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Effect of Pawpaw Seeds on Management of Alloxan-Induced-Diabetes in Rats

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Abstract

Ethanol extract of pawpaw seed was evaluated for a possible application in the management of diabetes. Alloxan was used to induce diabetes in rats, which were subsequently used to study the antihyperglycaemic effect of the extract. Four (4) treatment groups and two (2) control groups of 8 rats each were created using complete randomized design. Triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP), glutamic oxaloacetic transaminase (SGOT/AST), glutamic pyruvic transaminase (SGPT/ALT), alkaline phosphatase (ALP), urea and creatinine were studied using standard methods. A single dose of alloxan resulted in a significant increase ($p < 0.05$) in the serum TG, TC, LDL, AST, ALT, ALP, with a corresponding decrease in serum HDL, urea and creatinine. Following treatment with varying doses of the extract, there was a decrease in serum TG, TC, LDL, AST, ALT, ALP, and an increase in serum HDL, urea and creatinine. It could be inferred from the research outcome that crude extract of pawpaw seed (200 mg/kg extract) could be effective in the management of diabetes.

Keywords: pawpaw seed, diabetes, alloxan, insulin resistance, glibenclamide

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Introduction

Diabetes is a metabolic disease with features of hyperglycemia due to the deficiency of secretion or action of endogenous insulin (Alberti and Zimmet, 1998). It is a multifactorial disorder with no definite cause, and characterized by disturbances in the metabolism of carbohydrate, fat and proteins accompanied by long lasting complications which eventually leads to severe damage to the kidney, retina and many of the body's system, especially the blood vessels and the nervous system (Paik *et al.,* 1982; Taher *et al.,* 2016). This disease has been cited by Witting *et al.* (2011) as the fourth leading cause of hospitalization and death per year. Diabetes prevalence rate in Nigeria is about 94.3 % (Gojka, 2016) and this calls for greater attention.

Insulin therapy is mostly the common method for the management of diabetes. Nevertheless, regular intake of insulin and other synthetic drugs usually lead to certain drawbacks, such as fatty liver, insulin resistance, anorexia nervosa and brain damage (Piedrola *et al.,* 2001; Nissen and Wolski, 2007). These conditions have resulted in diabetologists seeking for other possible alternatives, especially supplements consisting of plant extracts for the optimization of the treatment of diabetes. Plant therapies are being produced because of the many secondary metabolites they contain (Twaij and Hasan, 2022). These metabolites, such as phenols and flavonoids, have been reported by researchers to possess antioxidant potentials, mopping up reactive oxygen species released as a result of oxidative stress induced in diabetic conditions (Shah and Khan, 2014; Umerie and Ekuma, 2016; Ubaoji *et al.,* 2019.

Pawpaw (*Carica papaya*) is herbaceous, belongs to the family of Caricaceae and native of the Americas (Daagema *et al.,* 2020). Currently, pawpaw is grown in many tropical and subtropical regions of the world, Africa inclusive, mainly due to their fruits. Studies have shown that this plant contains many vital minerals such as vitamin A, vitamin B, vitamin C, niacin, calcium, potassium, sodium, papain and chymopapain (Snake and Desmond, 1997; Aravind *et al.,* 2013; Oloruntola, 2019). Pawpaw seeds and leaves have also been reported to contain phenols and flavonoids (Maisarah *et al.,* 2014). Pharmacological studies review that the seeds and leaves of pawpaw possess nutraceutical and antioxidant properties (Kadiri *et al.,* 2015; Oloruntola, 2019)

Materials and Methods

Collection and preparation of plant samples

The seeds of *C. papaya* were collected from plants growing in Abakaliki, the capital of Ebonyi State, Nigeria during the rainy season. The fresh and clean seeds were air-dried under a shed. The samples were pulverized using pestle and mortar

The ethanol extraction was done using the Soxhlet apparatus to obtain about 0.5 kg of extract (Patel *et al.,* 2019). Fifty grams (50 g) of the powdered pawpaw seeds sample was measured using electronic weighing balance (Digital Precision Weighing Balance made in India), wrapped using whatman filter paper 46 x 57 cm, model 1001-917 Made in India and inserted in the tube of the soxhlet apparatus. A 250 ml of 70 % ethanol was poured inside the round bottom conical flask of the apparatus and was heated at 70 ^{0}C and extracted for 3 hours. The remaining ethanol in the extracts was evaporated to get crude extract. This was done in vacuum using a rotary evaporator (model Scale, REV200-P USA)

Experimental animals

A total of forty-eight (48) adult albino Wistar rats (male; weighed 150 g – 250 g; aged 16 – 19 weeks) were used for this experiment. The animals, upon arrival, were left to acclimatize for 7 days in the animal house of the Faculty of Science, Ebonyi State University, Abakaliki, Nigeria. The animals were randomly assigned to 6 groups of 8 rats, left under good laboratory conditions, and maintained under standard pellet diet and water *ad libitum*.

Acute toxicity test

This was evaluated using the up-and-down method of acute toxicity as described by the Organisation for Economic Co-Operation and Development OECD, (2008). Six (6) rats were randomly selected, weighed and divided into test and control groups of 3 rats each. The controls were vehicles, while the tests were given 2,000 mg/kg of the extract. Both groups were made to fast overnight before oral treatment of pawpaw seed through intraperitoneal injection and observed for a period of 48 hours.

Experimental design

Six treatment groups of 8 rats each were created using complete randomized design as shown Table 1. Each treatment group was allowed access to feed and water. Freshly prepared alloxan monohydrate was used to

induce diabetes in the rat as described by Sheriff *et al.* (2020). Day 1 of treatment started the third day following a successful injection of the alloxan and lasted for a period of four weeks.

Table 1: Different treatment groups

Group	Treatment
Group 1	Normal control; Not induced and received vehicle only
Group 2	Diabetic control; Infected with diabetes but received vehicle only
Group 3	Standard drug; Infected with diabetes and received 0.5 mg/kg glibenclamide
Group 4	Diabetic + 50 mg/kg extract
Group 5	Diabetic + 100 mg/kg extract
Group 6	Diabetic + 200 mg/kg extract

Groups 1 – 3 were the control groups, while groups 4 – 6 served as the study groups. Alloxan and extracts were administered to the rats through a single dose of intraperitoneal injection.

Blood collection

On the 29th day of the experiment, overnight fasted rats (16 h) were sacrificed by cervical decapitation as contained in the ethical norms of Ebonyi State University, Abakaliki. The blood flowing from the trunk was collected in plasma separation tubes, containing a clot activator. The collected blood was centrifuged at 2,000 x g for 10 min in a refrigerated centrifuge Beckman Coulter GS-15R USA at 4 ^{0}C to remove the blood clot. The resulting supernatant (serum) was stored in a polypropylene tube at 4 ^{0}C prior to analysis (Taher *et al.,* 2016).

Biochemical analysis

The serum level of triglyceride (TG) was determined using method by Bucolo and David (1973), total cholesterol (TC) according to method by Allain *et al.* (1974), high density lipoprotein (HDL) according to method by Lund-Katz *et al.* (2003), low-density lipoprotein (LDL) according to method by Nauck *et al.* (2002) and total protein (TP) as described by Dawnay *et al.* (1991). Kits were used to determine creatinine (Elabscience® Creatinine Assay Kit India), SGOT/AST, SGPT/ALT, ALP, as well as urea as described by Shah *et al.* (2013).

Statistical analysis

Analysis of variance (ANOVA) for Completely Randomized Design (CRD) was carried out using Statistical Package for Social Sciences (SPSS). A p-value of less than 0.05 ($p < 0.05$) was used to determine statistical significance.

Results

As shown in Figure 1, group 2 (diabetic control) showed a constant increase in blood glucose level from day 1 till day 28, although there was no significant difference ($p < 0.05$) in the values recorded. The blood glucose level for group 2 was significantly higher ($p<0.05$) than the blood glucose level of group 1 throughout the experiment. Again, it was observed that treatment with 0.5 mg/kg glibenclamide and the plant extracts resulted in a gradual and significant ($p<0.05$) decline in blood glucose.

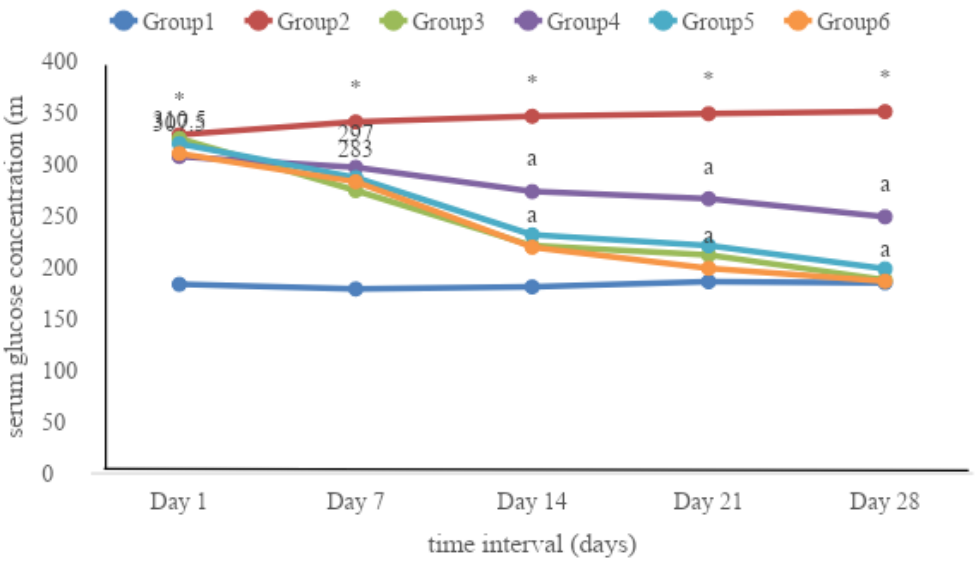

Figure 1: Effect of the extract on the blood glucose level in diabetic rats**.**

*Significantly different ($p<0.05$) when compared to the normal control (Group 1). [a]significantly different ($p<0.05$) when compared to the corresponding values of the negative control (Group 2)

Again, the findings presented in Figure 2 show clear trend of significant ($p<0.05$) increase in the level of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) in the negative control group (group 2) when compared to the normal control (group 1), whereas, pretreatment with glibenclamide and the extracts resulted in significant ($p<0.05$) decrease in TC, TG and LDL.

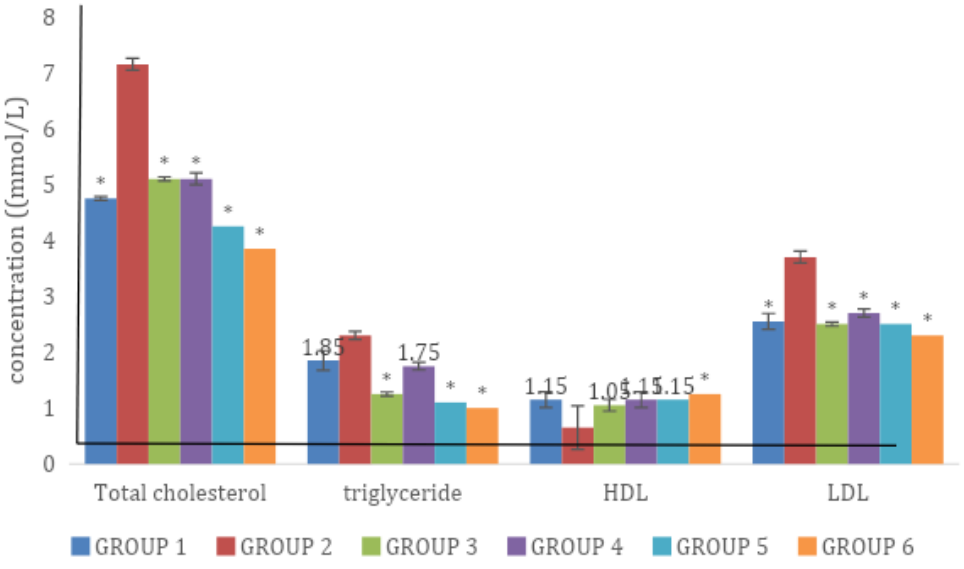

Figure 2: Effect of the extract on serum lipid profile of the rats.

*Significant difference ($p<0.05$) when compared to the negative control (Group 2).

Again, diabetes leads to an increased serum ALP, ALT. and AST as shown in Table 2. The ALP and ALT values recorded in group 2 (diabetic control) were observed to be significantly ($p < 0.05$) higher compared to the normal control. The group pretreated with 200 mg/kg extract produced serum ALP and ALT levels of 44.5 ± 0.75 U/L and 2.5 ± 0.58 U/L respectively. These values for ALP and ALT observed in group 6 were significantly lower than the values reported for group 3 (standard drug). No significant difference ($p < 0.05$) was recorded in the serum AST levels across all groups except for the group which had an ALT value that was significantly ($p < 0.05$) low.

Table 2: Effect of the extract on liver functions

GROUPS	ALP (U/L)	ALT (U/L)	AST (U/L)
Group 1	49.5^{b}	5^{bc}	2^{ab}
Group 2	54^{a}	11^{a}	4.5^{a}
Group 3	45.5^{bc}	7^{b}	2.5^{ab}
Group 4	50.5^{ab}	3.5^{bc}	2.5^{ab}
Group 5	47.5^{bc}	3.5^{bc}	2^{ab}
Group 6	44.5^{c}	2.5^{c}	1.5^{b}
SEM	0.75	0.58	0.33

Different letters indicate significant differences down the column ($p < 0.05$), where a > b > c Values are presented as mean±SEM. Key: SEM is Standard Error of Mean, ALP is Alkaline Phosphatase, ALT is Alanine Transaminase, AST is Aspartate Aminotransferase

In addition, renal function was ascertained by determining the serum urea and creatinine levels of the rats (Table 3). Alloxan-induced diabetic rats (group 2) recorded lower levels of urea and creatinine. The serum creatinine observed in group 2 (diabetic control) was significantly ($p < 0.05$) than the creatinine levels of the normal control. Upon pretreatment with glibenclamide and the extract, the urea and creatinine levels were restored to normal ranges. The highest values for urea and creatinine were recorded in the group pretreated with 200 mg/kg extract.

Table 3: Effect of the extract on serum urea and creatinine levels of the rats

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)
Group 1	4.45^{a}	72^{b}
Group 2	4.05^{a}	54.5^{c}

Group 3	5.15[a]	71.5[b]
Group 4	4.3[a]	65.5[b]
Group 5	4.45[a]	69[b]
Group 6	5.25[a]	89[a]
SEM	0.12	2.92

Different letters indicate significant differences down the column ($p < 0.05$), where a > b > c Values are presented as mean±SEM. Key: SEM is Standard Error of Mean.

Finally, the serum total protein (TP) decreased in diabetic control group (group 2) when compared to the normal control (group 1) (Figure 2). Pretreatment with the extract produced serum TP values that were not significantly different ($p \geq 0.05$) from the serum TP values observed in the groups treated with the standard drugs. Only the group treated with 200 mg/kg produced serum TP value was not significantly different ($p \geq 0.05$) from the normal control group.

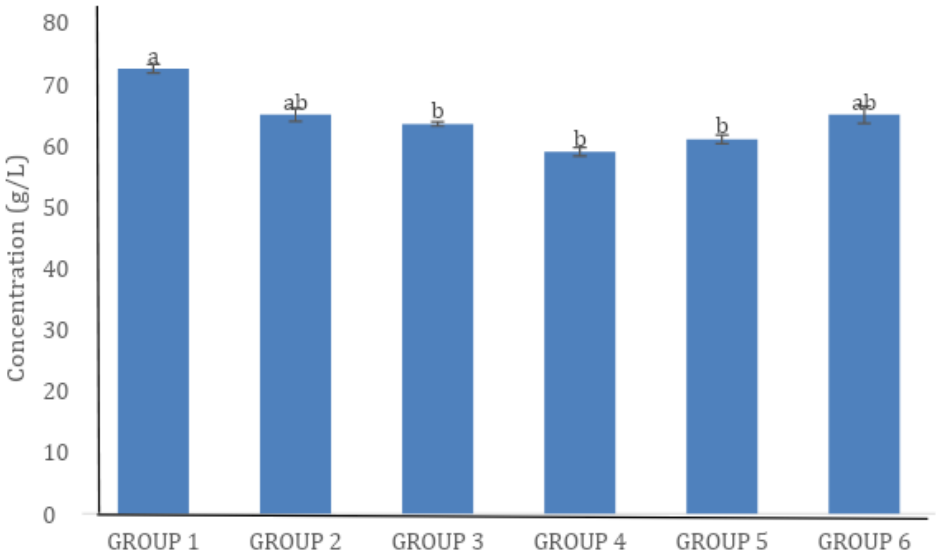

Figure 3: Effect of the extracts on serum total protein concentration of the rats

Discussion

Diabetes mellitus is a major metabolic disorder influencing most of the world population. Studies have shown that orthodox hypoglycemic agents such as sulfonylureas, thiazolidinediones and biguanides have failed to successfully provide the desired prolonged glycaemic control effect (Rasineni *et al.,* 2010;

Shah and Khan, 2014; Taher *et al.,* 2016). Consequently, plants have been resorted to in the treatment of diabetes because of their safety, effectiveness, affordability and the many phytochemicals contained in them with proven therapeutic effects (Oko, 2016).

Sequel to the above, the hypoglycaemic effect of ethanol extract of *C. papaya* seed in alloxan diabetic-induced rats was highlighted in this study. This was done by measuring the blood glucose level at different intervals and measuring various blood biochemical parameters. As expected, alloxan diabetic-induced rats showed some visible symptoms of diabetes such as frequent urination, constant thirst and hunger and high blood glucose level that was significantly different ($p < 0.05$) from the group that was not induced with diabetes (group 1) as seen in figure 1.

Alloxan induced diabetes through the destruction of the β-cells of the pancreas responsible for insulin secretion, thus, leading to hypoinsulinemia and subsequent hyperglycaemia (Zanatta *et al.,* 2007). Thus, the impact of glibenclamide and the extract on blood glucose, observed in this study, may have resulted from a possible upgrade of the activities of β-cells of the pancreas for a sufficient release of insulin. So, the extract might have led to the regeneration of the alloxan-destructed β-cells, which is probably because the pancreas contains quiescent cells that have the ability of regeneration (Banerjee *et al.,* 2005; Cano *et al.,* 2008).

Again, an increased risk of the development of atherosclerosis and coronary heart diseases, considered secondary complications of diabetes, is marked by the elevated levels of cholesterol, triglyceride and low-density lipoprotein in the serum (figure 2) (Bai, 2003; Kasetti *et al.,* 2010). Therefore, it is apparent from this study that glibenclamide and the extract administration, which led to reduction in TC, TG, LDL and increase in HDL, compared to the diabetic control, might be said to have ameliorated the damaging effect of alloxan on the lipid profile,(figure 2) thus, contributing to the reduction of atherogenic-related complications such as atherosclerosis and coronary heart infections.

The liver function test shown in Table 2 provides vital information on the liver activities, which include the ALP, ALT and AST (Ohaeri, 2001). An increased level of these enzymes in the serum, as seen in the diabetic control group, is an indication of liver injury, thus, showing spillage of these enzymes from the liver cytosol into the bloodstream (Shah and Khan, 2014). Nevertheless, treatment with glibenclamide and the extracts resulted in a significant decrease ($p<0.05$) in the serum concentration of these enzymes. The effect of the extract on the liver enzymes was in a dose-dependent manner, with the highest impact observed in the group given 200 mg/kg of the extract (group 6) (Table 2). This agrees with the "therapeutic window" phenomenon (Zakaria *et al.,* 2011), which is possibly related to the ability of the extracts to reduce the activities of the enzymes or to the optimal hepatoprotective effect of the extracts.

Again, low levels were witnessed in the diabetic control group (Table 3) compared to the normal group. This is in line with the conclusion of Harita *et al.* (2009) in their research that lower serum creatinine increases the risk of diabetes. Nevertheless, the extract was able to cause a significant ($p < 0.05$) increase in creatinine level, (Table 3) thereby restoring normal renal function. This finding is in line with earlier literature that diabetes can lead to renal dysfunction (Taher *et al.,* 2016).

Conclusion

Based on the foregoing, it could be concluded that ethanol extract of *C. papaya* seed produces hypoglycaemic effect. This was deduced from the observed concentration dependent-effects of the extract on total cholesterol, triglyceride, total protein, low density lipoprotein, high density lipoprotein, urea, creatinine and liver enzymes. This antihyperglycemic potential of the extract could be attributed to the many

phytochemicals that have been reported by scientists for this plant. Therefore, it is worthy to note that further studies are recommended to ascertain the exact mechanism of action of the extract in its antidiabetic property.

Ethics and animal welfare

Animal care and procedures were performed following the guidelines of good experimental practices according to the Code of Practice for Housing and Care of Animals Used in Scientific Procedures, approved by Faculty of Science, Ebonyi State University, Abakaliki Nigeria. Note: this manuscript does not contain clinical studies or patient data.

References

Alberti, K. G. and Zimmet, P.Z (1998). New diagnostic criteria and classification of diabetes-again. *Diabet. Med.* 15: 535 – 536.

Allain, C.C, Poon, L.S, Chan, C.S.G, Richmond, W and Fu, P.C (1974). Enzymatic Determination of Total Serum Cholesterol *Clinical Chemistry*, 20(4):470–475.

Aravind, G., Debjit, B., Duraivel, S. and Harish, G. (2013). Traditional and medicinal uses of *Carica papaya*. *J. Med. Plants Studies.* 1(1): 7 – 15.

Bai, V. N. (2003). Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. *J. Diabetes Res.* 4: 183 – 189.

Banerjee, M., Kanitkar, M. and Bhonde, R. R. (2005). Approaches towards endogenous pancreatic regeneration. *Rev. Diabet. Stud.* 2: 165 – 176.

Bucolo, c. and David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem*. 19(5): 476 – 482.

Cano, D. A., Rulifson, I. C., Heiser, P. W., Swigart, L. B., Pelengaris, s. and German, M. (2008). Regulated β-cell regeneration in the adult mouse pancreas. *Diabetes*. 57: 958 – 966.

Daagema, A. A., Orafa, P. N. and Igbua, F. Z. (2020). Nutritional potential and uses of pawpaw (*Carica papaya*): a review. *Eur. J. Nutr. Food Safety.* 12(3): 52 – 66.

Dawnay ABS, Hirst AD, Perry DE, Chambers RE. A Critical Assessment of Current Analytical Methods for the Routine Assay of Serum Total Protein and Recommendations for Their Improvement. *Annals of Clinical Biochemistry*. 1991; 28(6):556-567. doi:10.1177/000456329102800604

Gojka, R. (2016). WHO global report on diabetes: a summary. *Int. J. Non-Commun. Dis*. 1:1-11

Harita, N., Hayashi, T., Sato, K.K., Nakamura, Y., Yoneda, T., Endo, G., et al. (2009) Lower Serum Creatinine Is a New Risk Factor of Type 2 Diabetes. *Diabetes Care*, 32: 424-426

Kadiri, M., Ojewumi, A. W., Agboola, D. A. and Adekunle, M. F. (2015). Ethnobotanical survey of plants used in the management of diabetes mellitus in Abeokuta, Nigeria. *J. Drug Devl. Therapue*. 5(3): 13 – 23.

Kasetti, R. B., Rajasekhar, M. D., Kondeti, V. K., Fatima, S. S., Kumar, E. g., Sirasanagandla, S., Ramesh, B. and Apparao, C. (2010). Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food Chem. Toxicol*. 48(4): 1078 – 1084.

Lund-Katz, S., Liu, L., Thuahnai, S. T. and Phillips, M. C. (2003). High density lipoprotein structure. *Front. Biosci*. 8: d1044 – d1054.

Maisarah, A. M., Asmah, R. and Fauziah, O. (2014. Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica papaya*. *J. Nutr. Food. Sci*. 4(2): 267 – 274.

Nauck, M., Warnick, G. R. and Rifai, N. (2002). Methods of measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin. Chem*. 48(2): 236 – 254.

Nissen, S.E. and Wolski, K. (2007). Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *New England Journal of Medicine*, 356: 2457-2471.

Ohaeri, O. (2001). Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci. Rep.* 21: 19 – 24.

Oko, A.O., (2016). Evaluation of the Effect of *Albizia zygi*a (Stem Bark and Leaf) Extracts on the Glycemic Index of Rice. *African Journal of Basic & Applied Sciences* **8** (6): 361-366.

Oloruntola, O. D. (2019). Effect of pawpaw leaf and seed meal composite mix dietary supplementation on haematological indices, carcass traits and histological studies of broiler chicken. Bulletin Nat. *Res. Centre*. 43: 129 – 141.

Organization for Economic Cooperation and Development. (2008). OECD guidelines for the testing of chemicals, acute oral toxicity: up-and-down procedure No. 425. Paris: Available from: https://www.oecd-library.org/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure_9789264071049-en. (Accessed: 22nd May, 2021)

Paik, S. G., Blue, M. L., Fleischer, N. and Shin, S. I. (1982). Diabetes susceptibility of BALB/cBOM mice treated with streptozotocin: inhibition by lethal irradiation and restoration by splenic lymphocytes. *Diabetes*. 31(9): 808 – 815.

Patel, K., Panchal, N. and Ingle, P. (2019). Review of extraction techniques, extraction methods: microwave, ultrasonic, pressurized fluid, Soxhlet extraction, etc. *ACE J. Adv. Res. Chem. Sci.* 6(3): 6 – 21.

Piedrola, G., Novo, E., Escobar, F. and Garcia-Robles, R. (2001). White blood cell count and insulin resistance in patients with coronary artey disease. *Ann. Endocrinol* (Paris), 62: 7 – 10.

Rasineni, K., Bellamkonda, R., Singareddy, S. R. and Desireddy, S. (2010). Antihyperglycemic activity of *Catharanthus roseus* leaf powder in streptozotocin-induced diabetic rats. *Pharmacognosy Res.* 2(3): 195 – 201.

Shah, N. A. and Khan, M. R. (2014). Antidiabetic effect of *Sida cordata* in alloxan induced diabetic rats. *BioMed Res. Intern.* 2014: 1 – 15.

Shah, N. A., Khan, M. R. Ahmad, B., Noureen, F., Rashid, U. and Khan, R. A. (2013). Investigation on flavonoid composition and anti-free radical potential of *Sida cordata. BMC complemen. Altern. Med.* 13(1): 276.

Sheriff, O. L., Olayemi, O., Taofeeq, A. O., Riskat, K. E., Ojochebo, D. E. and Ibukunoluwa, A. O. (2020). A new model for alloxan-induced diabetes mellitus in rats. *J. Bangladesh Soc. Physiol,* 14(2): 56 – 62.

Snake, J. and Desmond, L. (1997). Cooking with papaya. Kentucky State University Cooperative. Extension program guide. 129atwood Res., Frankfort, K. Y. 40601 – 42355.

Taher, M., Zakaria, M., Susanti, D. and Zakaria, Z. (2016). Hypoglycemic activity of ethonlic extract of *Garcinia mangostana* Linn. In normoglycaemic and streptozotocin-induced diabetic rats. *BMC Complemen. Altern. Med.* 16:135-147.

Twaij, B.M and Hasan, M.N (2022). Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses *Int. J. Plant Biol.* 2022, *13*(1), 4-14

Ubaoji, K., Nwozor, K., Anaetoh, C., Ekuma, E., Ineh, M., Adibe, C., Nmoye, P., James, C., Nwaokike, G., Udechukwu, O. and Alaekwe, I. (2019). A biochemical evaluation of the anti-diabetic and antioxidant activities of *Tetrapleura tetraptera. Intern. J. Biomed. Clin. Sci.* 4 (1) 17 – 23.

Umerie, S. C. and Ekuma, E. T. (2016). Studies on the antioxidant potentials of *Croton spirale*

(*Codiaeum variegatum var. spirale*) (L.) leaf. *Intern. J. Agric. Biosci*. 5(6): 378 – 381.

Witting, D. R., Guariguata, L., Weil, C. and Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res. Clin. Pract.* 94(3): 311 – 321.

Zakaria, Z. A., Abdul-Hisam, e. e., Rofiee, M. S., Norhafizah, M., Somchit, M. N., The, L. K. and Salleh, M. Z. (2011). In vivo antiulcer activity of the aqueous extract of *Bauhinia purpurea* leaf. *J. Ethnopharmacol*. 137: 1047 – 1054.

Zanatta, L., de Sousa, E. and Cazarolli, L. H. (2007). Effect of crude extract and fractions from *Vitex megapotamica* leave on hyperglycaemia in alloxan-diabetic rats. *J. Ethnopharmacol*. 109(1): 151 – 155.