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ANTIDIABETIC PROPERTIES OF COMBINED METHANOL EXTRACTS OF PTERIDIUM AQUILINUM, MUCUNA PURIENS AND NEWBOLDIA LAEVIS (PMN) IN ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

The study of natural products has played a major role in the development of novel therapeutic substance with high efficacy. Mucuna puriens, Newboldia laevis and Pteridium aquilinum are known medicinal plants used in the treatment of many diseases. In this study, the use Mucuna puriens, Neuboldia laevis and Pteridium aquilinum as combined graded doses in the treatment of diabetes were evaluated. Diabetes was induced in wistar albino rats using intraperitoneal injection of 140mg/kg of alloxan. Thirty (30) albino rats were randomly divided in 5 groups of 6 rats each (n=6). 5mg/kg of glabenclamide was used as the standard control while different graded doses of the formulation at 200mg/kg and 400mg/kg were administered to the other groups with one untreated group serving as the diabetic control. The study lasted for a period of 21 days. The formulation significantly ($p<0.05$) lowered the elevated blood glucose level. The % reduction of the 400mg/kg of the combined dose was 59.19% compared to the standard drug 56.45%. There was also significant ($p<0.05$) decrease in the lipid, hematology indices and markers of cellular toxicity compared to the diabetic control (untreated group). The combined extracts showed more efficacy the glabenclamide.

This suggested that the polyherbal formulation enhances therapeutic action and is effective in the treatment diabetes.

KEYWORDS: Polyherbal formulation, Medicinal plants, Diabetes, amelioration,

INTRODUCTION

Diabetes mellitus is globally known as disease condition of both humans and animals with metabolic conditions characterized by high blood glucose resulting from defects in insulin secretion, insulin action, or both (Maxwell et al., 2015; Edwin et al., 2007). In human, beta cells of the islet of Langerhans produce this hormone known as insulin that controls the amount of sugar in the blood (Stephen et al., 2011). Diabetes is associated with disorders in carbohydrate, protein and fat metabolism. Patients with diabetes mellitus have increased oxidative stress and compromised antioxidant defense systems, which appear to contribute to the initiation and progression of diabetes associated complications (Raja et al., 2018).

World Health Organization reported that around 173 million adults are diabetic and over 8 million deaths are caused by this disease or its complications annually (Sunmonu and Afolayan 2013; Gloria and Anthony, 2015). This disease compromise the immunity and can make adults vulnerable to so many infectious disease like in the case of Covid 19 where greater percent of death are adults with underling chronic disease like diabetes (Hans, 2020).

The abundance of medicinal plants in our environment and the realization that they possess bioactive ingredients with therapeutic values has made the need for their studies imperative. The therapeutic applications of plant portend great opportunities for humanity. Research into medicinal plants will assist in ascertaining the efficiency of the flora as a remedy, and extend our frontiers of knowledge (George and Uwakwe, 2014).

Medicinal plants with hypoglycemic effects used in the management of diabetes mellitus have be reported (Malviya *et al.*, 2010). The use of plant could be effective in delaying the development of diabetic complications and correct the metabolic abnormalities (Malviya *et al.*, 2010). Lack of standardization of herbal medicinal plant does not prevent its prescription due to their relative safety and low costs (Majekodunmi et al., 2008), and medicinal plants can serve as good source of drugs and many of the currently available drugs have been derived directly or indirectly from them (Patel *et al.*, 2012). Some of the medicinal plants used in the management of diabetes are *mucuna puriens*, *newboldia laevis* and *pteridium*

aquilinum. Mucuna puriens (Fam. Leuminoseae) also known as velvet bean is commonly found in the Eastern part of Nigeria. *newbouldia laevis* (Bignoniacea) is a native to Tropical Africa which is commonly known as boundary tree and *pteridium aquilinum* (Dennstaedtiaceae) occurs in temperate and subtropical regions and is commonly known as eagle fern. These plants have enormous pharmacological properties and their combination could ameliorate the complications in diabetes mellitus. This study evaluated the antidiabetic properties of combined methanol extract of *mucuna puriens*, *newboldia laevis* and *pteridium aquilinum* in alloxan induced diabetes in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Alloxan monohydrate was procured from Sigma-Aldrich Chemical Co. (St Louis, MO, USA), while glibenclamide was a product of Blessed Pharmacy (Umuahia, Nigeria).

The assay kits used for biochemical analysis were products of Randox Laboratories (Ardmore, Co. Antrim, UK). All other reagents were of analytical grade.

Plant material

All the medicinal leaves (*Pteridium aquilinium*, *Mucuna puriens* and *Newbouldia laevis*) were collected from Michael Okpara University of Agriculture, Umudike farm, in Abia State, Nigeria. The plants were toxonomically identified by Dr. G. Omosun of Plant Science and Biotechnology, MOUAU, Nigeria. Voucher specimens have been kept in the herbarium of the Department (Voucher No. PSB 1467, PSB 1468, PSB1469).

Extract preparation

Fresh leaves of *Pteridium aquilinium*, *Mucuna puriens* and *Newbouldia laevis* were air dried at room temperature for two weeks. After drying, the leaves were pulverized to a fine powder and stored in a dry container. Five hundred gram (500g) of each powdered leaves will be maccrated in 2L of 95% ethanol inside a conical flask (separate flask for each pulverized leave) and were left for 72hours. Filtrations were carried out using a funnel and filter paper (Whatman No 1). Different extracts were concentrated in an evaporating dish over a hot Water bath (45^{o}c) and stored at 4^{o}c.

To prepare the formulation, the different extracts were mixed together in the ratio of 1:2:1 for *Pteridium aquilinium*, *Mucuna puriens* and *Newbouldia laevis* respectively and named PMN. Different dosages of 200mg/kg and 400mg/kg were prepared from the formulation (PMN).

Experimental animals

Thirty (30) male albino rats of the Wistar strain aged 8-12 weeks and weighing 120-170g were procured from animal house farm of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria. They were housed in standard transparent cages with wheat husk bedding, renewed every 24h and kept under controlled room temperature and humidity (18 to 29 °C; 30 to 70%) in a 12h light-dark cycle. Care of experimental animals was taken as per the guidelines approved by Animal use Ethics Committee of the University with Ethical number BCM/EC/01/98. Animals were acclimatized for two weeks to laboratory conditions before starting the study. The animals were given standard laboratory diet and water *ad libitum*.

Acute toxicity (LD_{50})

The acute toxicity of the *Pteridium aquilinium*, *Mucuna puriens, Newbouldia laevis* leaves and the formulation were determined using Lorke's method (1983).

Induction of diabetes

The induction of Diabetes was carried out according to a method used by Maxwell et al. (2015). single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (140 mg/kg body weight) in normal saline was given to 18hours fasted rats. At 48h after the injection, the fasting blood glucose level was estimated using a glucometer (GB Accu-Chek, Roche, Mannhein, Germany). Animals with glucose levels above 250mg/dl were considered diabetic and used for the study (Owolabi *et al*., 2011).

Experimental design

The rats were divided into five (5) groups of six rats each:

Group 1: Normal control rats were administered with 1 ml of distilled water once daily for 21 days.

Group 2: Diabetic control (untreated) rats were administered with 1 ml of distilled water once daily for 21 days.

Group 3: Diabetic rats were treated with glibenclamide (5 mg/kg body weight/day) for 21 days.

Group 4: Diabetic rats were treated with PMN mixture formulation (200 mg/kg body weight/day) for 21 days.

Group 5: Diabetic rats were treated with PMN mixture formulation (400 mg/kg body weight/day) for 21 days.

Estimation of fasting blood glucose level

During the 21 days of treatment, fasting blood glucose levels were measured by cutting the tail-tip of the overnight fasted rats and values were read using a glucometer (GB Accu-Chek, Roche, Mannhein, Germany) on days 0, 7, 14 and 21.

At the end of the 21 days, animals were fasted for 12 h and anaesthetized then sacrificed. Blood was collected by cardiac puncture into appropriate tube for haematological and biochemical assays.

Estimation of haematological parameters

Hb estimation by the cyanomethaemoglobin method described by Jain (1986), Platelets, WBC and RBC were estimated using a method described by Dacie and Lewis (1991).

Estimation of lipid profile

The serum total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were estimated using an automatic analyser as described by Tietz et al. (1994) and Tietz (1995).

Estimation of markers of cellular toxicity

The activities of alkaline phosphatise (ALP), aspartate and alanine transaminases (AST, ALT), Albumin, Total bilirubin, urea and creatine were estimated using Randox assay kits (Wroblewski and La Due 1956; Wright et al. 1972).

***In vivo* antioxidant assay**

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.* (1993), Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as adopted in Randox kit, Catalase activity was assayed by the method of Sinha (1972) and glutathione peroxidase activity was done according to the method of Paglia and Valentine (1967).

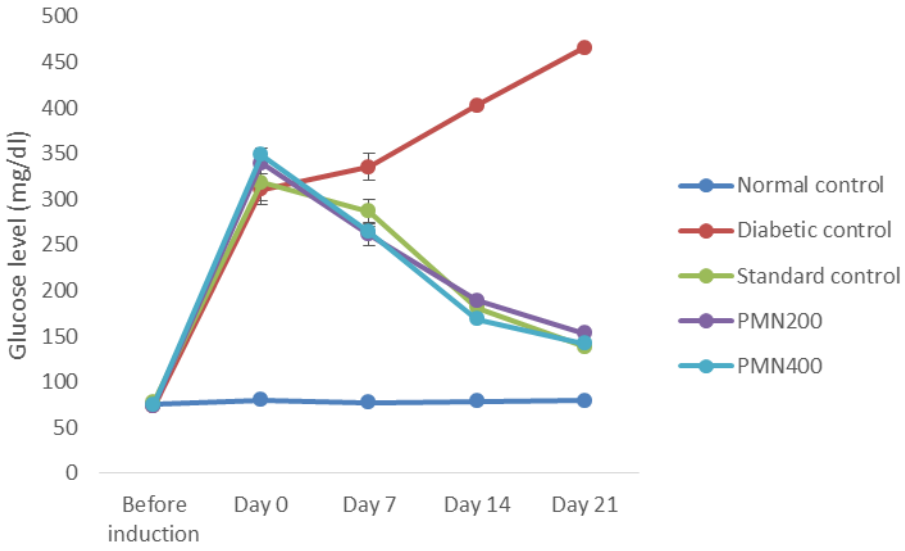
Statistical analysis

All statistical analyses were performed using SPSS version 20. The results are expressed as means±SEM (n = 6). A single-factor parametric one-way analysis of variance was used to determine significant differences among treatment means. Differences were separated using LSD test. A p value less than 0.05 was considered significant

RESULTS AND DISCUSSION

The acute toxicity of the formulation in rats recorded no mortality even at a high dose of 5000mg/kg body weight of the animal, thus LD_{50} could not be determined.

Table 1: Effect of the formulation (PMN) on blood glucose level in alloxan induced diabetes in rats.



The results are mean±SEM (n=6). PMN200 = 200mg/kg of combined *Pteridium aquilinium*, *Mucuna puriens*, *Newbouldia laevis* (1:2:1), PMN400 = 400mg/kg of combined *Pteridium aquilinium*, *Mucuna puriens*, *Newbouldia laevis* (1:2:1),

There was significant ($p<0.05$) decrease in blood glucose level of the treated groups compared to the diabetic control.

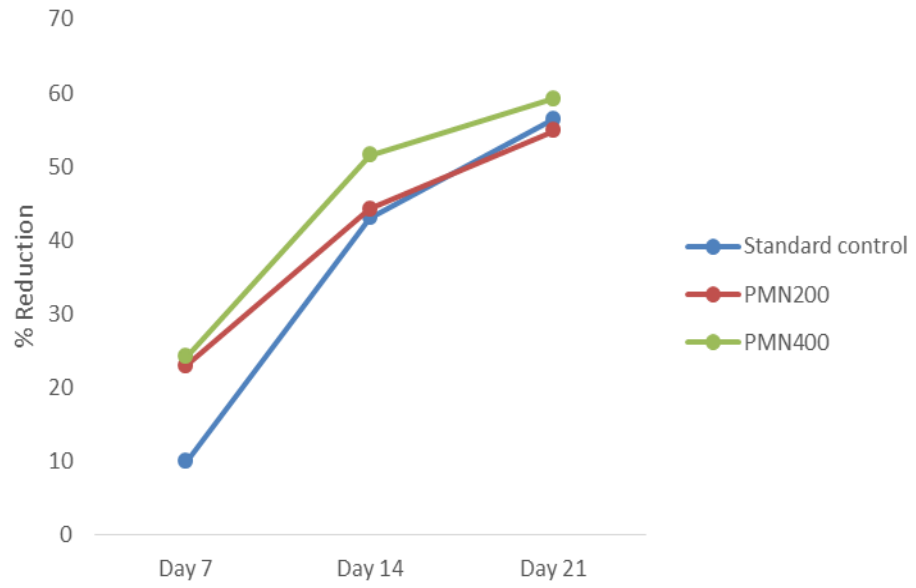


Figure 1: percentage reduction of blood glucose level by PMN in alloxan induced diabetes in rats.

The extract and standard drug showed significant percentage reduction in the elevated blood glucose level. The combined dose (PMN) at 400mg/kg showed highest percentage reduction (59.19%) compared to standard drug (56.45%).

Table 2: Effect of the formulation (PMN) on body weight in alloxan induced diabetes in rats.

Groups	Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21
1	Normal control	142.5±12.4^{b}	148.3±10.2^{a}	155.1±11.6^{ab}	163.4±11.9^{b}
2	Diabetic control	159.2±7.8^{a}	157.8±13.2^{a}	141.2±10.3^{b}	129.7±9.6^{c}
3	Standard control	148.2±9.9^{ab}	151.3±11.6^{a}	158.7±7.7^{b}	160.3±11.4^{b}
10	PMN200	165.8±10.7^{a}	167.4±11.5^{b}	172.3±9.2^{a}	176.2±10.5^{b}
11	PMN400	162.3±9.4^{a}	168.2±10.3^{b}	171.4±9.8^{a}	175.5±11.2^{a}

The results are mean±SEM (n=6). Different letters within each line shows significant ($p<0.05$) as determined by LSD range test.

There was significant ($p<0.05$) increase in body weight of the treated groups over the time period compared to the diabetic control. combined extracts at the doses of 400mg/kg has the highest increase in body weight.

Table 3: Effect of the formulation (PMN) on the different organ weight in alloxan induced diabetes in rats.

Groups	Treatment (mg/kg)	Liver (g)	Pancreas (g)	Lung (g)	Heart (g)	Kidney (g)
1	Normal control	0.78±0.11^{a}	0.21±0.06^{a}	0.12±0.05^{ab}	0.08±0.02^{a}	0.24±0.13^{a}
2	Diabetic control	1.47±0.25^{c}	0.52±1.19^{c}	0.19±0.03^{c}	0.12±0.04^{b}	0.38±0.36^{c}
3	Standard control	1.18±0.14^{b}	0.30±0.11^{b}	0.14±0.02^{b}	0.09±0.05^{a}	0.28±0.17^{b}
4	PMN200	0.79±0.13^{a}	0.31±0.16^{b}	0.13±0.04^{ab}	0.09±0.01^{a}	0.25±0.15^{a}
5	PMN400	0.7±0.15^{a}	0.27±0.08^{a}	0.09±0.02^{a}	0.08±0.03^{a}	0.21±0.09^{a}

The results are mean±SEM (n=6). Different letters within each row shows significant ($p<0.05$) as determined by LSD range test.

There was significant (<0.05) increase in the organs weight of the diabetic control. Combined graded doses of the extract (PMN) showed similar organs weight with the standard control. The formulation showed significant effect in maintaining the organs as the was non-significant different when compared with the normal control.

Table 4: Effect of the formulation (PMN) on the hematological indices in alloxan induced diabetes in rats.

Groups	Treatment (mg/kg)	Hb(g/dl)	WBC(x10 ^{9}/l)	RBC (10 ^{9}/l)	Palatetets (x10 ^{9}/l)
1	Normal control	18.22±0.33^{a}	10.27±1.12^{b}	9.02±0.35^{a}	787.8±15.60^{a}
2	Diabetic control	13.48±0.25^{b}	17.05±2.96^{d}	6.83±0.69^{b}	624.5±14.30^{c}
3	Standard control	17.86±0.44^{a}	9.92±1.65^{ab}	8.97±1.17^{a}	753.4±12.52^{b}
4	PMN200	17.85±0.51^{a}	9.94±1.62^{ab}	8.99±0.37^{a}	751.8±12.64^{b}
5	PMN400	18.11±0.23^{a}	8.65±1.18^{a}	9.23±0.47^{a}	769.5±12.30^{a}

The results are mean±SEM (n=6). Different letters within each row shows significant ($p<0.05$) as determined by LSD range test.

There was significant ($p<0.05$) decrease in the Hb, RBC and Platelets level of the diabetic control when compared with the treated groups. The significant increase in the WBC level of the diabetic control is an evidence of cellular toxicity. There was non-significant ($p>0.05$) different between FMN treated groups and the standard control.

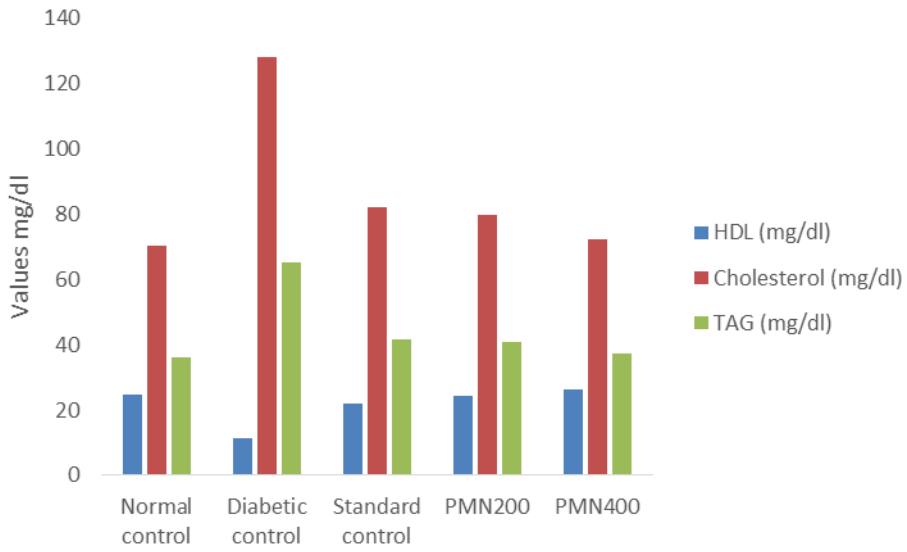


Figure 2: Effect of the formulation (PMN) on lipid profile level in alloxan induced diabetes in rats.

There was significant ($p<0.05$) increase in the TAG and Cholesterol with significant ($p<0.05$) decrease in the HDL of the diabetic control when compared with the treated groups. There was non-significant ($P>0.05$) difference in the HDL between combined graded doses and the standard control.

Table 5: Effect of the formulation (PMN) on the biomarkers of cellular toxicity in alloxan induced diabetes in rats.

Groups	Treatment (mg/kg)	ALP (u/l)	AST (u/l)	ALT (u/l)	Albumin (g/l)	Total bilirubin (g/l)	Creatinine (mmol/l)	Urea (g/l)
1	Normal control	142.3± 13.6^{a}	92.6± 2.8^{a}	46.0± 4.3^{a}	30.9± 1.08^{a}	7.8±1. 02^{a}	62.8±2. 13^{a}	5.2±0. 38^{b}
2	Diabetic control	328.5± 11.2^{c}	167.4± 1.6^{d}	186.3± 11.2^{e}	27.4± 0.82^{b}	22.0±3. 8^{c}	96.2±2. 09^{c}	16.6±2. 84^{c}
3	Standard control	189.2± 15.3^{b}	124.3± 2.9^{c}	102± 15.3^{d}	30.3± 0.50^{a}	10.6±0. 86^{b}	74.3±5. 34^{b}	4.8±0. 15^{ab}
10	PMN200	176.6± 11.4a^{b}	112.3± 2.3^{b}	89.5± 3.6^{c}	30.2± 0.8^{a}	10.3±1. 25^{b}	71.2±4. 8^{b}	5.4±0. 56^{b}
11	PMN400	158.2± 14.6^{a}	94.2± 3.3^{a}	76.7± 2.3^{b}	30.7± 1.2^{a}	8.4±1. 07^{a}	67.5±3. 12^{a}	4.2±0. 19^{a}

The results are mean±SEM (n=6). Different letters within each row shows significant ($p<0.05$) as determined by LSD range test.

The diabetic control showed significant ($p<0.05$) increase in the kidney and liver function parameters compared to the treated groups. 200mg/kg of the combined extracts (PMN) was

found to have equivalent potency with the standard control. The 400mg/kg of the combined extracts has more potency than the standard control.

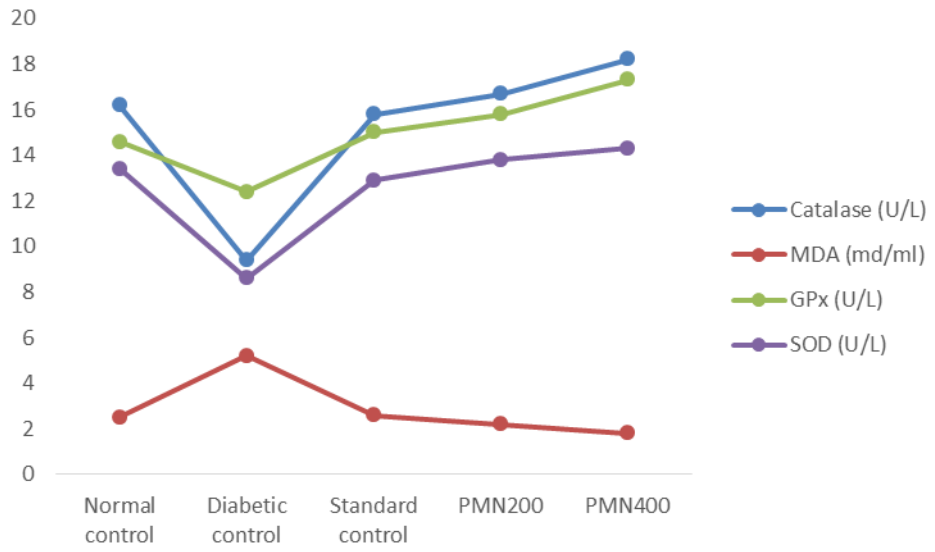


Figure 4: effect of individual and combined graded doses of *Mucuna puriens*, *Newbouldia laevis* and *Pteridium aquilinum* on the *in vivo* antioxidant level in alloxan induced diabetes in rats.

The diabetic control showed oxidative stress induced by alloxan which was evident in its significant ($p<0.05$) increase in the MDA and decrease in SOD, GPx and catalase when to the treated groups. The PMN showed great ability to maintain the endogenous antioxidant enzymes and decrease MDA level with equivalent potency with the standard control.

DISCUSSION

Diabetes is one of the endocrine disease that interfere with the rate at which the body metabolizes carbohydrate for energy, resulting to elevation in blood glucose level which can lead to the risk of developing serious health challenges. The antidiabetic properties of three medicinal herbs in combination were evaluated in this study. The individual hypoglycemic potency of the three selected plants has been evaluated in our previous works and by many authors from literature.

Herbal medicines have been recommended for the treatment of diabetes as a result of their less toxicity and fewer side effect than synthetic drug. The combination of herbal plants known as herbal formulation is a vital area of interest because it enhance therapeutic action and decrease the concentration of individual herbs. (Gloria and Anthony, 2015). The level of

toxicity of the herbs used in this study were well tolerated as there was no mortality at the highest dose used.

This study revealed that the combination (PMN) reduced the fasting blood glucose level in alloxain induced diabetes in rats similar to glibeclamide (Table 1 and fig1). Insulin enhances the rate at which glucose is transported into the muscle cell of animal and facilitates glucose utilization (Mohammed and Mutassium, 2016). The restoration of insulin output or insulin utilization is the basis for the lowering of blood glucose level in diabetes and antidiabetic drugs are known to posses such insulin restoration ability (Neelish et al., 2010). This is suggested to be the mechanism by which the extracts and their formulation decreased the elevated blood glucose level with the combination/formulation having the highest % reduction in blood glucose level.

There is high level of tissue wasting or weight loss in diabetes mellitus and the assessment of body weight is an important tool used in diabetes study to monitor response to treatment (Mayfield, 1998). There was decrease in body weight in the diabetes control but the combination of extract aid in weight gain (table 2).

The deleterious impact of foreign compound on blood compositions of animals can effectively be assessed using hematological parameters. There is a close association between anaemia and diabetes (Akindele et al., 2012; Colak et al., 2014). This statement was evident in the reduced Hb and RBC indices and increase in WBC found in the diabetic control of this study (table 4) contrary to the treated rats. The combined extract at the dose of 200mg/kg and 400mg/kg showed to be more potent in restoring RBC, Hb and Palatetets. Jarald et al. (2008) reported palatetets aggregation in diabetic patients with prolong reduced glucose level. The increase in palatelets by the glibeclamide and the extracts both individual and combined form indicated that the extracts could stimulate the synthesis of clothing factor for blood clothing.
It is of no doubt that diabetes is commonly associated with abnormalities in lipid profile and there is perfect coexistance between hyperglycemic and dyslipidemia in diabetic patients (Gloria and Anthony 2015). There was increase in cholesterol and TAG with decrease in HDL in the diabetic control. The extract and glibeclamide reduced cholesterol and TAG and increase HDL with the formulation showing more potency (fig 3).

There is always leakage of biomasker of cellular toxocity in health challenged patient and liver and kidney markers are release into the blood when damage occurs which could be

possible in diabetes (Edet et al., 2011). There was increase in ALP, AST, ALT total bilirubin, creatinme and urea in the diabetic control whereas the extract reduced the elevated biomarkers with the combined extract reducing the elevation to almost normal as shown in table 5.

There is increase level of MDA which is a marker for lipid peroxidation and decrease in catalase, GPX and SOD in the diabetic control unlike the treated animal where there was restoration and prevention of depletion of endogenous antioxidant with decrease MDA. Increase production of free radicals which mainfest as a result of oxidative stress is associated with diabetes mellitus and modulating oxidative stress and insulin resistance is essential therapeutic strategies for diabetes (Vijayalingam et al., 1996).

CONCLUSION

There was metabolic alteration leading to elevation of several biomarker of cellular toxicity and increase blood glucose level in the diabetic control of this study whereas the combination of extract (PMN) restored all the alteration with the 400mg/kg PMN having the highest potency. This study recommends usage of formulated herbal mixture in appropriate proportion for the treatment of diabetics mellitus.

Conflict of interest

The authors declare that no conflict of interest exists with respect to this work

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Authors contributions

Unegbu C.C. designed and supervised the work and did final correction on the manuscript. Onwusonye JC and Ajah O carried out the Laboratory analysis of the parameters analyzed.

Anyanwu O.O. carried out animal proper handling, Duru CA. carried out plant sampling and preparation. All the Authors wrote and read the manuscript.

REFERENCES

1. Akindele OA, Babatunde AI, Chinedu FM, Samuel OA, Oluwasola CA, Oluseyi AA. Rat model of food induced nonobese-type 2 diabetes mellitus; comparative pathophysology and histopathology. Intern J Physiol, Pathophysiol Pharmacol, 2012; 4: 51–58.
2. Arthur, J.R. and Boyne, R. Super oxide dismutase and glutathione-peroxides activities in neutrophils from selenium deficient and copper deficient cattle. *Life Sciences*, 1985; 36: 1569-1575.
3. Colak S, Geyi Koghu F, Aslan A, Deniz GY. Effffects of lichen extracts on haematological parameters of rats with experimental insulin-dependent diabetes mellitus. Toxicol Ind Health, 2014; 30: 878–887.
4. Dacie, J. V. and Lewis S. M. *Practical Haematology* ELBS with Churchill Livingstone. Longman group U. K, 1991; 7: 5-82.
5. Edet EE, Atangwho IJ, Akpanablatu MI, et al. Effect of Gongronema latifolium leaf extract on some liver enzymes and protein levels in diabetic and non-diabetic rats. J Pharm Biomed Sci, 2011; 1: 104–7.
6. Edwin E, Sheeja E, Dhanabal SP, Suresh B. Antihyperglycaemic activity of Passiflora mollisima Baily. Indian J Pharm Sci, 2007; 64: 570–1.
7. George, G.S and Uwakwe, A.A. hypolycemic properties of some local herbs ectracts in streptozotocin induced diabetic wister albino rats: 10SR: Journal of Dental and Medical Sciences, 2014; 13(1): 01-07.
8. Gloria, A.O and Anthony, J.A. antidiabetikc effect of combined spices of Allium sativa. Zingiber officinale and capsicum frutescent in alloxan induced diabetic rats. Frontiers in life science, 2015.
9. Hans, H.P.K older people are act highest risk frolm COVID-19 but all must act to prevent community spread: a statement, WHO reginal office for Europe, 2020. www.euro.who.int retrieved 14/04/2020.
10. Jain, N. C. *Schalm's veterinary Haematology*, Lea and Fabriger, Philadelphia, 1986; 4: 564 – 572.
11. Jarald E, Joshi SB, Jain DC. Diabetes and herbal medicines. Iran J Pharmacol Ther, 2008; 7: 97–106.

12. Lorke, D. A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, 1983; 55: 275 -287.
13. Majekodunmi SO, Adegoke OA, Odeku OA. Formulation of the extract of the stem bark of Alstonia boonei as tablet dosage form. Trop J Pharm Res, 2008; 7(2): 987-994.
14. Malviya, N., Jain, S. and Malviya, S. Antidiabetic potential of medicinal plants. *Acta Pol Pharm*, 2010; 67(2): 113–118.
15. Maxwell I.E., Aruh, O.A and Isaac, U.A antidiabetic, antilipidemic and antioxidant activities of Gouania longipetala methanol leaf extract in alloxan induced diabetic rats. Pharmacentical biology, 2015; 53(4): 605-614.
16. Mayfield J. Diagnosis and classification of diabetes mellitus; new criteria. Am Fam Phys, 1998; 58: 1355–62.
17. Mohammed, M.U. and Mutassim, M.A. Role of insulin and other related hormones in energy metabolism: A review cogent food and Agriculture, 2016; 2(1): 1267591.
18. Neelish M, Sanjah J, Sappa M. Antidiabetic medical plants. Acta Pol Pharm Drug Res, 2010; 67: 113–18.
19. Owolabi, O.J., Amaeclina, F.C and Okoro, M. Effect of ethanol leaf extract of newbouildia laevis on blood glucose level of diabetic rats. Tropical journal of pharmaceutical research, 2011; 10(3): 249-254.
20. Paglia, D. E. and Valentine, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 1967; 70: 158-169.
21. Patel, I.K., Prasad, S.K. Kumar, R. and Hemalatha, S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*, 2012; 2(4): 320—330.
22. Raja, S.B., Sameh, K., Wissem, A.W., Khaoula, A. and Riadh, K. Antidiabetic, antihyper lipemic and antioxidant influence of the sp;ice cinnamon (Cinnamonum Zeylanicumon) in experimental rats. Brazilian journal of pharmacentical sciences, 2018; 54(2): e17576.
23. Sinha, A. Colorimetric Assay of Catalase. *Analytical Biochemistry*, 1972; 47: 389-394.
24. Stephen, O.M., Ademole A.O., SOlomon, U. and Ohiowatoyin A.O. evaluatikon of the anti-diabetic properties of mucuna purens seed extract. Asian pacific journal of tropical medicine, 2011; 632-636.
25. Sunmonu TO, Afolayan AJ. Evaluation of antidiabetic activity and associated toxicity of Artemisia afra aqueous extract in Wistar Rats. Evid based Complement Altern Med, 2013. doi:10.1155/2013/929074

26. Tietz NW, Prude EL, Sirgard-Anderson O. *Textbook of clinical chemistry*. London, UK: WB Saunders, 1994.
27. Tietz NW. *Clinical guide to laboratory tests*. Philadelphia, PA, USA: WB Saunders, 1995; 3.
28. Vijayalingam S, Parthiban A, Shanmugasundaram KR, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. Diabetic Med, 1996; 13(8): 715-719.
29. Wallin, B., Kosengreen, B., Shertzer, H.G. and Camejo, G. Lipoprotein oxidation and measurement of TBARS formation in a single microlitre plate; Its use for evaluation of antioxidants. *Journal of Analytical Biochemistry*, 1993; 208: 10-15.
30. Wright PJ, Leatherwood PD, Plummer DT. Enzymes in rat urine: alkaline phosphatase. Enzymologia, 1972; 42: 317–327.
31. Wroblewski F, La Due JS. Serum glutamic pyruvic transaminase SGP-T in hepatic disease: a preliminary report. Ann Intern Med, 1956; 45: 801–811.