformin for a period of four weeks. Effects of fasting blood glucose, lipid profile, antioxidant enzymes, insulin resistance, β cell function and inflammatory markers were evaluated and histological studies carried out. The results indicated decreased in body weight with significant ($p<0.05$) increase in fasting blood glucose, total cholesterol, triglyceride and low density lipoprotein cholesterol in all diabetic rats and diabetic rats exposed to crude with significant ($p<0.05$) decrease in the mentioned parameters in rats treated with *Allium sativum* extract plus metformin. There was significant ($p<0.05$) increase in SOD activity, GSH concentration and MDA level in all diabetic rats and diabetic rats exposed to crude oil compared to diabetic rats treated with ASE plus metformin. Inflammatory markers (IL-6, TNFα, VEGF), Insulin resistance and β cell dysfunction were significantly ($p<0.05$) low in diabetic rats treated with ASE plus metformin. Histological examination of pancreas revealed changes in the cyto-architecture of pancreas of all diabetic rats. Treatment with the extract of *Allium sativum* plus metformin had improved the structure of pancreas. Exposure to crude oil therefore affects diabetes management with metformin by increasing hyperglycemia, oxidative stress indices, inflammatory markers and insulin resistance. Therefore, treatment with *Allium sativum* extract plus metformin would help improve or maintain glucose metabolism and homeostasis in people living in areas of crude oil exposure.

Keywords: *Allium sativum*, Antioxidants, β-cell function, Crude Oil, Diabetes Mellitus, Metformin, Pancreas, Oxidative stress.

1.0 INTRODUCTION

Crude oil contains diverse array of chemical substances that produce definite biochemical, physiological and anatomical changes in the living system (Osibemhe *et al.*, 2024). Petroleum hydrocarbons are important substances widely used as herbicides, insecticides, solvents, and intermediates for the production of rubber and plastics. Each petroleum hydrocarbon has its own peculiar properties that confer toxicities on specific target organs producing adverse health effects which manifest predominantly in a wide array of non-communicable diseases (NCDs) including cancer, endocrinal disruption, diabetes mellitus, reproductive problems and neurological pathologies (Osibemhe *et al.*, 2024).

Diabetes mellitus is one of the world's major public health issue affecting over 638 million people world-wide; refers to a group of metabolic diseases characterized by persistent or recurrent hyperglycemia due to inadequate insulin secretion, inadequate utilization or both (IDF, 2023). For decades, it was believed that diabetes mellitus results from genetic influence only, but many patients presenting symptoms of diabetes had no family history of the disease (IDF, 2023). Then researchers reported that over 70 percent of diabetes results from environmental influence or factors including physical inactivity, poor or unhealthy diet, and environmental pollutants such as heavy metals, fine particles and petroleum hydrocarbons. For instance, study by Jardel *et al.* (2022) found an association between oil spill clean-up related hydrocarbon exposure and diabetes mellitus. Another study by Kim *et al.* (2022) reported increased stroke and cardiovascular disease prevalence in southern United State. Also, Wesley *et al.* (2019) reported insulin resistance in mice exposed to volatile benzene. Taken together, these studies indicated the role of petroleum hydrocarbons in the development of non-communicable diseases (NCDs).

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In oil exploration areas of Nigeria, diabetes mellitus occurs more prevalent with a diffuse, severe, and aggressive pattern of distribution (Maduka and Paul, 2021). Despite the management and treatment options undertaken by patients in the area, the number of morbidity and mortality is still climbing. Hence the present study to identify whether exposure of diabetic rats to petroleum hydrocarbons affects diabetes treatment/management with metformin and, would there be a substance that is quite affordable, accessible and less expensive that would help to improve the quality of life of people living in oil exploration areas (Maduka and Paul, 2021, Ismail *et al.*, 2021; Lei *et al.*, 2015).

Since ancient times, spices and herbs have been used for the treatment and prevention of diseases and are well patronized by over 80% of the people world-wide due to their acceptability, ease of access, and are affordable with less side effects (Tudu *et al.*, 2022). *Allium sativum L.* is one of the most widely used spices/herbs being used in ancient times and in the present era for the treatment of several ailments. It belongs to the family Liliaceae, commonly called Garlic in English and *Tafarnuwa* in Hausa (Nigeria). It is a fragrant herb and tuber-derived spice widely used as a flavoring ingredient and therapeutic purposes of many diseases in the world. Its anti-carcinogenic, antihypertensive, immune-modulatory, anti-oxidant, antiviral, anti-microbial, anti-diabetic, and anti-hyperlipidemic effects were reported (Tudu *et al.*, 2022). Metformin was the drug of choice in this research due to its insulin sensitizing, insulin secretion, and antioxidant effects.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals, drug and crude oil

Streptozotocin was a product of Henan Tianfu Chemical Co., Ltd, China. Bonny Light Crude oil for the study was from Ebedei Oil Terminal, Delta State, Nigeria. Metformin was purchased from Albarka Yazid Medicine Store, Dutsin-Ma.

2.1.2 Experimental animals

A total of fifty four (54) albino rats weighing between 150-180g were purchased from animal house, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were fed normal rat chow and allowed access to clean water *ad libitum*. The rats were kept in wire mesh cages for a period of two weeks to acclimatize before the commencement of the experiment.

2.1.3 Plant Material

Allium sativum was purchased from Chake Market, Katsina, Katsina State Nigeria. It was identified and authenticated at herbarium of the Department of Biology, Federal University Dutsin-Ma, Katsina State.

2.2.0 Methods

2.2.1 Plant Material Extraction

Allium sativum was sliced in to small pieces, dried at room temperature for five days, grounded to powder with pestle and mortar, and then kept in sealed plastic containers until required. Exactly 200 g of the powdered plant was macerated into 600 ml of distilled water for 48 h, and then filtered with Whatman No. 1 filter paper. The extract was concentrated in water bath at 40°C and subsequently kept in sealed plastic container until required (Osibemhe *et al.*, 2018).

2.2.2 Induction of Diabetes

Diabetes was induced by a single intra-peritoneal injection of 65 mg/kg streptozotocin (Henan Tianfu Chemicals Ltd. China) dissolved in citrate buffer to overnight fasted rats using insulin syringe. Diabetes mellitus was confirmed after 7 days of streptozotocin injection by the observation of fasting blood glucose (FBG) ≥8.00mmol/L using ACCU CHEK glucometer (Osibemhe *et al.*, 2024; Osibemhe *et al.*, 2018).

2.2.3 Animal Grouping and Experimental Protocol

The rats were randomly grouped into 9 groups of 6 rats each as follows:

Group 1: Control group, received water (non-diabetic).

Group 2: Untreated diabetic rats (negative control)

Group 3: Diabetic rats treated with 250 mg/kg b.w metformin (positive control).

Group 4: Diabetic rats exposed to 5% v/v crude oil treated with 250mg/kg b.w metformin.

Group 5: Diabetic rats exposed to 1% v/v crude oil treated with 250mg/kg b.w metformin.

Group 6: Diabetic rats exposed to 5% crude oil, treated with metformin (250mg/kg b.w.) + 200mg/kg b.w of extract.

Group 7: Diabetic rats exposed to 1% crude oil treated with 250 mg/kg metformin + 200 mg/kg b.w of extract.

Group 8: Untreated diabetic rats exposed to 5% v/v crude oil.

Group 9: Untreated diabetic rats exposed to 1% v/v crude oil.

Exposure and treatment lasted for 28 days.

2.2.4 Body Weight

The body weight of all the animals before induction and within the period of treatment were weekly recorded using digital weighing balance M-METLAR (QC PASS K03).

2.2.5 Blood Sample Collection and Clinical Chemistry

After the last treatment, rats were fasted overnight, body weight and fasting blood glucose measured, they were then anaesthetized by dropping individual rat in a plastic

jar containing a cotton wool saturated with diethyl ether vapor. The rats were then removed from the jar and blood samples collected through cardiac puncture into labelled plastic specimen bottles containing heparin. They were then centrifuged at 4000 g for ten minutes to obtain plasma which were pipetted in to specimen test tubes for other biochemical analyses.

2.2.6 Determination of Biochemical Parameters

Plasma total cholesterol, triglyceride, and high density lipoprotein were estimated with the method of Allain *et al.* (1974) as reported by Osibemhe *et al.* (2018). Low density lipoprotein cholesterol was estimated using Fredewald *et al.* (1972) formula reported by Muhammad *et al*. (2018). Catalase activity (CAT) and superoxide dismutase (SOD) activities were estimated according to the methods of Aebi (1984) and Fridovich (1989) as reported by Muhammad *et al.* (2018). GSH was determined using the method of Ellman (1959) as reported by Omoboyowa *et al.* (2017). Assessment of β-cells function and insulin resistance were with the method of Wesley *et al.* (2019). Insulin, vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) were determined using ELISA kits according to manufacturers' instructions.

2.2.7 Statistical analysis:

The data obtained were expressed as mean ± standard error of mean, and analyzed using SPSS version 22 software. The significance among groups was determined by One Way Analysis of Variance using LSD post hoc test for multiple comparison. Means regarded significant at $P<0.05$.

2.2.8 Histopathology

Tissue sample of the pancreas was fixed in 10 per cent formalin. The formalin fixed specimen was embedded in paraffin, sectioned and stained with haematoxylin and eosin. The histological sections were examined under microscope and then photographed at magnification 400.

3.0 RESULTS

3.1 Observation for toxic symptoms

Exposure of rats to crude oil caused reduced reaction to noise, slow movement and resting at the corner of cages. It was also observed that crude oil exposed rats had watery stool and hair loss which was reduced and/ or restored to normal after five days of extract plus metformin administration.

3.2 Body Weight Changes in Experimental rats

The effect of treatment with aqueous extract of *A. sativum* (200mg/kg b.w.) plus metformin (250mg/kg b.w.) on body weight of STZ-induced diabetic rats are presented in Table 1. Results indicate that STZ injected rats had a drastic decrease in body weight compared to normal rats (control). Treatment with aqueous extract of *A. sativum* plus metformin resulted to improved body weight compared to untreated diabetic rats (negative control) and untreated diabetic rats exposed to crude oil. There was improvement in body weight of diabetic rats treated with *A. sativum* extract (200mg/kg) plus metformin (250mg/kg) after the second week of treatment. It was similar for metformin treated group (positive control).

3.3 Effect of Crude Oil Exposure on Fasting Blood Glucose of Experimental Rats

The effect of *A. sativum* extract and metformin administration on fasting blood glucose of control and experimental rats (Table 2). The result showed significant ($p<0.05$) increase in fasting blood glucose of untreated diabetic rats (negative control), untreated diabetic rats exposed to crude oil compared to normal control rats. In positive control rats also, exposure to crude oil had affected the fasting blood glucose (FBG). Treatment of crude oil exposed rats with extract plus metformin resulted in decreased FBG after second week of treatment. This is supported by the histological observations on plate 1. From the plate, all diabetic rats showed changes in cyto-architecture of pancreas after streptozotocin was injected (Plate 1b-i). Treatment with extract and standard drug however, had resulted in improving and or maintaining the cyto-architecture of pancreas with small histological changes (plates 1b and 1g). Diabetic rats exposed to crude oil, treated with standard drug (metformin) had necrosis in the islet cells of the pancreas (plates 1c and h). Normal control rats had normal appearance of the pancreas (Plate 1a).

3.4 Plasma Lipid Profile of Experimental Rats Exposed to Crude Oil

The effect of treatment with *A. sativum* extract and metformin on plasma lipid profile are presented in Table 3. The results indicate significant ($p<0.05$) increase in the levels of triglyceride and low density lipoprotein with a significant ($p<0.05$) decrease in high density lipoprotein cholesterol of untreated diabetic rats (negative control). Treatment with *A. sativum* extract and metformin caused an increase in the level of high density lipoprotein cholesterol, with significant ($p<0.05$) decrease in plasma cholesterol, triglyceride and low density lipoprotein concentrations.

3.5 Antioxidant Profile of Experimental Rats Exposed to Crude Oil

The antioxidant profile of control and experimental rats is presented in Table 4. From the result, there was a significant ($p<0.05$) increase in the level of malondialdehyde (MDA) in untreated diabetic rats (negative control), untreated diabetic rats exposed to

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crude oil compared to normal control and *A. sativum* extract plus metformin treated rats. The activities of catalase (CAT) and superoxide dismutase (SOD) were significantly high in negative control and untreated diabetic rats exposed to crude oil compared to control rats and *A. sativum* extract plus metformin treated rats.

3.6 Fasting Plasma Insulin, HOMA-IR and HOMA-β of Rats Exposed to Crude Oil

Evaluation of crude oil exposure on glucose handling in experimental rats found high insulin resistance and β cell dysfunction in negative control, untreated diabetic rats exposed to crude oil, and positive control (Table 5). Treatment with *Allium sativum* extract plus metformin caused significant decrease in insulin resistance.

3.6: Inflammatory markers of Experimental Rats Exposed to Crude Oil

Inflammatory markers of crude oil exposed rats are presented in Table 6. Result showed statistically significant ($p<0.05$) increase in the level of VEGF, IL-6 and TNFα in negative control, untreated diabetic rats exposed to crude oil and positive control rats compared to normal rats. Treatment of rats with *Allium sativum* extract plus metformin caused a significant decrease in the level of all inflammatory markers despite crude oil exposure similar to normal control rats.

Table 1: Body Weight of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium sativum* Extract

	Weekly Weight (g)				
Group	Initial	1	2	3	4
Group 1	132.4±2.6^{a}	148.2±5.2^{b}	169.6±7.2^{c}	196.0±6.6^{d}	205.6±7.1
Group 2	130.8±2.1^{a}	137.7±2.4^{b}	125.1±1.7^{c}	108.1±6.4^{d}	105.4±2.3
Group 3	134.2±2.5^{a}	174.1±4.9^{b}	147.6±5.4^{c}	136.8±2.8^{d}	126.8±5.8^{d}
Group 4	131.5±2.4^{a}	146.3±3.3^{b}	121.5±6.9^{c}	160.1±3.2^{d}	154.6±3.7^{d}
Group 5	178.1±2.8^{a}	181.9±4.1^{a}	184.0±3.1^{a}	196.9±3.9^{b}	192.9±1.6^{b}
Group 6	134.5±1.6^{a}	139.8±4.3^{a}	127.4±6.4^{b}	147.6±7.3^{c}	146.8±6.1^{c}
Group 7	155.6±4.0^{a}	151.2±7.4^{a}	161.6±3.8^{b}	190.6±2.0^{c}	200.7±5.9^{d}
Group 8	134.0±1.6^{a}	146.4±2.1^{b}	124.8±2.4^{c}	110.9±2.6^{d}	106.8±1.8^{d}
Group 9	179.1± 3.8^{a}	175.4±1.6^{a}	159.9±3.2^{b}	152.6±2.2^{b}	156.3±2.4^{b}

Values are Mean± SEM for six animals per group. Different superscript in the same row are significantly different at $p<0.05$. ASE=*Allium sativum* extract. Group 1: Control; group 2: Negative control; group 3: positive control; group 4:5% standard; group 5: 1% standard; group 6:5% AE + Metformin; group 7: 1% ASE+ Metformin; group 8: 5% untreated; group 9: 1% untreated.

Table 2: Fasting Blood Glucose of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium Sativum* Extract.

	Weekly Fasting Blood Glucose (mmol/L)				
Treatment	Initial	1	2	3	4
Group 1	4.8±0.14^{a}	4.86±0.12^{a}	4.82±0.13^{a}	4.9±0.18^{a}	4.84±0.12^{a}
Group 2	4.4±0.26^{a}	24.1±3.66^{c}	22.6±3.14^{c}	18.8±2.68^{b}	21.9±0.03^{c}
Group 3	4.7±0.26^{a}	23.3±2.26^{c}	8.1±1.56^{ab}	8.3±0.91^{ab}	7.9±0.61^{a}
Group 4	4.6±0.18^{a}	23.7±2.41^{c}	12.1±0.78^{b}	14.3±0.45^{b}	13.6±0.31^{b}
Group 5	4.7±0.25^{a}	19.7±2.46^{c}	14.9±2.35^{bc}	12.7±2.64^{b}	14.2±1.34^{b}
Group 6	4.4±0.20^{a}	21.9±3.41^{c}	21.1±2.84^{c}	13.3±2.80^{b}	11.6±1.90^{b}
Group 7	5.3±0.37^{a}	28.9±1.29^{c}	17.1±1.74^{c}	13.9±4.62^{b}	7.6±0.41^{a}
Group 8	4.2±0.17^{a}	17.1±1.93^{c}	19.3±1.05^{c}	17.7±0.70^{c}	19.9±0.13^{c}
Group 9	4.2±0.31^{a}	12.8±1.42^{b}	17.6±1.54^{c}	18.2±3.94^{c}	18.8±1.69^{c}

Values are Mean± SEM for six animals per group. Different superscript in the same row are significantly different at $p<0.05$. ASE=*Allium sativum* extract. Group 1: Control; group 2: Negative control; group 3: positive control; group 4:5% standard; group 5: 1% standard; group 6:5% AE + Metformin; group 7: 1% ASE+ Metformin; group 8: 5% untreated; group 9: 1% untreated.

Table 3: Lipid Profile of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium sativum* Extract.

Treatment	CHOL. (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AI
Group 1	37.54±2.25^{c}	41.32±1.96^{c}	8.00±1.63^{a}	20.08±2.42^{c}	0.71±0.06^{c}

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Group 2	72.68±3.08[a]	86.58±2.25[a]	5.50±0.26[b]	43.65±1.61[a]	1.19±0.11[b]
Group 3	35.92±4.32[c]	67.60±2.77[b]	5.75±0.98[b]	21.49±1.01[c]	1.07±0.08[b]
Group 4	38.8±1.56[c]	34.08±1.16[d]	5.5±0.25[b]	30.25±1.88[b]	0.79±0.07[c]
Group 5	32.68±2.64[c]	48.15±2.03[c]	6.12±0.49[b]	16.9±0.88[c]	0.89±0.14[c]
Group 6	45.03±2.16[b]	67.00±4.26[b]	7.63±1.08[a]	17.02±1.40[c]	0.94±0.05[c]
Group 7	34.92±2.56[c]	62.75±3.72[b]	8.93±2.00 [a]	24.05±1.02[c]	0.84±0.06[c]
Group 8	77.80±2.24[a]	78.60±2.68[a]	2.75±0.42[c]	49.98±1.06[a]	1.46±0.08[a]
Group 9	70.43±3.91[a]	72.73±4.29[a]	4.16±1.56[b]	16.66±1.04[c]	1.18±0.10[b]

Values are Mean± SEM for six animals per group. Different superscript in the same row are significantly different at p<0.05. ASE=*Allium sativum* extract. Group 1: Control; group 2: Negative control; group 3: positive control; group 4:5% standard; group 5: 1% standard; group 6:5% AE + Metformin; group 7: 1% ASE+ Metformin; group 8: 5% untreated; group 9: 1% untreated.

Table 4: **Antioxidants and MDA Level of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium sativum* Extract.**

	Parameters			
Group	SOD (u/ml)	GSH (µ/ml)	MDA (nmol/ml)	CAT (u/ml)
Group 1	10.04±0.54[a]	16.8±0.19[a]	58.8±1.58[c]	3.04±0.36[b]
Group 2	10.05±0.94[a]	16.20±0.54[a]	163.5±1.53[a]	6.15±0.48[a]
Group 3	7.70±0.44[a]	13.50±0.62[a]	51.3±0.98[c]	5.45±0.23[a]
Group 4	4.63±0.21[b]	6.93±0.31[b]	98.6±1.0[b]	3.10±0.33[b]
Group 5	3.48±0.35[b]	5.00±0.24[b]	118.5±2.85[b]	2.05±1.49[b]
Group 6	6.18±0.38[b]	10.03±0.24[b]	42.40±1.10[c]	3.60±0.41[b]
Group 7	4.68±0.61[b]	13.20±0.66[a]	55.72±0.78[c]	1.63±0.21[c]
Group 8	8.25±0.18[a]	14.38±0.90[a]	141.7±2.67[a]	3.57±0.08[b]
Group 9	5.33±0.16[b]	6.93±0.26[b]	159.2±2.01[a]	1.53±0.26[c]

Values are Mean± SEM for six animals per group. Different superscript in the same row are significantly different at p<0.05. ASE=*Allium sativum* extract. Group 1: Control; group 2: Negative control; group 3: positive control; group 4:5% standard; group 5: 1% standard; group 6:5% AE + Metformin; group 7: 1% ASE+ Metformin; group 8: 5% untreated; group 9: 1% untreated.

Table 5: Insulin Resistance and β Cell Function of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium sativum* Extract.

Treatment	FBG (mmol/L)	Insulin(mIu/ml)	HOMA-IR	HOMA-β
Normal control	4.84±0.05	118.6±1.02[a]	25.52±0.25[a]	1757.2±2.41[a]
Negative control	21.9±0.07	133.5±5.18[a]	118.13±2.43[b]	145.15±2.06[d]
Positive control	7.90±0.62	139.2±4.36[a]	49.30±1.53[a]	632.40±2.48[b]
5%+Standard treat.	8.30±0.31	175.37±3.93[b]	64.58±1.10[a]	730.50±5.64[b]
1% Standard treat.	14.2±1.34	167.32±2.33[c]	136.92±2.89[b]	312.75±2.14[c]
5% ASE+Std	11.6±1.91	116.01±2.06[a]	59.80±1.43[a]	363.80±2.28[c]
1% ASE+Std	8.1±0.31	141.80±1.85[c]	51.05±2.42[a]	605.66±1.95[b]
5% Untreated	19.9±0.13	163.8±1.85[b]	162.45±4.12[b]	192.70±1.65[d]
1% Untreated	18.6±1.96	169.06±3.05[b]	139.03±2.90[b]	223.40±1.16[d]

Values are Mean± SEM for six animals per group. FBG: Fasting Blood Glucose; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; HOMA-β: Homeostasis Model Assessment of β cell.

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Table 6: Cytokine Levels of Diabetic Rats Exposed to Crude Oil Treated with Metformin and *Allium sativum* Extract.

	Parameters		
Treatment	VEGF (pg/ml)	IL6 (pg/ml)	TNF (pg/ml)
Control	542±10.98[a]	79.93±1.44[b]	301.99±8.31[b]
Negative cont.	684.04±12.52[a]	107.80±3.85[ab]	454.41±10.71[ab]
Positive cont.	668.86±5.61[a]	124.46±7.36[a]	394.20±7.22[ab]
5%+standard	867.57±13.34[a]	102.89±4.38[ab]	541.84±10.28[ab]
1%+standard	606.53±11.92[a]	82.17±3.49[b]	428.08±4.64
5%+ASE +stand	811.76±14.70[a]	93.35±1.68[b]	419.93±8.64[ab]
1%+ASE+ stand	703.58±9.37[a]	60.58±1.40[b]	248.18±4.46[c]
5% Untreated	751.16±18.11[a]	96.00±1.63[ab]	603.57±13.86[a]
1% Untreated	1028.16±8.00[a]	126.98±1.58[a]	522.96±5.48[a]

Values are Mean± SEM for six animals per group. Different superscript in the same column are statistically significant at $p<0.05$.

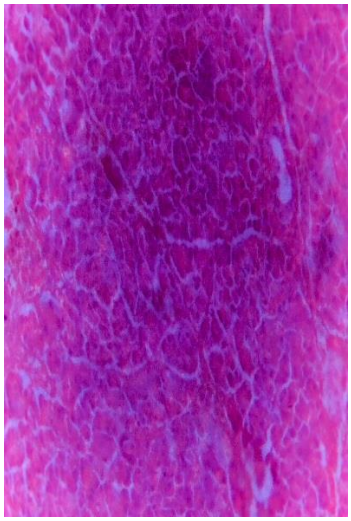

Plate 1a: normal features of the epithelial tissues of the pancreas

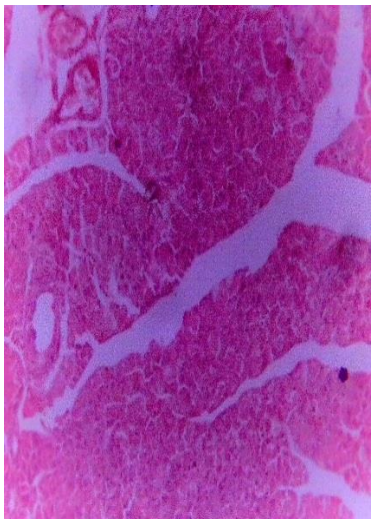

Plate 1b: slight changes to the pancreatic epithelial tissue

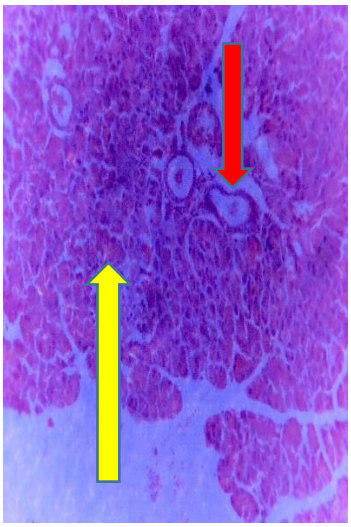

Plate 1c: necrosis of the changes pancreas

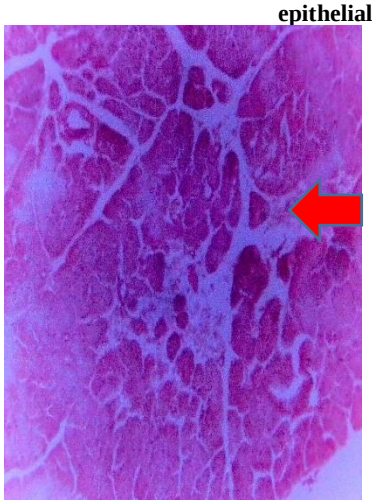

Plate 1d: Necrosis of pancreas

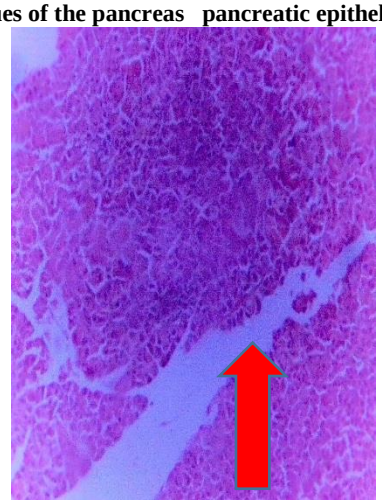

Plate 1e: Tissue necrosis

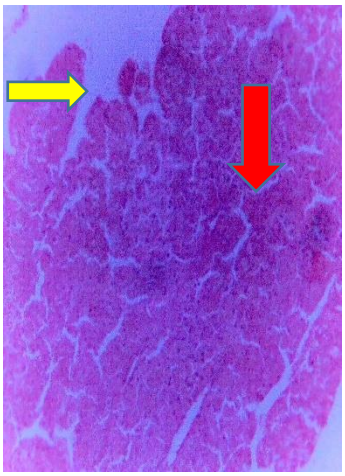

Plate 1f: Tissue necrosis

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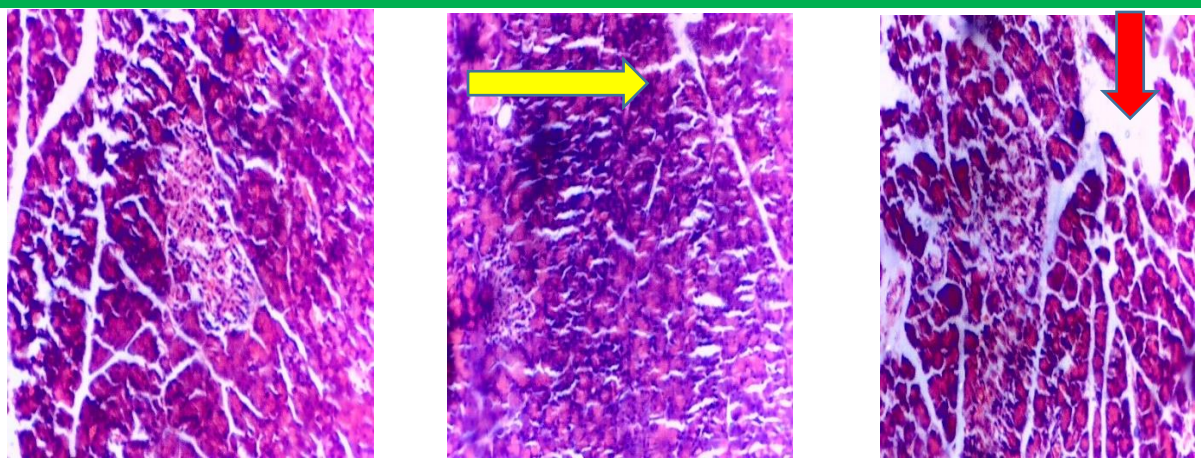

Plate 1g: Normal features **Plate 1h: Necrosis of pancreas** **Plate 1i: Intense necrosis**

Plate 1: Histological examination of pancreas of control and experimental animals treated with 200 mg/kg body weight extract and 250 mg/kg body weight metformin exposed to 5% crude oil for four weeks. Normal control group (1a); diabetic rats treated with garlic extract plus standard drug exposed to 5% crude oil (1b); diabetic rats treated with standard drug exposed to 5% crude oil (1c); diabetic rats treated with standard drug (1d); diabetic control group (1e); diabetic rats exposed to 5% crude oil without treatment. The red arrow indicates necrosis, yellow arrow indicates inflammation or congestion.

4.0 DISCUSSION

Streptozotocin is considered one of the agents that induce diabetes mellitus as it causes serious disturbances in glucose metabolism by degeneration of pancreas beta cells leading to glycemic dysregulation (Osibemhe *et al.*, 2018). In the present study, an effort to elucidate the implication of crude oil exposure on management of diabetes mellitus was investigated. This study may likely be the first to report the implication of crude oil/petroleum hydrocarbon exposure on management of diabetes mellitus. It reports the additive effect of combined therapeutic intervention with metformin and *A. sativum* extract on diabetic rats exposed to crude oil. It is well known that crude oil exposure foster serious disturbance in glucose metabolism by activation of phagocytes to liberate arachidonic acid, peroxide enzymes to produce alcohoxyl and peroxyl radicals; liberation of ions of transition metals as well as conversion of xanthine dehydrogenase to xanthine oxidase producing systemic damage and rupture of energy metabolism (Ita *et al.*, 2014; Jardel *et al.*, 2022; Kim *et al.*, 2022).

In this study, it was observed that exposure to crude oil had caused the rats to respond so low to noise, with slow movement and to rest at the edge of cages. The reduced reaction to noise may suggest the depressant effect of the oil on central nervous system. This is in line with the observation made in a study by Azeez *et al.* (2012) who found reduced reaction to noise in rats exposed to crude oil/petroleum hydrocarbons. The depressant effect of crude oil might be attributed to the effect of oil in impairing metabolism of tryptophan leading to increased synthesis of serotonin thereby altering mood of the rats. It may also be that exposure to crude oil interferes with the absorption and or utilization of niacin whose deficiency is associated with depression, diarrhea and dementia. It was also observed that rats that were injected with streptozotocin had a watery stool and hair loss which were reduced or restored to normal after five days of *A. sativum* extract plus metformin administration. This may suggest the immune enhancement effect of the extract. This agree with a study reported by Tadele and Zerssa (2021). It is also in line with a report from a study review by Ozma *et al.* (2022) and Rauf *et al.* (2022). It also agree with a study reported by Ashfaq *et al*. (2021) who stated the immune modulatory action of garlic phytoconstituents. The immune enhancement effect could be attributed to the vitamins, minerals and polyunsaturated fatty acids contents of *A. sativum*. Vitamins C, E and K play vital roles during the healing process and serve as anti-inflammatory agents (Verma *et al.*, 2023). It could also be due to the effect of sulfur compounds present in *A. sativum* that helps synthesis of endogenous antioxidant enzymes such as SOD, GSH and CAT. The mineral contents of the *A. sativum* including Cu, Zn, Mn and Se serve as cofactors for the synthesis of antioxidant enzymes or as cofactors for normal function of enzymes (Kumar *et al.*, 2021). From this, *A. sativum* can be said to have nutritional and therapeutic role in management of diabetes. Result of this study found a drastic drop in body weight of streptozotocin induced diabetic rats compared to normal control rats. The decrease in body

weight found in STZ injected rats could be due to selective damage/injury of pancreatic beta cells of islet of Langerhans by streptozotocin which caused hypoinsulinemia with a consequent decrease in peripheral uptake and utilization of glucose and increase in gluconeogenesis (Osibemhe *et al.,* 2024; Osibemhe *et al.,* 2018). This caused increased degradation of structural proteins thereby affecting the body weight of the rats. This is in line with a study conducted by Osibemhe *et al.* (2018) who stated decrease in body weight of rats that were injected with STZ. During this study, the observed increase in weight found in diabetic rats exposed to crude oil treated with both *A. sativum* extract plus metformin after the first week of administration may be a function of anti-diabetic effect of the extract and metformin indicating synergistic effect. *Allium sativum* possess ability to reduce hyperglycemia and improve glucose metabolism which may lead to improve glucose uptake and utilization, decreased gluconeogenesis and increased glycogenesis which spares structural proteins from degradation thereby maintaining body weight (Fatemeh *et al.,* 2023). The study agree with the findings from a study conducted by Ashraf *et al.* (2011).

The decline in FBG of diabetic rats treated with both standard drug and *A. sativum* extract might be due to fiber present in the extract (Tudu *et al.,* 2022). It could also be due to the viscous nature of the extract; inhibition of intestinal glucose absorption or inhibition of α-amylase and α-glucosidase enzymes that are linked to hypoglycemic effect of the plant. The findings of this study is in line with previous studies reported by Liu *et al.* (2006) and Eidi *et al.* (2006) who reported a lower serum glucose in rats administered garlic extract orally. It also agree to study by Ashraf *et al.* (2011); Ashraf *et al.* (2021) who found a synergistic effect of combined effect of *A. sativum* extract and the drug metformin. It also agree with a study by Mahesar *et al* (2010) in alloxan induced male rabbits. The result is also in agreement with findings of Faroughi *et al* (2018) who reported a drop in FBG of pre-diabetic patients treated with garlic from 11.1 mmol/L to 6.3mmol/L for four weeks. It however contrasts the findings of Liu *et al.* (2006) who found no hypoglycemic effect quickly but took some weeks before needed result was seen. This may be due to differences in the method of preparation, the duration of administration and dose consumed.

Crude oil has so many components that are well absorbed in to the body and are known to bio-accumulate leading to sequence of events causing pathologies. This may augment the effects of diabetes on various organs and tissues thereby potentiating morbidity and mortality burden Okoye *et al.,* 2021; Osibemhe *et al.,* 2024).

Alteration in lipid metabolism has been hypothesized in animals with diabetes mellitus. In this study, there was a significant increase in lipid profile of untreated diabetic rats and diabetic rats exposed to crude oil, treated with metformin compared to control rats. This is a result of increased degradation of lipids and increased mobilization of free fatty acids from peripheral deposits due to insulin deficiency. The insulin deficiency activates hormone sensitive lipase which becomes in active in the presence of insulin (Osibemhe *et al.,* 2018). Other hormones such as glucagon and catecholamines become active, which in turn stimulate lipolysis in adipose tissue (Muhammad *et al.,* 2017). This agrees with a study reported by Muhammad *et al.* (2018) on diabetic rats. It is also in line with a study conducted by Omoboyowa *et al.* (2017). Administration of *A. sativum* extract plus standard drug showed decrease in low density lipoprotein (LDL) with increase in HDL despite crude oil ingestion/exposure. The reduction in lipid profile parameters demonstrate the hypolipidaemic potential of both ASE extract and standard drug. With respect to this effect, the rate of lipolysis may be reduced due to improved normoglycemic effect of both *A. sativum* extract and standard drug which inhibits hormone sensitive lipase thereby reducing lipolysis. It may also be that the extract delays glucose absorption which activates lipogenesis and inhibit lipolysis. In addition, fiber could bind and decrease uptake of cholesterol from small intestine which binds with bile salts causing increased degradation of cholesterol to bile salts and its expulsion from body. This agrees with a report of Hussein *et al* (2017).

Free radicals contribute to the pathogenesis of diabetes mellitus and related complications (Fatemeh *et al.,* 2023; Osibemhe *et al.,* 2018). Plant based antioxidant are known to delays or inhibit the onset of diabetes mellitus. Superoxide dismutase (SOD) is an enzyme that destroys superoxide radical (O_2^{*-}) converting it to hydrogen peroxide (H_2O_2) which is subsequently destroyed catalase thereby protecting from oxidative stress. The result of this study showed a significant decrease in the activity of CAT, SOD and reduced glutathione level of diabetic rats treated with the extract compared with untreated diabetic rats. This may be because of the increasing oxidative stress burden that the rats suffer which stimulate production of more of these enzymes causing increase in the concentration of those enzymes in diabetic rats (Omoboyowa *et al.,* 2017).

Insulin level has been hypothesized to be low in type 1 diabetes and could be normal, low or even high in type 2 diabetes (Osibemhe *et al.,* 2018). In this study, insulin level was high in rats treated with ASE plus standard

drug. Similarly, glucose level was the same for diabetic control and untreated diabetic rats exposed to crude oil. The rise in insulin content in rats treated with ASE plus standard drug might be due to secretion of insulin, and insulin sensitizing effect of ASE. Ashraf *et al.* (2011) reported increase in insulin secretion in diabetic patients given/ treated with *A. sativum*. The pancreatic beta cell function was expected to reduce in STZ injected rats and crude oil exposure potentiates reduction of beta cell function (Osibemhe *et al.,* 2024; Osibemhe *et al.,* 2018). However, insulin resistance was high in untreated diabetic rats exposed to crude oil and untreated diabetic rats. It was comparatively higher in crude oil exposed rats treated with standard drug compared with *A. sativum* extract plus standard drug treated rats. The higher insulin resistance observed could be unconnected to the effect of crude oil ingestion which raises oxidant stress markers which affect insulin receptors and its downstream signaling pathways thereby causing insulin resistance. Crude oil contains heavy metals and volatile organic compounds that may have participated in this development (Jardel *et al.,* 2022; Okoye and Awunor, 2022; Kumar *et al.,* 2022). Treatment with ASE in addition to standard drug had helped improve sensitivity of insulin receptors as *A. sativum* has been proven to have detoxifier, antioxidant, anti-inflammatory, insulin receptor sensitizing and insulin secretion effects (Osibemhe *et al.,* 2024).

Generally, both hyperglycemia and crude oil exposures have been postulated to cause increase in expression of inflammatory markers which derives complex processes of diabetic complications. Cytokines are generally glycoproteins and polypeptides secreted by leucocytes into the extracellular compartment, and in diabetes, there is increased expression due to vascular changes and proteolytic cascades mediated by ROS, which are interlinked to act as intra and inter cellular signals (Fatemeh *et al.,* 2023; El-Saber *et al.,* 2020). In hyperglycemia, protein kinase c (PKC) becomes activated due to increase in cytosolic phosphotidyl inositol-4,5-biphosphate levels which activates protein kinase C. The increased expression of PKC causes down regulation or decreased levels of eNOS, thereby causing decreased blood flow and hypoxia which stabilizes hypoxia inducible factor-α (HIFα), up regulates VEGF and therefore angiogenesis (Fatemeh *et al.,* 2023). Further, increased expression of NF kappa β increases pro-inflammation markers expression. In this study, the level of inflammation markers especially TNFα was significantly ($p<0.05$) high in diabetic rats exposed to crude oil compared to normal rats. Also, there was found increase in value of IL-6 in all groups compared to normal rats. The rise in TNFα value may be due to the effects of hyperglycemia on stimulating chronic inflammation which in turn caused impaired insulin signaling associated with reduced transcription of GLUT-4; a protein that facilitates entry of glucose into skeletal muscles. It may also be due to the effect of crude oil in activating NADPH oxidase which is known to raise oxidant stressors leading to increased TNF-α levels. The reduced levels of TNFα found in rats treated with *A. sativum* extract compared with other groups could be due to the effect of the extract in up regulating PI3K/Akt signaling pathway that derives glucose uptake, thereby suppressing diabetic complications. The findings of this study is in line with the findings of Colin *et al.* (2011) who found that ASE reduced the level of TNFα in rats given *A. sativum* extract for 14 days. The increased level of TNFα generally causes phosphorylation of serine and or threonine residues by kinases thereby causing reduced insulin signaling (Tudu *et al.,* 2022).

Angiogenesis is a pathophysiological process of blood vessels formation in oxygen deficient tissues (Colin *et al.,* 2011). In this study, VEGF level was increased (non-significantly) ($p>0.05$) in all diabetic rats compared to normal rats. The drop in VEGF of rats that received ASE plus standard drug in the presence of crude oil might be due to the effect of ASE in inhibiting stabilization of HIF-1α, thereby reducing VEGF level. It may also be that both ASE and standard drug inhibited nuclear translocation of HIF-1α, and HIF-1 dimerization which consequently reduced VEGF. This agree with anti-inflammatory effect of *A. sativum* reported by Tudu *et al.* (2022).

Conclusion

Crude oil exposure affects treatment/management of diabetes by up regulating pathways of inflammation, oxidative stress and hyperglycemia. Intake or use of *A. sativum* may help reduce or suppress toxicity profile of crude oil ingestion/exposure. Thus people living in a polluted environment would have an improved living by consumption of *A. sativum* due to its detoxifier, anti-inflammatory, hypoglycemic, hypolipidemic, and antioxidant effects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with publication of this research.

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