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INHIBITORY EFFECT OF *ARTOCARPUS ALTILIS* LEAF POWDER SUPPLEMENTED DIET ON KEY ENZYMES RELEVANT TO ACUTE TYPE-2 DIABETES IN ALLOXAN INDUCED DIABETIC RATS.

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Abstract

The anti-diabetic effects of *Artocarpus altilis* (*A. altilis*) leaf powder was investigated by evaluating the ameliorating effect on glucose concentration and its inhibitory properties of *A. altilis* leaf powder towards carbohydrate hydrolyzing enzymes, i.e., α-glucosidase, α-amylase and glucose 6 phosphate dehydrogenase. Forty (40) adult albino Wistar rats (male) were used for the study. The animals were divided into five groups of eight rats each.

Hyperglycemia was induced in the rats using alloxan. A diet was formulated using 5% *A. altilis* leaf powder. The animals were fed with the formulated diet for four (4) weeks; blood samples were collected from the animals and analyzed for glucose levels using *Accu* – Check glucometer. The data were compared statistically using analysis of variance (ANOVA). There was a remarkable decrease in blood glucose in both the treated and the pre-treated group. The supplementary diet showed a significant decrease ($P \leq 0.05$) alpha amylase, alpha glucosidase and G6PD inhibitory activity.

The decrease in the blood glucose level of the rats following the administration of the supplemented diet suggests that the plant leaf possesses anti-diabetic, anti-hyperglycemic and hypoglycemic effects on alloxan induced diabetic rats. The presence of phytochemicals, vitamins, and other nutrients such as proteins, lipids, carbohydrates, ash, and other components in the leaf must have acted synergistically to potentiate the anti-diabetic role of the plant leaf.

Keywords: *Diabetes1, Atocarpus altilis2*

Introduction

Diabetes mellitus (DM) is a metabolic disease with multiple etiology and many systemic complications, according to a recent estimate by the World Health Organization and International Diabetic Federation, There were 382 million people in the world with diabetes in the year 2013 and this is projected to increase to 592 million by 2035.[1] This disease is associated with reduced life expectancy, significant mortality, and diminished quality of life. In 2005, an estimated 1.1 million people died from diabetes and diabetes complications.[2] Several studies have reported on the hypoglycemic property of *A. altilis* in animal models of diabetes.[3] The use of the leaf is the common practice in communities that have acclaimed to its hypoglycemic efficacy. The changes in the intensity of the hypoglycemic effect of *A. altilis* over a predetermined time course will be informative in establishing its antidiabetic and medical value in prolonged usage.

In addition, the available drugs (insulin, sulphonylureas, biguanides, e.t.c.) used in the management of diabetes mellitus has been characterized with side effects.[4] In view of this, there is a need to search for alternative remedy. Ojo *et al*.[5] reported that several plants species has been ascribed with normoglycaemia with little or no side effect. An example of plant that may be useful in this category is *A. altilis* (breadfruit) leaf powder. It belongs to a family of *Moraceae*. *A. altilis* parts have been used in traditional medicines. [6] All the medicinal plants used in the management of diabetes mellitus must have the abilities of inhibiting α-amylase and α -glucosidase.

α-Amylase inhibitors are also called starch blockers. This is because they contain substances that prevent dietary starch from being absorbed into the body system, which may be useful in the management of diabetes. α -Amylase may exert blood glucose lowering effect through inhibition of salivary and pancreatic amylase.[7] In other words, α -glucosidase inhibitors are suppressor of postprandial hyperglycaemia in diabetic mellitus patients by inhibiting the activity of α -glucosidase in the intestine, this reduces glucose absorption by delaying carbohydrate digestion and increasing digestion time.[8] Therefore, the aim of this study was to evaluate the glucose concentration, α -amylase and α -glucosidase as well as glucose 6 phosphate dehydrogenase inhibitory activities of *A. altilis* leaf powder.

Methods

Materials And Methods

2.1. Chemical and Reagent

α -Amylase, α -glucosidase, para-nitrophenyl- α -D-glucopyranoside (pNPG), starch, maltose, dinitrosalicylic acid, ferric chloride, Griess reagent, Folin-Ciocalteu reagent and aluminum chloride were obtained from Sigma–Aldrich, Germany. All other chemicals and reagents were of analytical grades.

Result

Alloxan is a drug of choice used to induce diabetes in animals. In this study, we find out *A. altilis* leaf powder supplemented diet to have significantly decrease fasting blood glucose level (Table. 1), when administered for a period of one week to diabetes rats (p value < 0.05).

Alpha-amylase inhibition potential of the *A. altilis* leaf powder supplemented diet was determined (Table 2). The inhibition of *α*- amylase in the diabetic control group was significantly low when compared to the normal control group, however, treatment with *A. altilis* leaf powder supplemented diet significantly restored the activity to normal, which is similar to the standard drug-treated group, however, the inhibitory potential of *A. altilis* leaf powder supplemented diet in the pre-treated group shows a significantly (P < 0.05) increased value, when compared to all other groups.

Alpha-glucosidase inhibitory potential of *A. altilis* leaf powder was also determined (Figure 3). There was a significant (P < 0.05) increase in the activity of this enzyme in the serum of the normal control when compared to the diabetic control, although the activity of this enzyme in liver and the kidney of the normal control was insignificantly (P < 0.05) higher than that of the diabetic control. However, feeding with *A. altlis* leaf powder restored the values to near normal, the pre-treated group also showed a significantly (P < 0.05) high values when compared to the normal control. Similarly, treatment with standard drug significantly (P < 0.05) increases the values when compared to the normal control group.

G6PD activity was significantly decreased in diabetic animals compared with NDM controls (Fig. 4). Treatment with *A. altilis* leaf powder normalized the decrease in G6PD activity of the diabetic control group. However, pre-treated group showed a significantly ($P < 0.05$) high activity when compared to diabetic group, there was also a significant ($P < 0.05$) increase in the group treated with standard drugs.

Discussion

Diabetes can be induced by pharmacological, surgical, or genetic manipulation in several animal species especially in rodents.[9] The majority of studies have used pharmacological models in which alloxan is frequently used for induction of diabetes.[10] This drug exerts its diabetogenic action through reactive oxygen species, which cause rapid destruction of pancreatic β-cells.[11] In the present study, alloxan was used to induce diabetes in animal as previously reported.

DM causes increase in blood glucose concentration.[12] Blood glucose in our body is derived from three sources, which include intestinal absorption of dietary carbohydrates, glycogenolysis, and gluconeogenesis.[13]. Due to insulin deficiency (secretion or action), gluconeogenesis rises and subsequently liver production of glucose increases.[14] It has been suggested that in insulin-dependent diabetes, glucose uptake into skeletal muscle and adipose tissues is compromised.[15] Moreover, in experimental diabetes models, intestinal carbohydrate digestion and absorption are altered which cause increasing of glucose uptake from the gut.[16]

In this light, our study revealed that the diabetic status was associated with an increase in blood glucose concentration, as there was an observed significant increase in the blood glucose of untreated-diabetic rats (Table 1). The *A. altilis* leaf powder supplemented diet mitigated the increase in blood glucose concentration but the group pre-treated with *the A. altilis leaf powder supplemented diet* had a greater impact in controlling the increased blood glucose concentration caused by DM similar to the reference drug (100 mg/kg metformin). Anti-hyperglycemic activity of *A. altilis* leaf may be due to possession of high levels of polyphenolic compounds and presence of flavonoids and tannins in this leaf which have been shown to be involved in the stimulation of the ß-cells and the subsequent secretion of preformed insulin.[17] The results are in agreement with.[18] who reported the hypoglycemic effect of *Vernonia amygdalina* when administered to diabetic rats.

The effect of *A. altilis* leaf supplemented diet on the activities of α-amylase and α-glucosidase was evaluated. The supplemented diet showed potent inhibition of α-amylase activity. Inhibitors of α-glucosidase delay the breaking down of carbohydrate in the small intestine and diminish the postprandial blood glucose excursion in a person suffering from diabetes.[19] One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level.[20] This is an attempt to search for alternatives from medicinal plants with increased potency and lesser adverse effects than existing drugs.[21]

This result is in agreement with previous reports which indicated that excessive inhibition of pancreatic α- amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon and therefore mild α-amylase inhibition activity is desirable.[22] This may be achieved by hindering the absorption of glucose through inhibition of the carbohydrate hydrolysing enzymes, α- amylase and α-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial increase of plasma glucose.[23] The mechanism of action is through the inhibition of the last step in carbohydrate digestion, namely the conversion of disaccharide to monosaccharide (glucose) and a consequent decrease in the rate of entry of glucose into the systemic circulation. [24] The pre-treated group showed a similar inhibition compared with the standard drug.

As for α-glucosidase, both the treated and the pre-treated group exhibits strong inhibition towards the activity of the enzyme. This is in line with reports of

Kwon *et al*,[25] which natural *α*- glucosidase inhibitors from plants had been shown to have a strong inhibition activity against *α*-glucosidase and therefore can be potentially used as an effective therapy for postprandial hyperglycemia with minimal side effects.

To further confirm the hypoglycemic ability of *A. altilis* leaf, glucose-6-phosphate dehydrogenase (G6PD) levels was evaluated (Table 4). In oxidative stress, upregulation of G6PD has been reported in the liver.[26] In this experiment, there was a significant reduction in Glucose-6-phosphate dehydrogenase (G6PD) activity in the liver of diabetic rats. Administration of our plant leaf powder via the supplemented diet resulted in a significant restoration of G6PD activity compared with the diabetic rats treated with metformin. The reduction witnessed in G6PD activity may probably be due to insulin deficiency as this enzyme activity depends on insulin. G6PD is the key enzyme in pentose phosphate pathway which plays a pivotal role in maintaining normal blood glucose levels. Reduction in G6PD activity in liver is associated with obstruction in glucose utilization which results in hyperglycemia.[27] The reversal of the changes in this enzyme activity achieved by the *A. altilis* leaf powder supplemented diet, revealed improvement in the formation of NADPH, favouring lipogenesis and the use of an alternative channel to dispose excess glucose via the HMP pathway. These findings are similar to that of Ramachandran *et al*.[28]

Conclusion

The results obtained in this research indicate that pre-treatment of diabetes with a. *altilis* leaves could be effective in treating the diabetes in a way comparable to metformin. It can be concluded that *A. altilis* leaf powder exerts an inhibitory effect on, blood glucose concentration, α glucosidase, α-amylase, and enhanced the activity of G6PD in diabetic rats. These results further support the traditional use of plants in medicine based on their inhibitory activity of glucose absorption in the body system. However, further pharmacological and biochemical investigations are considered necessary to find out the active constituent and its system of action to understand the bioactive and ameliorative potential of the plant.

References

1. International Diabetes Federation (IDF) (2006): Diabetes Atlas third Edition.
2. Maritim A. C., Sanders R. A, Watkins J. B . Diabetes, oxidative stress, and antioxidants. (2003). *J Biochem Mol Toxicol*; 17(1): 24-38
3. Sairam S., Urooj A. (2004). Safety evaluation of *Artocarpus altilis* as pharmaceutical agent in wistar rats. *J Toxicol*: 2014: 980404
4. Visweswara Rao P., Madhavi K., Dhananjaya Naidu M., Gan S. H. (2013). *Rhinacanthus nasutus* improves the levels of liver carbohydrate, protein, glycogen, and liver markers in streptozotocin-induced diabetic rats. *Evid Based Complement Altern Med*; 2013: 102901
5. Ojo O. A., Basiru A., Olayide I., Fadaka A. O., and Olasehinde O. R. (2016). Ethyl acetate fraction of bark of Bridelia ferruginea inhibits carbohydrate hydrolyzing enzymes associated with type 2 diabetes (α-glucosidase and α-amylase). *Adv Biorese*; 7(3): 126-33
6. Shai L. J., Masoko P., Mokgotho M.P., Magano S.R., Mogale A.M., and Boaduo N. (2010). Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South Afr J Bot*; 76: 465-70
7. Kazeem M. I., Raimi O. G., Balogun R. M., Ogundajo A. L.(2013). Comparative study on the a-amylase and a-glucosidase inhibitory potential of different extracts of *Blighia sapida Koenig. Am J Res Commun*; 1(7): 178-92
8. Johnston C. S., Steplewska I., Long C. A., Harris L. N., Ryals R. H. Examination of the antiglycemic properties of vinegar in healthy adults. *Ann Nutr Metab* 2010; 56: 74-9
9. Bliss M. (2007). The Discovery of Insulin. Chicago, USA: university of chicagi press;.
10. Shirwaikar A., Rajendran K., Barik R. (2006). Effect of aqueous bark extract of *Garugapinnata roxb* in streptozotocin-nicotinamide induced type-II diabetes mellitus. *J Ethnopharmacol.*;107: 285–290
11. Szudelski T (2001). The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. *Physiol Res*. 50: 537–546

12. Morino, K., Petersen, K. and Shulman, G. (2006): "Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction"; *Diabetes*; 55 (2): S9–15
13. Giugliano D., Ceriello A., Esposito K. (2008). Glucose metabolism and hyperglycemia. *Am J ClinNutr*. 2008; 87: 217S–222S.
14. Liu S. H., Chang Y. H., Chiang M. T. (2010). Chitosan reduces gluconeogenesis and increases glucose uptake in skeletal muscle in streptozotocin-induced diabetic rats. *J Agric Food Chem.*; 58:5795–5800
15. Gonzalez E, McGraw T. E., (2006). Insulin signaling diverges into Akt-dependent and -independent signals to regulate the recruitment/docking and the fusion of GLUT4 vesicles to the plasma membrane. *MolBiol Cell*. ;17: 4484–4493
16. Goto T., Horita M., Nagai H., Nagatomo A., Nishida N., Matsuura Y., Nagaoka S., (2012). Tiliroside, a glycosidic flavonoid, inhibits carbohydrate digestion and glucose absorption in the gastrointestinal tract. *MolNutr Food Res*. 56: 435–445
17. Monalisa and Chinmay, (2014): Phytoconstituent analysis and comparative bioefficacy assessment of breadfruit leaf and fruit extracts for antipathogenic potentiality. *American Journal of Phytomedicine and Clinical Therapeutics.* 2: 077-087
18. Mazunder U. K., Cropta M, Manikanda L, Haldar P. (2003). Evaluation of antiinflammatory activity of Vernonia spp. leaves extract in rats. *Phytomedicines*, 10:185-188
19. Kwon Y. I, Apostolidis E, and Shetty K, (2007). "Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension," *Journal of Food Biochemistry*; 31(3), 370–385
20. Mccue P., Kwon Y.-I., and Shetty K., (2005). "Anti-amylase, antiglucosidase and anti-angiotensin I-converting enzyme potential of selected foods," *Journal of Food Biochemistry*. 29 (3), 278–294
21. Matsui T. Ogunwande I. A., Abesundara K. J. M., and Matsumoto K. (2006). "Anti-hyperglycemic potential of natural products". *Mini-Reviews in Medicinal Chemistry*. 6(3): 349–356
22. Apostolidis E., Kwon Y. I., and Shetty K., (2007) "Inhibitory potentialof herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension," *Innovative Food Science and Emerging Technologies*, 8(1): 46–54.
23. Rhabasa-Lhoret R., Chiasson J. L. (2004). α-Glucosidase inhibitors. In International Textbook of Diabetes Mellitus, vol. 1. 3rd edition. Edited by Defronzo R. A., Ferrannini E., Keen H., Zimmet P., U.K: John Wiley & Sons Ltd; 901–914
24. Joubert P. H., Venter H. L., Foukaridis G. N. (1990): The effect of miglitol and acarbose after an oral glucose load: A novel hypoglycaemic mechanism. *Br J Clin Pharmacol* 30:391–396
25. Lee J. W., Choi A. H., Ham M. (2011). G6PD up-regulation promotes pancreatic beta-cell dysfunction. *Endocrinology*, 152: 793–803
26. Bhat M., Kothiwale S. K., Tirmale A. R., Bhargava S.Y., Joshi B.N. (2011). Antidiabetic properties of Azardiracta indica and Bougainvillea spectabilis: *In vivo* studies in murine diabetes model. *Evid Based Complement Alternat Med*. 10.1093/ecam/nep033
27. Ramachandran, B., Kandaswamy M., Narayanan V., and Subramanian S. (2003). Insulin mimetic effects of macrocyclic binuclear oxovanadium complexes on streptozotocin-induced experimental diabetes in rats. Diabetes. *Obes. Metab*., 5: 455-461

TABLE 1: Blood glucose level of *A. altilis* leaf powder supplemented diet treated and pre-treated DM rats

Treatment	Blood glucose concentration (mg/dL) on Weekly basis				
Group	**Initial Glucose**	**Week One**	**Week Two**	**Week Three**	**Week Four**
1	77.3±2.91	79.7±4.00	81.4±3.75	80.1±2.38	82.1±1.10
2	518±7.30	459.0±1.50	409.3±1.18	399.5±1.68	345.5±1.00
3	503±8.10	335.3±3.25	314.4±1.68	238.8±1.42	229.1±0.78
4	210.3±10.55	191.7±1.32	173.0±7.11	161.3±5.71	155.7±7.02
5	529.7±36.14	358.1±2.33	303.1±5.6	215.4±3.91	154.3±15.5

Results are expressed as means ±SD of four determinations (n=4); values in the same row with different superscripts are significantly different at P ≤ 0.05.
1= Non-Diabetic control group
2=Diabetic control group
3= Treated group fed with *A. altilis* leaf powder supplementary diet
4= Pre-treated group fed with *A. altilis* leaf powder supplementary diet
5= Treated group with standard drug (Metformin

Table 2.0 : Effects of *A. altilis* formulated diet on α-Amylase activity in serum, liver, kidney of alloxan-induced diabetic rats.

Groups	Serum	Kidney	Liver
1	14.2 ± 0.8^{b}	**3.5 ± 2.8**^{bc}	**8.82 ± 1.0**^{b}
2	27.2 ± 0.0^{a}	**18.2 ± 0.5**^{a}	**12.5± 0.4**^{a}
3	10.9 ± 0.5^{b}	**6.3 ± 0.0**^{b}	6.9 ± 0.2^{b}
4	2.4 ± 1.1^{c}	0.6 ± 0.0^{c}	**1.7 ± 9.7**^{c}
5	12.7 ± 0.0^{b}	20.5 ± 0.5^{a}	5.06 ± 0.0^{b}

Results are expressed as mean*s* ±SD of four determinations (n=4); values in the same row with different superscripts are significantly different at P ≤ 0.05.
1= Non-Diabetic control group
2=Diabetic control group
3= Treated group fed with *A. altilis* leaf powder supplementary diet
4= Pre-treated group fed with *A. altilis* leaf powder supplementary diet
5= Treated group with standard drug (Metformin)

Table 3.0: Effects of *A. altilis* formulated diet on Alpha glucosidase activity in serum, liver, kidney of alloxan-induced diabetic rats.

Groups	Serum	Kidney	Liver
1	14.7 ± 1.2^{a}	14.3 ± 9.7^{a}	37.0 ± 0.0^{a}
2	6.8 ± 0.5^{c}	6.8 ± 0.5^{b}	10.6 ± 0.0^{b}
3	19.5 ± 1.0^{a}	9.4 ± 1.3^{b}	14.7 ± 1.0^{b}
4	15.8 ± 3.1^{ab}	15.8 ± 3.1^{a}	29.3 ± 6.7^{a}
5	15.7 ± 1.3^{ab}	12.8 ± 0.0^{a}	6.8 ± 0.1^{b}

Results are expressed as mean*s* ±SD of four determinations (n=4); values in the same row with different superscripts are significantly different at P ≤ 0.05.
1= Non-Diabetic control group
2=Diabetic control group
3= Treated group fed with *A. altilis* leaf powder supplementary diet
4= Pre-treated group fed with *A. altilis* leaf powder supplementary diet
5= Treated group with standard drug (Metformin)

Table 4.0: Effects of *A. altilis* formulated diet on G6PD activity in serum, liver, kidney of alloxan-induced diabetic rats.

Groups	Serum	Kidney	Liver
1	108.6 ± 2.4^{a}	138.7 ± 112.6^{ab}	4.8 ± 0.3^{d}
2	74.72 ± 2.4^{c}	64.0 ± 0.18^{abc}	2.8 ± 0.3^{e}
3	92.76 ± 3.7^{b}	183.8 ± 2.9^{a}	3.1 ± 0.0^{e}
4	101.51 ± 0.4^{a}	121.5 ± 0.4^{ca}	16.6 ± 1.5^{d}
5	82.95 ± 0.9^{b}	47.95 ± 0.9^{bc}	21.8 ± 1.7^{d}

Results are expressed as means $\pm$SD of four determinations (n =4); values in the same row with different superscripts are significantly different at $P \leq 0.05$.
1= Non-Diabetic control group
2=Diabetic control group
3= Treated group fed with *A. altilis* leaf powder supplementary diet
4= Pre-treated group fed with *A. altilis* leaf powder supplementary diet
5= Treated group with standard drug (Metformin)