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Effect of crude oil pollution on phytochemical and anti-diabetic potentials of *Oxytenanthera abyssinica* (rhizomes) in normal and alloxan induced diabetic rats

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ABSTRACT

Objectives: This study is aimed at investigating the effect of crude oil pollution on the phytochemical content and anti-diabetic potentials of *Oxytenanthera abyssinica* (rhizomes) in normal and alloxan induced diabetic rats using standard methods.

Method: The ability of the extracts to reduce blood glucose level in normal rats was done using 200 and 400 mg/kg b.w of non crude oil- polluted *Oxytenanthera abyssinica* extract (NCOPOAE) and 200 and 400 mg/kg b.w of crude oil-polluted *Oxytenanthera abyssinica* extract (COPOAE) respectively. Alloxan monohydrate at 130 mg/kg b.w was used to induce diabetes to rats and was treated with 100, 200 and 400 mg/kg b.w of NCOPOAE and 100, 200 and 400 mg/kg b.w of COPOAE respectively while phytochemical analysis was done following standard method.

Results: Oral glucose tolerance test revealed significant decrease in plasma glucose concentration ($p<0.05$) in groups of rats fed 200 and 400 mg/kg b.w of NCOPOAE at intervals of 30, 60 and 120 minutes while that of COPOAE, none significantly decreased the plasma glucose concentration, when compared with those of control groups. The extracts did not have any significant effect in alloxan induced diabetic model.

Conclusion: These results could indicate that crude oil pollution could affect the synthesis of phytochemicals in plants therefore affecting the ethnomedicinal potencies. It also shows that *O. abyssinca* (rhizomes) could be more potent in management of type -2 diabetes where the cells are resistant to insulin.

KEYWORDS: *Diabetes, Oxytenanthera abyssinica, rhizomes, crude oil-polluted Oxytenanthera abyssinica, non crude oil-polluted Oxytenanthera abyssinca.*

1. INTRODUCTION

Pollution has been one of the most problems faced by living organisms, ranging from human beings, aquatic and wild lives. However pollutants such as crude oil have introduced a lot of harmful chemicals to the environments which have been shown by researchers to be damaging to the ecosystem. Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide and may affect five times more people than it does now in the next ten years (Ada, 2012). It has been concisely, described as the cause of common metabolic disorder of fat and carbohydrate metabolism, which is as a result of complete or relative lack of insulin characteristic of hyperglycaemia (Sharon and Marvin, 1975; Walter, 1977). Diabetes is categorized into two types: types 1 and 2. Type 1 diabetes is also called insulin dependent diabetes mellitus. In this condition, the body does not produce insulin; therefore, patients with type 1 diabetes receive daily insulin injections. Type 2 diabetes is also known as non-insulin-dependent diabetes mellitus, it's the most regular diabetes condition and accounts for 90 %–95 % of the disease. In this condition the body does not produce enough amounts of insulin or does not properly utilize the produced insulin. while 9 out of 10 people of people suffering from type 2 diabetes mellitus don't know that they have pre-diabetes (Guill´en *et al.*, 2015). Reasons for this rise include the increase in inactive way of life, consumption of energy-rich diet, obesity, higher life span, etc (Yajnik. 2001). Synthetic blood glucose reducing agents and insulin can produce severe side effects. However, in ethnomedicine, plants have proved to be important alternative to control and treat diabetes mellitus with reduced or no side effects in clinical settings (Gupta *et al.*, 2005; Balamurugan *et al.*, 2014). Ethnobotanical reports reveled that approximately 800 plants may exhibit anti-diabetic properties (Patel *et al.*, 2012). Some secondary metabolites such as phenolics, flavonoids, steroids, triterpenoids, alkaloids, glycans and mucilages with hypoglycemic activity, have been extracted from traditional medicinal plants by using different organic solvents (Gupta *et al.,* 2006). *Oxytenanthera abyssinica* (bamboo) is a tropical drought resistant plant that grows in open grassland, lowlands and highlands, although frequently on hills or along intermittent watercourses (Sharma and Sarma, 2013). They are spread largely in the tropics and also occur naturally in subtropical and temperate zones of all continents except

Europe. Ethnomedically, the rhizome is used in the treatment of dysentery and the leaves are marketed for treating diabetes, colics and rheumatism. *O. abyssinica* has wide applications in various countries. In Nigeria the leaves are used in the management of diabetes, in Ethiopia the root is applied in the treatment of skin diseases while in Senegal leaf decoctions are used to treat polyuria, oedema and albuminuria (Louppe *et al*., 2008).

2. MATERIALS AND METHODS

2.1. Chemicals

Chemicals and reagents used for this study were of analytical grade while alloxan monohydrate used to induce diabetes was a product of Sigma – Aldrich Chemical Co., USA.

2.2. Collection of Plant Materials

The rhizomes of *O. abyssinica* were collected from Owerezukala of Orumba Local Government Area of Anambra State while the crude Oil polluted *O. abyssinica* rhizome samples were collected from Akirika Ndoki in Ukwa East Local Government Area of Abia State Nigeria. The rhizomes were authenticated in Nnamdi Azikiwe University Awka by the taxonomist.

2.3. Preparation of Plant Extracts

The rhizomes of *O. abyssinica* were air dried at room temperature and reduced to powder by milling. Two hundred gram (200 g) of the powder was extracted with 80% methanol, filtered with Whatman No. 1 filter paper. The filtrate was concentrated in vacuum in a rotary evaporator.

2.4. Experimental Animal Models

Seventy two (72) male albino rats weighing 140 – 200 g were purchased from animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept for two weeks in the animal house of Department of Biochemistry for acclimatization.

2.5. Phytochemical Analysis of The Extract

Qualitative and quantitative phytochemical tests were carried out on the methanol extract of *O. abyssinica* rhizomes using standard phytochemical tests as described by Harbone (1973), Trease and Evans (1989). The following phytochemicals were assayed: Reducing sugar, glycosides, carbohydrate, flavonoids, phenol, tanins, alkaloid, terpenoids, steroids and saponins.

2.6. Acute Toxicity Study

The LD_{50} of the extracts were determined in mice using the Lorke method (1983). The animals were administered with the extracts and monitored for 24 hours for signs and symptoms and LD_{50} was calculated.

2.7. Postprandial Test

Postprandial test measures the body's ability to metabolize carbohydrates and produce insulin. Thirty (30) albino rats were randomly assigned to five groups; 1, 2, 3, 4 and 5 with each consisting of six rats. They were fasted for about 18 hrs with access to only water (Egwim, 2005). Glucometer (ACCU-CHEK, Roche Diagnostics) was used to estimate their initial blood sugar level. After which Group 1 received normal saline and served as the control. Group 2 and 3 received 200 and 400 mg/kg b.w of NCOPOAE while groups 4 and 5 received 200 and 400 mg/kg b.w of COPOAE respectively. After 30 min, each rat was administered orally with 40 % (w/v) glucose at a dose of 1ml /100 g bw. Blood glucose levels were monitored at 30, 60, and 120 min. intervals and reported as the average glucose level of each group.

2.8. Induction of Diabetes

Diabetes was induced by intraperitoneally injecting 130 mg/kg b.w of alloxan monohydrate (Sigma–Aldrich Chemical Co., USA) reconstituted in cooled normal saline after the rats were subjected to fasting overnight. After 3 days of alloxan administration, glucose level was measured in the blood collected from the tail vein by using an Accu-check glucose meter (Roche Diagnostics Co., USA). Thirty six (36) rats with blood glucose level of 200 mg/dl and above were considered diabetic and used for the study. They were randomly grouped into seven groups of six rats each with group 1 serving as the control group.

2.9. Statistical Analysis

Data were reported as mean ± standard deviation of triplicate determination. One – way analysis of variance (ANOVA) and student T- test were used to analyze the data results using statistical package for social science (SPSS) version 20. Group means obtained after each treatment, were compared with controls and difference were considered significant when the results were $p<0.05$.

3. RESULTS

3.1. Acute Toxicity

The median lethal dose (LD_{50}) of this study showed that non crude oil-polluted extract (NCOPOAE) had LD_{50} of 5000 mg/kg b.w while that of the crude oil-polluted extract (COPOAE) was calculated to be 3807.8 mg/kg b.w.

3.2. Effect of Crude Oil Pollution on the Phytochemical Content of O. *abyssinica* Rhizomes

The result of this study showed that there was significant reduction ($p<0.05$) in the phytochemical content of *O. abyssinica rhizomes* from crude oil polluted area of Ukwa East Local Government of Abia State compared to the *O. abyssinica rhizomes* gotten from non crude oil polluted area of Owerezukala of Orumba Local Government Area of Anambra State. (Table 1.0)

3.3. Effect of NCOPOAE and COPOAE Rhizomes on Oral Glucose Tolerance Test in Normal Albino Rats.

The plasma glucose levels of group 1 rats (control), increased from the FBS concentration and peaked at 60 minutes after oral administration of 40% glucose and gradually decreased to pre - prandial level, while group 2, 3, 4 and 5 test rats fed different doses of NCOPOAE and COPOAE one hour before administration of 40% glucose peaked at 30 minutes before returning to pre -prandial level with NCOPOAE significantly reduced ($p<0.05$) the blood glucose level compared to COPOAE. (Figure 1).

3.4. Result of the Effect of NCOPOAE and COPOAE Rhizomes on Alloxan Induced Diabetic Albino rats.

Figure 2, shows the result of the effect of NCOPOAE and COPOAE on alloxan induced diabetic rats. It shows that, there was a significant increase in mean plasma glucose concentration ($p<0.05$) on day 0 in all the tested rats compared to FBS while on the 3rd day of the study, the test rats did not show any significant difference ($p>0.05$) in mean plasma glucose concentration compare to day 0 of the study. Only group 4 rats survived the 6th day of the treatment having a significantly increase in plasma mean glucose concentration ($p<0.05$) compared to FBS, day 0 and 3.

Table 1.0: Quantitative Phytochemical Constituents of NCOPOAE and COPOAE.

Phytochemicals	NCOPOAE	COPOAE
Flavonoids (mg/dl)	781.80± 28.10	339.10±16.90
Phenols (mg/100g)	2360.20 ±13.10	23481.10±96.20
Tanines (mg/100g)	29.65 ± 0.70	29.57±7.90
Alkaloid (mg/dl)	2416.60 ±11.00	2093.75±10.82
Glgcosides (mg/ 100g)	0.01 ± 0.00	0.01±0.00
Terpenoids (mg/100g)	90.60±7.60	34.70±2.40
Steroids (mg/100g)	1.16±0.00	0.65±0.10
Flavonol (rut. Equiv)	0.03±0.00	0.01±0.00
Saponins (mg/100g)	1.40± 0.41	1.30 ± 0.40
Carbohydrate (mg/100g)	1.31± 0.21	1.13 ± 0.12

Values are mean ± SD of triplicate determinations

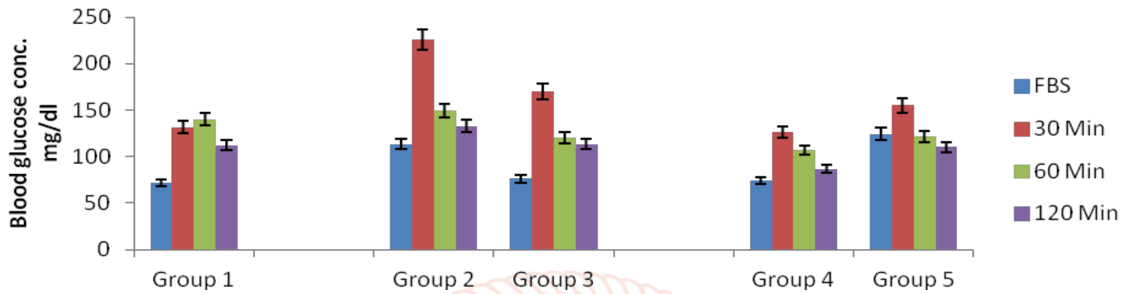

Fig 1: OGGT plasma glucose concentrations in test and normal groups.
Data represented as mean ± SEM

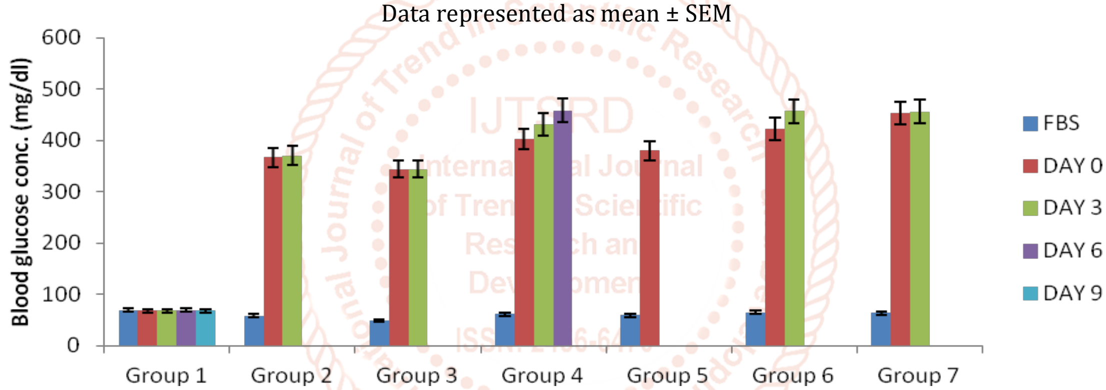

Fig 2. Alloxan induced plasma glucose concentrations in test and normal group
Data represented as mean ± SEM.

DISCUSSION

The median lethal dose (LD_{50}) of this study revealed that non crude oil polluted extract (NCOPOAE) had LD_{50} of 5000 mg/kg b.w while that of the crude oil polluted extract (COPOAE) was calculated to be 3807.8 mg/kg b.w. The LD_{50} differences observed in this study could be linked to the toxic components of crude oil such as polycylic aromatic hydrocarbons (PAHs) and aromatic hydrocarbon (AHCs) present in COPOAE. However, crude oil has been reported to contain several poisonous compounds, which accumulates in the body and induces toxic symptoms and could result to death (Heintz *et al*., 1999). Secondary metabolites have been implicated in conferring protection against environmental stress in plants (Seigler, 1998). The results of the quantitative analysis of this study indicated that NCOPOAE contains more quantities of these phytochemicals than COPOAE which correlates with the observations that crude oil pollution affected the quantity of secondary metabolites in plants, (Iwuala and Odjegba, 2017). The lower concentration of phytochemicals observed in COPOAE could be related to limited carbon assimilation that may consequently affect carbon allocation for secondary metabolites synthesis as the synthesis of the basic skeleton for the active secondary metabolite is dependent on carbon assimilation during photosynthesis (Zobayed *et al*., 2007). Net photosynthesis decreases when plants are under stress such as high temperature, high salinity, heavy metal and oil pollution (Murch *et al*., 2003), which are associated with crude oil pollution.

Conventionally, diabetes has been treated with orthodox medicine which functions as hypoglycaemic agent or insulin modulators (Ogbonnia *et al*., 2008). From this study (Figure 1), groups 2 and 3 of NCOPOAE responded more than COPOAE to the uptake of glucose within 30 minutes. This result could be as a result of components of crude in COPOAE inhibiting the activity of α- glucosidase enzyme present in the lining of the intestine thereby preventing the breakdown of sugar (Konno *et al*., 2001). The groups treated with NCOPOAE significantly reduced plasma glucose concentration ($p<0.05$) within the interval of 60 and 120 minutes more than group 4 and 5 treated with 200 and 400 mg/kg COPOAE. Some drugs such as rosiglitazone exert their effects on the stimulation of glucose uptake by the muscles and adipose tissues (Hong *et al*., 2007). This could explain the reduced glucose concentration observed in groups treated with NCOPOAE. In alloxan induced diabetes model,

(Figure 2), NCOPOAE and COPOAE did not have significant change on the plasma glucose concentration (p>0.05) on the tested groups at day 3 and 6 compared to day 0 of diabetes conferment. This could be as a result of the extract not being able to stimulate the production of insulin by β-islet of the pancreas (Oguanobi *et al*., 2012). The hypoglycaemic properties of NCOPOAE compared to COPOAE seems to indicate that components of crude oil could have reduced the ethanomedicinal values of the extracts while the reductions of glucose concentration by NCOPOAE in oral glucose tolerance test (OGTT) suggests that the extract could be used in the management of type -2 diabetes where the cells are resistant to insulin.

CONCLUSION

The low LD_{50} value of (3807.8 mg/kg b.w.) observed in the crude oil polluted *O. abyssinica rhizomes* indicates slight toxicity which could be as a result of crude oil pollution. The crude- oil polluted extract also revealed low phytochemical contents compared to the non crude oil- polluted extract. This could reduce the ethnomedicinal value of the polluted extract as the secondary metabolites are the major bio-compounds responsible for plants potency in ethnomedicine. The study showed also, that the extracts had good postprandial glucose effect which suggests that the extract could be used in the management of type -2 diabetes where the cells are resistant to insulin.

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