e-ISSN :3027-2068:Print ISSN:3026-9830

https:www.ijbsmr.com

***Int. J.Bio. Sc. Mol. Res.* December,2023Vol.1(4) 194-203**

Antidiabetic and Antioxidant Potentials of Methanol Leaf Extract of *Napoleonae Imperialis* In Streptozotocin Induced Diabetic Rats

***Mba, O.J[1], Omodamiro, O.D[2], Aja, O.A[3], Azubuike, N.J[4], Okafor, P.N[5]**

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.

[2]Department of Pharmacology and Toxicology, School of Pharmacy, Kampala International University, Kampala, Uganda.

[3]Biochemistry Research Unit, Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic Unwana, Ebonyi State, Nigeria.

[4,5]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

***Corresponding author e-mail: mbajoseph227@gmail.com.**

Abstract

Background: This study aimed to evaluate the antidiabetic and antioxidant potentials of methanol leaf extract of *Napoleonae imperialis* in streptozotocin induced diabetic rats. **Methodology**: Forty two male albino rats of mean weight 140 g were used for the study. The animals were grouped into seven of six rats each. Group 1 is the normal control, Group 2 is the negative control (diabetic untreated group), Group 3 is the positive control, Groups 4, 5 and 6 were the test groups that were orally given 250, 500 and 1000 mg/kg body weight of the extract, while group 7 is the group that received the extract only (500 mg/kg b.wt). **Result:** The results showed that there was significant ($p < 0.05$) decrease in the blood glucose concentration and significant ($p < 0.05$) increase in the body weight of the test groups, normal control and the group that received the extract only when compared with the diabetic untreated group. The result also showed that there was significant ($p<0.05$) increase in the concentration of reduced glutathione, catalase, superoxide dismutase, vitamin C and vitamin E and a significant ($p < 0.05$) decrease in malondialdehyde in the test groups, drug control and the group that received the extract only when compared with the diabetic untreated group). **Conclusion:** The results of this study indicate that methanol leaf extract of *Napoleonae imperialis* possesses antidiabetic and antioxidant potentials and thus could be utilized in managing diabetes and diseases associated with rise in free radicals.

Keywords: *Napoleonae imperialis*, streptozotocin, glibenclamide, antidiabetic, antioxidants.

1. Introduction

Diabetes mellitus (DM) is a chronic endocrine disorder of multiple etiologies distinguished by hyperglycemia leading to deficiencies in insulin production, insulin activity, or both of them (ADA, 2014). The medical analysis of diabetes is frequently shown by the occurrence of signs such as polyuria, polydipsia, polyphagia, unpredicted loss in weight, and is established by standard hyperglycemia (CDC, 2015). Diabetes impairments can be categorized as microvascular complications such as nervous system impairment (neuropathy), kidney impairment (nephropathy) and eye injury (retinopathy), and macrovascular complications such as heart ailment, stroke, and peripheral vascular disease (Afroz *et al.*, 2019).

It has been managed with medicinal plants for a very long time and many herbs have been shown to possess anti-diabetic property (Alkofahi *et al.,* 2017). Some local herbs have been utilized as a therapy for the treatment of diseases for centuries before the commencement of contemporary medicine. Factually, ancient medicinal plants have revealed to exert pharmacological effect, for example, *Galega officinalis* with the bioactive compound metformin (Martin-Timon *et al.,* 2014).

Furthermore, in modern studies, drugs gotten from herbs have shown to be less toxic and more compatible with biological systems due to their minor side effects, in comparison with the synthetic ones;therefore, massive attention of scientific study is concentrating on producing novel medicines centered on natural or nature-identical compounds of herbal source for the management of severe ailments such as diabetes (Mallick *et al.,* 2007). *Napoleonaea imperialis* is a small, evergreen tropical West African tree in the family Lecythidaceae, native to Africa (Mba *et al.,* 2020). The plant's local name is Napoleonae hat, and it grows to about 6 m in height, with a thick, low-branching crown, and spreads from Benin, Nigeria, Gabon and the Democratic Republic of Congo, southwards to Angola. The attractive flowers of the plant have two inner rows of petals, and differ in colour, generally creamy yellow beside the edge, and the middle stretching from red to apricot to purple (Ojinnaka and Okpala, 2012). Extracts gotten from the leaves show bactericidal property, and comprises of glycosides, tannins, phenols, alkaloids, terpenoids, saponin, flavonoids, reducing sugar, carbohydrate and steroids (Mba *et al.,* 2020).

Napoleonae imperialis plant. Source: (Ojinnaka and Okpala, 2012).

Orthodox drugs for the treatment of diabetes in most cases are costly and mainly out of reach of the poor, especially, in Africa. In addition, a good number of them have side effects on human system. It has been shown that natural products and herbal plants that are abundant in nature, especially in Africa, have very good potential for management and treatment of diabetes when correctly processed and applied (Odugbemi *et al.,* 2010). The need for identification of more plants with antidiabetic and antioxidant properties led to this present study.

2. Materials and Methods

2.1 Plant material

Fresh leaves of the plant *Napoleonae imperialis* was obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and was identified by Prof. Garuba Omosun, a Taxonomist of the Plant Science and Biotechnology Department, Michael Okpara Uiversity of

Agriculture, Umudike. The fresh leaves were washed and dried under shade at room temperature, and were milled to fine powder using an electric blender (QLink, Model QBL, Taiwan) and stored in air tight containers.

2.2 Extraction

The powdered leaves of *Napoleonae imperialis,* 500 g, was soaked in four litres of methanol for 48 hours, after which the extract was filtered using a Whatman no. 1 filter paper and the filtrate was air dried at a temperature of 40^{o}C and stored in the refrigerator for further use as methanol leaf extract of *Napoleonae imperialis*. During the experiment, the crude extract was diluted with distilled water (a portion of 20 g of the methanol leaf extract of *N. imperialis* was dissolved in 200 ml of water to give the concentration of stock as 0.1g/mL or 100 mg/mL) just before administration to the animals.

2.3 Animals

Healthy looking male albino rats of mean weight of 140 g were used for the study. All the animals were kept in metabolic cages in the animal house under normal room conditions, and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum.*

2.4 Induction of diabetes

All the rats were fasted overnight before the administration of streptozotocin. Diabetes was induced in the rats by intra peritoneal injection of streptozotocin dissolved in 0.1 M sodium citrate buffer of pH 4.5, at the dose of 65 mg/kg body weight. After the injection, they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycemic shock. The development of diabetes was confirmed after one week of streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dL were considered as diabetic and used in the experiment.

2.5 Experimental design

Forty two (42) male albino rats of mean weight 140 g were used for the study. The animals were grouped into seven (7) of six (6) rats each. Group 1 was the normal control that received feed and water only, group 2 was the negative control group (diabetic untreated group) and group 3 was the positive control (drug control that received 5 mg/kg body weight of the standard drug (glibenclamide). Test groups (4), (5) and (6) were orally given 250, 500 and 1000 mg/kg body weight of methanol leaf extract of *Napoleonae imperialis*, while group 7 only, received the methanol leaf extract of *Napoleonae imperialis* (500 mg/kg b.wt). All the rats used in this study were initially subjected to diabetes by single intra-peritoneal induction of 65 mg/kg body weight of streptozotocin, except the normal control (group 1) and the group that received the extract only (group 7). Treatment was given daily for 28 days, after which the animals were anaesthetized under mild ether anesthesia. Blood samples were collected into plain bottles for the analyses.

2.6 Biochemical analyses

After 28 days following the treatments, fasting blood glucose and body weight was measured at weekly intervals to check the blood glucose level and body weight. The serum was obtained by centrifuging the blood at 3000 rpm for 10 minutes. The blood glucose level was determined using ACCU-CHEK Active Glucometer (Roche Diagnostic), by glucose oxidase-peroxidase method. Lipid peroxidation (MDA) was estimated according to the method of Wallin *et al.* (1996). Superoxide dismutase (SOD) activity was assayed according to the method of Arthur and Boyne (1985). Catalase (CAT) activity was assayed using the procedure described by Naskar *et al.* (2001). Reduced glutathione (GSH) was estimated by the method of Exner *et al.* (2000), while Vitamin C and Vitamin E concentrations were measured by the method of Omaye *et al.* (1979).

2.7 Statistical analysis

The data obtained were analyzed statistically, using one-way Analysis of Variance (ANOVA) to determine the significant difference between the group means at 95% level of confidence, using SPSS statistical package (version 22.0). Values at $p \leq 0.05$ were considered significant.

3. Results

3.1 Result of methanol leaf extract of *Napoleonae imperialis* on fasting blood sugar (FBS) in streptozotocin induced diabetic rats

There was significant ($p<0.05$) decrease in the fasting blood glucose level from week one to week four in the treated groups that orally received 250, 500 and 1000 mg/kg body weight of the extract, drug control (5 mg/kg body weight glibenclamide), and the group that received the extract only (500 mg/kg body weight), when compared with the diabetic untreated group,indicating that *Napoleonae imperialis* leaf-extract and the standard drug (glibenclamide) lowered the sugar level.

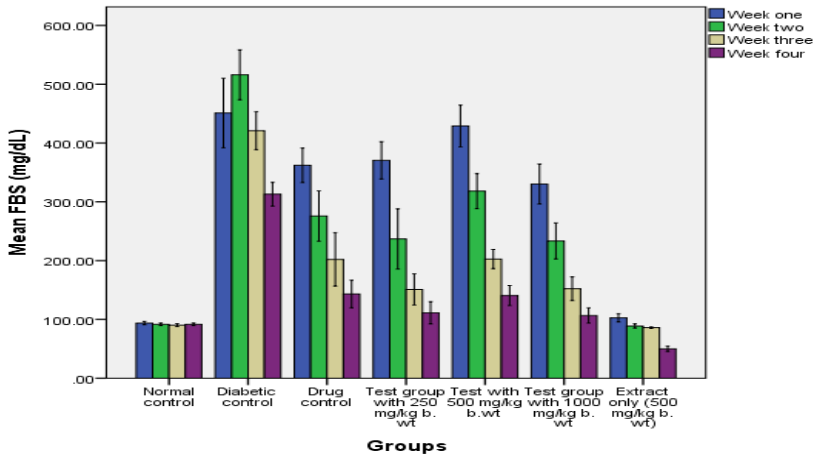

Figure 3.1: Effect of *Napoleonae imperialis* methanol leaf extract on fasting blood sugar concentration in Streptozotocin-induced diabetic rats.

3.2 Result of methanol leaf extract of *Napoleonae imperialis* on the body weight in streptozotocin induced diabetic rats

There was significant (p<0.05) decrease in the body weight of the streptozotocin induced diabetic untreated control when compared with the normal control group, the drug control that received 5 mg/kg body weight and the group that received the extract only (500 mg/kg b.wt). Oral administration of the leaf extract at of dose of 250, 500 and 1000 mg/kg body weight showed a significant (p<0.05) increase in the body weight when compared to the untreated diabetic group.

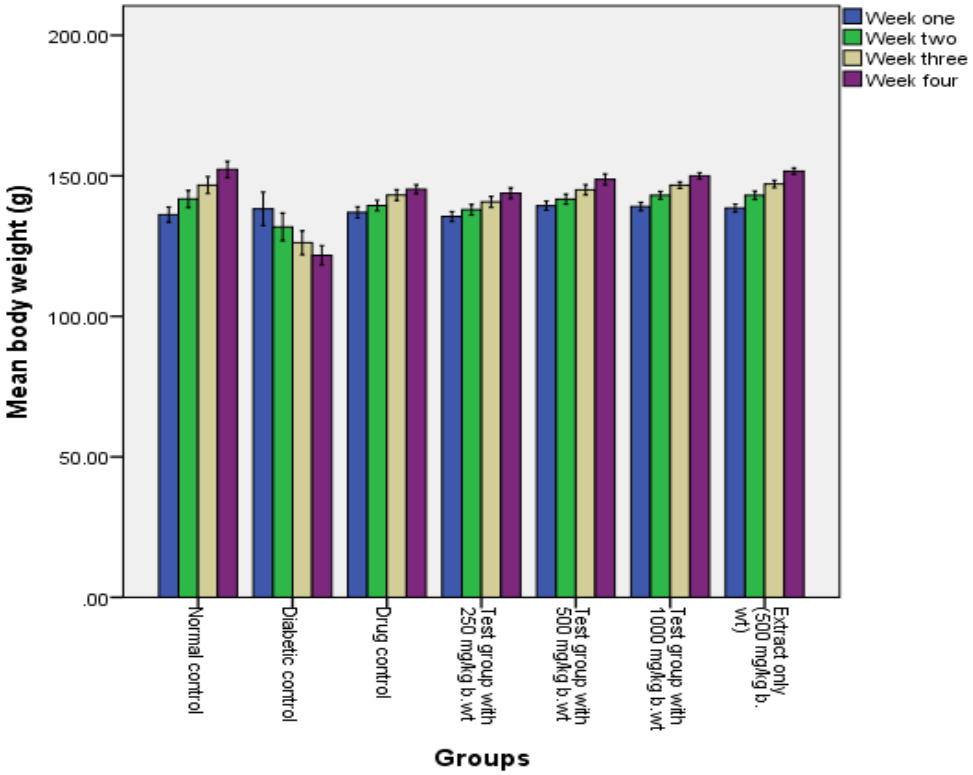

Figure 3.2: Effect of *Napoleonae imperialis* methanol leaf extract on the body weight in Streptozotocin-induced diabetic rats.

3.3 Result of methanol leaf extract of *Napoleonae imperialis* on antioxidants in streptozotocin induced diabetic rats.

Table 3.1 below, shows that there was statistically significant (p< 0.05) decrease in the diabetic untreated control group ,when compared with the normal control, the drug control that received 5 mg/kg b.wt of glibenclamide, the test groups that received 250, 500, and 1000 mg/kg b.wt of the extract, and the group that received the extract only (500 mg/kg b.wt) for GSH, SOD, catalase, vitamin C and E, while there was significant (p < 0.05) increase in the diabetic untreated control group, when compared with the normal control, the drug control that received 5 mg/kg b.wt of glibenclamide, the test groups that received 250, 500, and 1000 mg/kg b.wt of the extract, and the group that received the extract only (500 mg/kg b.wt) for MDA.

Table 3.1: Serum antioxidant profile of STZ-induced diabetic rats administered methanol leaf extract of *Napoleonae imperialis.*

Groups	GSH (mg/dL)	SOD (µ/mg)	CAT(µ/mg)	MDA (mg/mL)	Vit C (mg/dL)	Vit E (mg/dL)
1	2.19±0.24^{a}	11.48±0.02^{a}	1.08±0.03^{a}	0.24±0.03^{bc}	1.72±0.04^{a}	1.42±0.12^{a}
2	0.92±0.05^{b}	10.94±0.02^{e}	0.15±0.01^{b}	0.60±0.05^{a}	1.13±0.06^{b}	1.07±0.02^{b}
3	2.29±0.31^{a}	11.37±0.03^{bcd}	1.07±0.03^{a}	0.20±0.03^{c}	1.92±0.03^{a}	1.51±0.04^{a}
4	1.94±0.53^{a}	11.45±0.01^{ab}	1.06±0.02^{a}	0.26±0.04^{bc}	1.83±0.12^{a}	1.39±0.11^{a}
5	2.04±0.19^{a}	11.42±0.03^{abc}	1.09±0.03^{a}	0.37±0.06^{b}	1.84±0.08^{a}	1.55±0.09^{a}
6	2.42±0.05^{a}	11.33±0.03^{d}	1.08±0.02^{a}	0.30±0.33^{bc}	1.90±0.05^{a}	1.64±0.09^{a}
7	2.08±0.22^{a}	11.34±0.02^{cd}	1.19±0.09^{a}	0.24±0.05^{bc}	1.85±0.08^{a}	1.64±0.10^{a}

Values are mean ± standard deviation (SD). Means with different superscripts along the column are statistically significantly different at $p < 0.05$ (n = 6). Group 1 = normal control, group 2 = diabetic control (disease control), group 3 = drug control (5 mg/kg b.wt of glibenclamide), group 4 = diabetic rats + 250 mg/kg b.wt, group 5 = diabetic rats + 500 mg/kg b.wt, group 6 = diabetic rats + 1000 mg/kg b.wt, group 7 = extract group only (500 mg/kg b.wt).

4. Discussion

Induction of rats with streptozotocin (STZ), led to a significant decrease in body weight in all the test groups, when compared with the normal control. However, a statistically significant increase in body weight was observed in the diabetic rats treated with the methanol leaf extract of *Napoleonae imperialis,* when compared with the negative control, from week two to week four. Significant decrease in body weight was observed in the diabetic untreated control groups, which continued until the conclusion of the research work. The significant decrease in total body weight seen in the negative control animals, in spite of their improved feed consumption,was accredited to the STZ-induced diabetes, which is associated with a significant decline in body weight (*Perinyar et al., 2009).*

The statistically significant increase in the concentration of fasting blood glucose was observed in STZ induced diabetic rats, as compared to normal rats, could be as a result of destruction of beta cells of the pancreas. Administration of the leaf extract at different concentrations showed statistically significant (p<0.05) reduction in blood glucose, when compared with the diabetic untreated control, at the expiration of the 28 days of treatment. This study showed the ability of the methanol leaf extract to reduce blood glucose level, which might have been due to the presence of the bioactive compounds such as flavonoid, which has been reported to stimulate insulin secretion, and saponins which increased hepatic glucokinase in an insulin like manner, as earlier reported by (Courtois *et al.,* 2003). The accumulated evidence suggested that modulation of insulin secretion and or insulin action, could be involved in the antidiabetic effect of the leaf extract. This evidence was supported by (Mba *et al.,* 2020), who revealed that *Napoleonae imperialis* leaves contains phytochemicals like flavonoids, steroids, terpenoids, phenols, tannins, glycosides, alkaloids, saponins, which might stimulate insulin secretion or which could protect

the intact functional β-cells from further deterioration, or still, due to regeneration of STZ damaged β-cells. Ampa *et al.*(2013), for example, revealed that administration of flavonoids to STZ-induced diabetic rats, resulted in an elevation of insulin and glucokinase activity in plasma samples associated with a drop in the glucose-6-phosphatase action. The study of Mba *et al*.(2023), earlier suggested that *Napoleonae imperialis* leaves might have the potential to become the lead extract in the improvement of new types of antidiabetic pharmaceuticals that are able to reduce blood glucose levels, without increasing adiposity.

Furthermore, a lot of researches have shown that free radicals and oxidative stress, were among the instrumental features in the causation and progression of numerous ailments, including cancer and diabetes (Eleazu *et al.,* 2014). Naturally, free radicals are removed by antioxidant enzymes such as superoxide dismutase (SOD) and catalase, which defend the body from oxidative stress (Sharma *et al.,* 2011). The decrease in the action of these redox enzymes could result in a rise in superoxide anion, hydrogen peroxide, and hydroxyl radicals, leading to widespread lipid peroxidation in the diabetic patients (Vertuani and Manfredini, 2004). In this study, the observed rise in oxidative stress in the diabetic animals might have been as a result of a decline of the antioxidant enzymes responsible for scavenging the free radicals produced during the metabolism of STZ. Hyperglycemia resulted in increased oxidative stress in diabetic patients, which included extensive lipid peroxidation (Aloh *et al.,* 2015). The aldehyde groups of MDA made during lipid peroxidation might function as a catalyst between sugar and protein moieties, which accelerated the production of glycated proteins (Balasubraimanian *et al.,* 2009), It was likely that the glycation drive could have inhibited the antioxidant enzymes, resulting to the diseased situation. It has been revealed that the leaf extract of *Napoleonae imperialis* contains high flavonoids and phenolic compounds that have hydroxyl groups in their structure increasing their antioxidative effect (Williams *et al.,* 2004). These compounds could be important in enhancing the redox status in the diabetic rats treated with the leaf extract (Barky *et al.,* 2017), as evidenced in the present study.

Conclusion

In this study, methanol leaf extract of *Napoleonae imperialis* demonstrated hypoglycemic potential, and exhibited significant improvement in blood glucose level, various parameters like body weight and antioxidant activity, as well as potential to protect pancreatic islets of Langerhans, by decreasing the concentration of lipid peroxidation products, and increasing the activity of antioxidants, which might be valuable in diabetes treatment.

References

ADA (2014). Standards of medical care in diabetes, *Diabetes Care*, 37(3) :14 - 80.

Afroz, A., Zhang, W., Wei, W., Loh, A.J., Jie, D.X., Lee, D.X. and Billah, B. (2019). Macro- and micro- vascular complications and their determinants among people with type 2 diabetes in Bangladesh, *Diabetes Metabolic Syndrome,* 13(5): 2939 - 2946.

Alkofahi, A.S., Abdul-Razzak, K.K., Alzoubi, K.H. and Khabour, O.F. (2017). Screening of the antihyperglycemic activity of some medicinal plants of Jordan. *Pakistan Journal of Pharmaceutical Sciences*, 30: 907 - 912.

Aloh, G.S., Obeagu, E.1., Odo, C.E., Kanu, U.G. and Mba, O.J. (2015). Effect of methanol extract of *Napoleonae imperialis* on free radical scavengers and lipid profile of wistar albino rats. *European Journal of Pharmaceutical and Medical Research*, 2(2): 140 - 150.

Ampa, L., Watchara, K. and Tanaree, J. (2013). Anti-hyperglycemic properties of *Moringa oleifera* Lam. Aqueous leaf extract in normal and mildly diabetic mice. *British Journal of Pharmacology and Toxicology*, 4(3): 106 - 109.

Arthur, J.R. and Boyne, R. (1985). Superoxide dismutase and glutathione peroxidase activities in neutrophil from selenium deficient and copper deficient cattle. *Life Sciences*, 36**:** 1569 - 1575.

Balasubraimanian,T., Lal, M.S., Mahananda, S. and Chatterjee, T.K. (2009).Antihyperglycaemia and antioxidant activities of medicinal plant *Stereospermum suaveolens* in streptozotocin-induced diabetic rats. *Journal Dietary Supplements*, 6(3): 227 - 251.

Barky, A.E., Hussein, S.A., Alm-Eldeen, A.E. and Mohamed, A.M. (2017). Saponins and their potential role in diabetes mellitus. *Diabetes Management*, 7: 148.

CDC (2015). Diabetes Report Card 2014, Centers for Disease Control and Prevention, *US Department of Health and Human Services, Atlanta, GA. https://www.health.ny.gov.*

Courtois, P., Jijaki, H., Ladriers, L., Oguzhan, B., Sener, A. and Malaisses, W. (2013). Pharmacodynamics, insulinotropic action and hypoglycemic effect of nateglinide and glibenclamide in normal and diabetic rats. *International Journal of Molecular Medicine*, 11: 105 - 109.

Eleazu, C.O., Okafor, P.N. and Ijeh, I.I. (2014). Biochemical basis of the use of cocoyam (*Colocassia esculenta*) in the dietary management of diabetes in streptozotocin induced diabetes in rats. *Asian Journal of Tropical Disease*, 4:705 - 711.

Exner, R., Wessner, B., Manhart, N. and Roth, E. (2000). Therapeutic potential of glutathione. *Wien Klin Wochenschr*, 112: 610 - 616.

Mallick, C., Chatterjee, K., GuhaBiswas, M. and Ghosh, D. (2007). Antihyperglycemic effects of separate and composite extract of root of *Musa paradisiaca* and leaf of *Coccinia indica* in streptozotocin induced diabetic male albino rat. *African Journal of Traditional Complementary and Alternative Medicine*, 4: 362 - 371.

Martin-Timon, I., Sevillano-Collantes, C., Segura-Galindo, A. and Canizo-Gomez, F.J. (2014). Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? *World Journal of Diabetes*, 5: 444 - 470.

Mba, O.J., Aloh, G.S., Nwachukwu, K.C. and Michael, P.O. (2020). Phytochemical Characterization, Acute Toxicity Studies of the Methanol Extract of *Napoleonae imperialis* Leaves. *Journal of Genetics and Cell Biology*, 3(3): 194 - 198.

Mba, O.J., Aloh, G.S. and Uhuo, E.N. (2020). Ameliorative Effect of the Methanol Extract of *Napoleonae imperialis* Leaves against Methotrexate induced Renal Damage in Albino Rats. *Asian Journal of Research in Biochemistry*, 6(4): 10 - 20.

Mba, O.J., Omodamiro, O.D., Okafor, P.N. and Maduagwu, E.N. (2023). Hepatoprotective effects of methanol leaf extract of *Napoleonae imperialis* in streptozotocin induced diabetic albino rats. *World Journal of Pharmaceutical Research*, 12(5): 85 - 99.

Naskar, S., Mazumder, U.K., Pramanik, G., Gupta, M., Kumar, R.B., Bala, A. and Islam, A. (2011). Evaluation of antihyperglycemic activity of *Cocos nucifera* Linn. on streptozotocin induced type 2 diabetic rats. *Journal of Ethnopharmacology*, 138:769 - 773.

Odugbemi, T.O., Odunayo, R., Akinsulire, I., Aibinu, E. and Fabeku, O. (2007). Medicinal Plants Useful for Malaria Therapy in Okeigbo, Ondo State, Southwest Nigeria. *African Journal of Traditional Medicine*, 4(2):191 - 198.

Ojinnaka, C.M. and Okpala. D.C. (2012). A Molluscicidal triterpenoid saponin from the fruits of Napoleonae P. Beauv (Lecythidaceae). *Journal of Applied Sciences and Environmental Management,* 16(2):213 - 216.

Omaye, S.T., Turnbull, J.D. and Sauberlich, H.E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. *Methods of Enzymology*, 62:3 - 11.

Perinyar, S.S., Balu, P.M., Sathiya, M.P. and Muragesan, K. (2009). Antihyperglycemic effect of mangiterin in streptozotocin induced diabetic rats. Journal of Health Science, 55(2): 206 - 214.

Sharma, M., Siddique, M.W., Akhter, M.S., Shukla, G. and Pillai, K.K. (2011). Evaluation of antidiabetic and antioxidant effects of Seabuckthorn (*Hippophae rhamnoides* L.) in streptozotocin nicotinamide induced diabetic rats. *The Open Conference Proceedings Journal*, 2: 53 - 58.

Vertuani, A.A. and Manfredini, S. (2004). The antioxidants and pro-antioxidants network: an overview. *Current Pharmaceutical Design,* 10(14) 1677 - 1694.

Wallin, B., Rosengren, B., Shertzer, H.G. and Camejo, G. (1993). Lipoprotein oxidation and measurement of TBARS formation in single microlitre plate; it's use for evaluation of antioxidants. *Analytical Biochemistry*, 208: 10 - 15.

Williams, R.J., Spencer, J.E. and Rice-Evans, C. (2004). Flavonoids: antioxidants or signaling molecules. *Free Radical Biology and Medicine,* 36(7):838 - 849.

Funding

The project was jointly funded by the authors.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical issues

The authors declare that there were no ethical issues involved.

Author Contributions

Mba, O.J, Okafor, P.N and Omodamiro, O.D conceptualized, supervised the project and wrote the work. Mba, O.J and Azubuike, N. J conducted the experiment. Aja, O. A. and Azubuike, N.J procured the samples. Mba, O.J, Okafor, P.N and Omodamiro, O.D carried out the proof-reading, plagiarism and grammar check. Aja, O.A carried out the statistical analysis. All the authors read the work and approved it before publication.