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Antidiabetic Potentials of Jute Leaf (*Corchorus Olitorius*) On Type-2 Diabetic Rats

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Abstract

The use of functional foods and their bioactive constituents have been considered as a new approach in the prevention and management of type-2 diabetes and its complications.This study sought to evaluate the antidiabetic potentials of jute leaf (*Corchorus olitorius*) on low dose streptozotocin/high fat diet induced diabetic rats. In this study, average food intake, average weight, blood glucose, intestinal alpha-glucosidase, pancreatic alpha-amylase, lung angiotensin-1-converting enzyme activities, lipid peroxidation in pancreas, total cholesterol and triglyceride were measured. NTPDase, 5′-nucleotidase and adenosine deaminase activities were also examined in the platelets of diabetic rats. The results showed that there was a significant increase ($P<0.05$) in the blood glucose, α-amylase, α-glucosidase, angiotensin-1-converting enzyme activities, lipid peroxidation in pancreas, total cholesterol and triglyceride levels in diabetic rats when compared to the normal (control) rats. However, the supplementation of 10% of jute leaf showed a significant reduction ($P<0.05$) of these biochemical parameters when compared to the diabetic control group. Furthermore, the dietary inclusion of this vegetable also significantly increased ($P<0.05$) ATP, ADP and AMP hydrolyses except for adenosine hydrolysis in the platelets of diabetic rats when compared to the diabetic control group. In conclusion, the supplementation of vegetable diet in type-2 diabetic rats showed antihyperglycemic, antihyperlipidemic and antiperoxidative effects. It also exhibited modulatory effects on purinergic enzymes involved in the prevention of platelet abnormality and consequent vascular complications in diabetic state. Thus, this vegetable could be a good source of functional food for dietary intervention in the management of type-2 diabetes and its associated complications.

Keywords: jute, antihyperglycemic, antihyperlipidemic, antiperoxidative, type-2 diabetic rats

INTRODUCTION

Type 2 diabetes is a complex metabolic disorder associated with developing insulin resistance, impaired insulin signaling and β-cell dysfunction, abnormal glucose and lipid metabolism, sub-clinical inflammation and increased oxidative stress; these metabolic disorders lead to long-term pathogenic conditions including micro- and macro-vascular complications, neuropathy, retinopathy, nephropathy, and a consequent decrease in quality of life and an increase in the rate of mortality (Santaguida *et al*.,2008). Among the multiple risk factors underlining the incidence and progression of type 2 diabetes, diet is the main modifiable factor. Both experimental and epidemiological evidences have shown that consumption of vegetables rich in phenolic compounds and possess high antioxidant capacity may have inverse relationship with the incidence and prevalence of type-2 diabetes (Bahadoran *et al*., 2013). Dietary control remains one of the most desirable avenues for the prevention and management of chronic degenerative diseases such as type 2 diabetes and cardiovascular diseases. The growing number of diabetics coupled with the harsh side effects of some synthetic drugs has led to the increasing search for alternatives which are relatively cheap with minimal side effects. Green leafy vegetables and fruits have been reported to have some health benefits.

Consequently, jute leaf is a green leafy vegetable popularly used as food and in traditional medicine for the management of diabetes mellitus. However, there is dearth of information on the possible mechanisms of action by which these vegetables exert their health benefits. Therefore, this study sought to investigate the possible mechanisms of action of this vegetable in type-2 diabetic model and its associated diabetic complications.

METHODS AND PROCEDURES

Bioassays Animals

Adult male Wistar rats (150–200g) from the Central Animal House of the Federal University of Santa

Maria, RS, Brazil, were used in this experiment. The animals were maintained at a constant temperature (23±1°C) on a 12-h light/dark cycle with free access to water and to a standard commercial chow (Supra, Porto Alegre, RS, Brazil). All animal procedures of Animal Ethics Committee from the Federal University of Santa Maria were followed. They were acclimatized under these conditions for two weeks prior to the commencement of the experiments.

High-fat fed-diet with low dose of streptozotocin-induced diabetes (type-2 diabetic rat model) bioassay

All the rats were provided with formulated basal diet and water *ad libitum*, prior to the dietary manipulation. After two weeks of acclimatization, a fraction of the rats were placed on normal control (NC) diet [(wheat flour (30%), soybean oil (5%), casein (15.5%), sucrose (10%), cornstarch (35%), vitamins and minerals (4.5%), while the high-fat diet (HFD)-fed rats were allocated into a dietary regimens consisting of (wheat flour (30%), Fat (35%) soybean oil (5%), casein (15.5%), sucrose (10%), vitamins and minerals (4.5%), as a percentage of total feed weight]. After 2 weeks of dietary manipulation, the rats fed with HFD were injected intraperitoneally (i.p.) with low dose of streptozotocin (STZ) (35 mg/kg) for the induction of diabetes, according to a modified method described by Srinivasan *et al.,* (2005).

The rats were randomly divided into five (5) groups comprising five animals each per group as given below;

- Group 1 were normal rats received citrate buffer (pH 4.5) (1 ml/kg, i.p) on basal diet (wheat flour (30%), soybean oil (5%), casein (15.5%), sucrose (10%), corn starch (35%), vitamins and minerals (4.5%).
- Group 2 were diabetic control rats on high fat diet [(wheat flour (30%), Fat (35%) soybean oil (5%), casein (15.5%), sucrose (10%), vitamins and minerals (4.5%)].
- Group 3 were diabetic rats on high fat diet and received acarbose (50mg/kg/bwt) orally.
- Group 4 were diabetic rats on high fat diet and 10% Jute leaf
- Group 5 were normal rats on basal diet and 10% of Jute leaf

Streptozotocin (STZ) was freshly prepared in citrate buffer (0.1 M, pH 4.5) and injected intraperitoneally (i.p.) at a single dose of 35 mg/kg body weight in rats fed high fat diet (Srinivasan *et al.,* 2005). Diabetic state was checked 72 hours after induction with STZ. Blood samples were taken by tail vein puncture and glucose level was monitored using auto-analyzer (Accucheck Auto-coding ™). Animals with blood glucose ≥ 200 mg/dl after 72 hours were considered diabetic and were used in the study. The non-diabetic animals received 1ml of 0.1M citrate buffer intraperitoneally. The diabetic rats were randomly divided into five groups consisting each of five rats and were further placed on dietary regimen containing 10% vegetable supplementation [as a percentage of total feed weight], for the period of thirty days. During the experiment, fasting blood glucose was monitored at 5 days interval. The experiment lasted for 30 days and the rats were decapitated after an overnight fast. The blood was rapidly collected by direct heart puncture and the pancreas, lungs and the small intestine were isolated, rinsed in 1.15% KCl, blotted with filter paper and weighed. The organs were then minced with scissors in 4 volumes of ice cold 0.1M phosphate buffer, pH 6.9 and homogenized in a potter-Elvehjem homogenizer. The homogenates were later centrifuged at 12000xg for 15 minutes at 4°C to obtain Post Mitochondrial Fraction (PMF) which were stored at 4°C and later used for biochemical assays.

The serum was assayed for blood glucose, lipid profile (Total cholesterol and Triglyceride) commercially available kits (Randox Laboratories UK). The pancreas was assayed for the α-amylase activity (Worthington, 1993), the small intestine was assayed for α-glucosidase activity (Apostolidis *et al.,* 2007) and the lung was assayed for the ACE activity (Cushman and Cheung, 1971). Pancreatic malondialdehyde (MDA) content was determined as described by Okhawa *et al.,* (1979).

α-Amylase Assay

Pancreatic homogenate of 50 µl was added to 250 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was incubated at 25°C for 10 min. Then, 50 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 2 ml of distilled water, and absorbance measured at 540 nm. The α-amylase activity was expressed as enzyme activity as shown in the appendix (Worthington, 1993).

α-Glucosidase Assay

Briefly, 15 µl of intestinal α-glucosidase (EC 3.2.1.20) solution in 0.1 M phosphate buffer (pH 6.9) was incubated at 37°C for 10 min. Then, 40 µl of 5 mM *p*-nitrophenyl-α-*D*-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 37°C for 10 min, before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase activity was calculated and expressed as shown in appendix (Apostolidis *et al.,* 2007).

Angiotensin-I Converting Enzyme (ACE) Assay

The ACE assay was done using a slightly modified method of Cushman and Cheung (1971). Lung´s homogenate of 50 µl was added to tris buffer. The enzymatic reaction was initiated by adding 150 µl of 8.33 mM of the substrate Bz–Gly–His–Leu in 125 mM Tris–HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37 ^{o}C, the reaction was arrested by adding 250 µl of 1 M HCl. The Gly–His bond was then cleaved and the Bz–Gly produced by the reaction was extracted with 1.5 ml ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 ml of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm.

Determination of MDA Contents

The lipid peroxidation assay was carried out using the modified method of Okhawa *et al.* (1979). Lipid peroxidation expressed as units/mg protein.

Determination of Serum Total Cholesterol and Triglyceride Concentrations

Serum cholesterol and triglycerides were determined using RANDOX kit procedure.

Determination of NTPDase and 5^{1}-Nucleotidase Activity

This reaction was initiated by the addition of ATP or ADP to a final concentration of 1.0 mM. Briefly, 160 µl of the medium containing 5.0 mM KCl, 1.5 mM $CaCl_2$, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose and 45 mM Tris–HCl buffer, pH 8.0, was added to the platelet containing the enzyme in a final volume of 200 µl. The platelet (10–20µg protein) was added to the reaction mixture and pre-incubated for 10 min at 37°C. Then, 20 µl of nucleotide (ATP or ADP or AMP) was added. The reaction medium was then incubated for 20 minutes and stopped by the addition of 200µl 10% trichloroacetic acid. The samples were chilled on ice for 10 min, and 100 µl samples were taken for the assay of released inorganic phosphate (Pi) (Chan *et al*., 1986). 200 µl of the aliquot of ATP or ADP were transferred into another test tube. 200 µl of distilled water was then added. Finally, 3ml of aliquot of malachite green was added and the absorbance read at 630nm after 15 minutes. Enzyme activities were expressed as nmol of inorganic phosphate released per min per milligram of protein (nmolPi.min^{-1}mg^{-1}of protein).

Adenosine Deaminase Activity Determination (ADA)

Adenosine deaminase activity was measured spectrophotometrically in platelet by the method of Giusti *et al*., (1974). The reaction mixture was vortexed and incubated for 30 minutes at 37^{0}C and read at 620 nm. Ammonium sulphate of 75 umol/l was used as ammonium standard. The ammonia concentration is directly proportional to the absorption of indophenol at 620 nm. The specific activity is reported as U/l.

DATA ANALYSIS

The results of replicate readings were pooled and expressed as mean ± standard deviation. One way analysis of variance was used to analyze the results and Duncan multiple tests was used for the post hoc (Zar, 1984). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis

RESULTS AND DISCUSSION

Table1: Average Feed intakes of high-fat diet and low-dose STZ- induced type-2 diabetic rats fed vegetable supplemented diets

Groups	Treatments	Average Feed Intake (g/rat/day)
I	Normal rats (Basal)	17.79 ± 3.2^{a}
II	HFD fed rats + STZ (35 mg/kg) Control rats	15.28 ± 2.8^{a}
III	HFD fed rats + STZ (35 mg/kg) + Acarbose	16.32 ± 3.0^{a}
IV	HFD fed rats + STZ (35 mg/kg) + Jute leaf (10%)	15.42 ± 2.9^{a}
V	Normal rats + Jute leaf (10%)	16.95 ± 2.9^{a}

Values represent mean ± standard deviation (n = 5).

Table 2: Average weight (g/rat) of type-2 diabetic rats fed vegetables supplemented diets.

Groups	Weight (g/rat) 1st day	30th day	Weight gain/loss (%)
I	200.5±11.7	222.4±13.9	10.9^{b}
II	210.6±13.8	176.3±9.8	-16.3^{c}
III	217.2±12.8	240.5±15.4	10.7^{b}
IV	212.7±11.2	235.6±12.3	10.8^{b}
V	205.2±10.7	231.2±17.5	12.7^{a}

Values represent mean ± standard deviation (n = 5).
Values with the same superscript alphabet on the same row are not significantly (P>0.05) different .

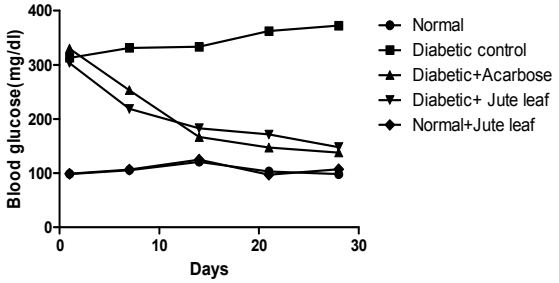
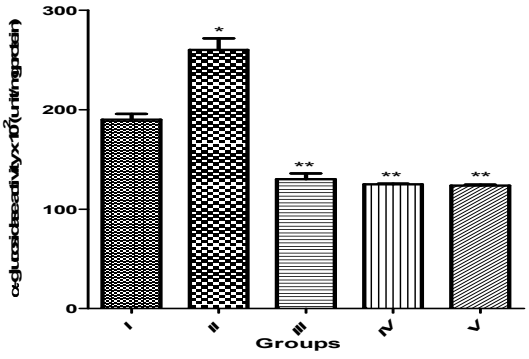


Figure 1: Effect of Jute leaf on blood glucose of diabetic rats.

As shown in Figure 2, significant (P<0.05) increase in the pancreatic α-amylase activity was observed in type-2 diabetic control rat group when compared with the normal control rat group. However, significant (P<0.05) decrease in α-amylase activity was observed in both the treated type-2 diabetic rat groups and vegetable supplemented normal rat groups when compared with type-2 diabetic and normal control rat groups. However, acarbose treated type-2 diabetic rat group had the highest reduction in α-amylase activity.

Furthermore, there was significant (P<0.05) increase in the intestinal α-glucosidase in type-2 diabetic control rat group when compared with the normal control rat group. Moreover, significant (P<0.05) decrease was observed in intestinal α-glucosidase activity of both the treated type-2 diabetic rat groups and vegetable supplemented normal rat groups when compared with type-2 diabetic and normal control rat groups. Lung angiotensin-I-converting enzyme (ACE) activities was significantly (P<0.05) elevated in type-2 diabetic control rat group when compared with the normal control rat group and other groups. However, the ACE activity in the lungs was significantly (P<0.05) reduced in all the rat groups when compared to the diabetic rats (Figure 3). Furthermore, there was no significant difference among the groups except for the diabetic rats.



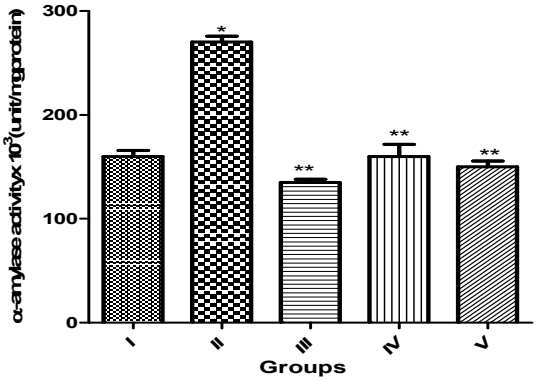
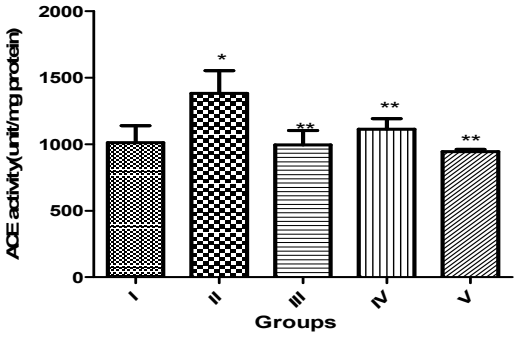


Figure 2: Effect of Jute leaf on Alpha amylase and alpha glucosidase activities of Diabetic Rats.



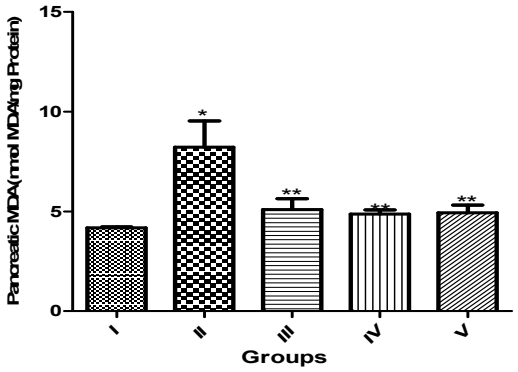


Figure 3: Effect of Jute leaf on angiotensin-1-converting enzyme (ACE) activity and lipid peroxidation in pancreas of diabetic rats.

Effect of jute leaf on lipid peroxidation in pancreas of diabetic rats was also shown in Figure 3. The result showed that there was significant increase (P<0.05) in lipid peroxidation of the diabetic rat group when compared to the normal control and other treated groups. However, there was no significant difference between the normal control and the vegetable supplemented normal rats.

The result of the serum lipid profile [(triglyceride (TG) and total cholesterol (TC)] showed that there

were significant (P<0.05) elevation in the levels of plasma TG and TC compared to the normal control rat group (Figure 4). However, the levels of serum TG and TC were significantly (P<0.05) reduced in both metformin and acarbose and vegetable treated type-2 diabetic rats when compared with the type-2 diabetic rats.

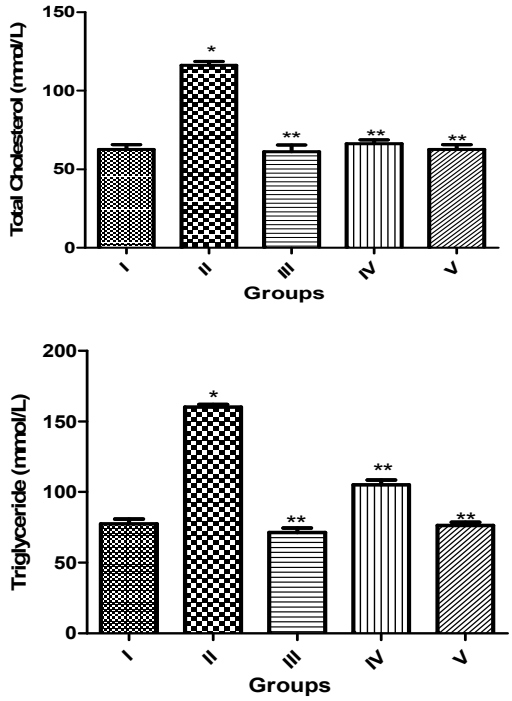


Figure 4: Effect of Jute Leaf on Serum Total Cholesterol and Triglyceride levels of Diabetic Rats
*Values are significantly (P<0.05) different from normal group
**Values are significantly (P<0.05) different from control group

The results shown in Figure 5-6 revealed the effects of Jute leaf on the activities of ectonucleotidases. There were significant difference (P<0.05) in the activities of both NTPdase and 5′-nucleotidase in diabetic rats when compared to the normal rats as shown in the rate of ATP and ADP hydrolysis (NTPdases) and AMP hydrolysis (5′-nucleotidase). However, the vegetable treated rats showed significantly elevated levels of activities (P<0.05) when compared to the diabetic rats.

Conversely, the result obtained in Figure 6 showed that there was a significant (P<0.05) elevation of adenosine deaminase (ADA) activity in diabetic control group when compared to the normal control group. However, a significant (P<0.05) reduction in the adenosine deaminase (ADA) activity was observed in the vegetable treated diabetic rats when compared to the diabetic rats.

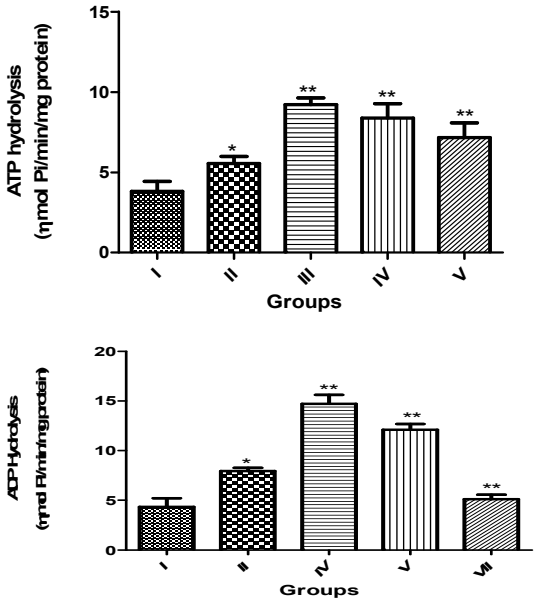


Figure 5: Effect of Jute Leaf on ATP and ADP Hydrolyses in platelets of Diabetic Rats.
*Values are significantly (P<0.05) different from normal group
**Values are significantly (P<0.05) different from control group

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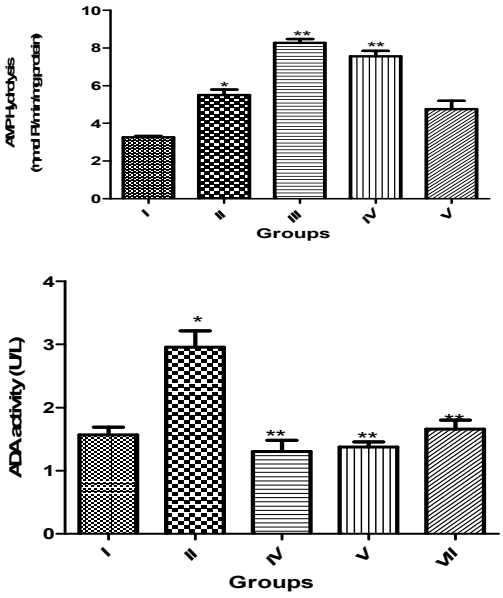

Figure 6: Effect of Jute Leaf on AMP Hydrolysis and Adenosine Deaminase (ADA) Activity of Diabetic Rats.
*Values are significantly (P<0.05) different from normal group
**Values are significantly (P<0.05) different from control group

In this study, the effect of jute leaf was investigated using the type 2-diabetic rat model by high-fat

feeding and streptozotocin injection. Chemical, like streptozotocin (STZ) exhibits its diabetological action through the production of free radicals causing damages to β-cells of the islets of Langerhans. STZ may also exert its differential wreck action to hepatocytes, nephrons and cardiomyocytes (Rosholt *et al*., 1994). The combination of high fat diet (HFD) and low doses of STZ resulted in characteristic of type 2 diabetes mellitus; HFD induces insulin resistance while low doses of intraperitoneal STZ induce moderate impairment of insulin secretion (Zhang *et al*., 2003). Furthermore, although high-dose STZ severely impairs insulin secretion mimicking type1diabetes, however, insulin resistance will be developed in these animals when fasting blood glucose increased in diabetic rats.

The blood glucose lowering effect of jute leaf may indicate that these vegetables may possess antidiabetic agents which could control hyperglycemia. This is in consonance with earlier reports that green leafy vegetables possess antidiabetic properties (Zhang *et al*., 2010; Balamurugan and Ignacimuthu, 2011).

One therapeutic approach for treating in the early stage diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Chen *et al*., 2006).

As observed in this study, the significant increase in pancreatic α-amylase and intestinal α-glucosidase activities in type-2 diabetic rats agree with earlier works where elevated activities of the carbohydrate hydrolyzing enzymes were reported in type-2 diabetic animals and human subjects (Shankaraiah and Reddy, 2011; Ademiluyi and Oboh, 2013). However, the increased pancreatic α-amylase and intestinal α-glucosidase activities in type-2 diabetic rats may have contributed to the observed elevation in blood glucose level. Furthermore, the increased intestinal α-glucosidase and pancreatic α-amylase activities in type-2 diabetic rats were observed to be reduced in vegetables treated type-2 diabetic rats. The observed reduction in the activities of the carbohydrate hydrolyzing enzyme activities (pancreatic α-amylase and α-glucosidase) of vegetables treated type-2 diabetic rats could be linked to the inhibition of the enzymes by phenolics present in the vegetables (Saliu *et al*., 2012).

Regulation of the renin-angiotensin system may retard or prevent glomerular hypertension, which is a major factor in the progression of diabetic nephropathy. The reduced activity of ACE observed in vegetable treated rats may be linked to the inhibition of ACE by the phenolics in the vegetables (Saliu *et al*., 2012). Lipid peroxidation is well known as an important parameter of oxidative stress (Puntel *et al.,* 2005). The increase in pancreatic malondialdehyde (MDA) in type-2 diabetic rats may have originated from hyperglycemia leading to oxidative and cellular damage. This inhibition of lipid peroxidation might be due to the high antioxidant potentials of the polyphenolic constituents of jute leaf as reported in earlier works (Oboh *et al*., 2012).

The hypertriglyceridaemia observed in these type-2 diabetic rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic VLDL and decreased TG uptake in peripheral tissues. Hypercholesterolaemia may be attributed to the increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition (Srinivasan *et al*., 2005). However, the levels of serum TG and TC were significantly reduced in the vegetable treated type-2 diabetic rats. Moreover, it can be conjestured that the lipid lowering effects of these vegetable supplemented diets could be due to the inhibition of hepatic cholesterol, triglyceride and fatty acid synthesis by the phenolic constituents of the vegetables investigated (Balamurugan and Ignacimuthu, 2011). An enhanced increment in NTPDase and 5^1-nucleotidase activities in platelets of type-2 diabetic rats with vegetable suplementation indicates that the consumption of both vegetables interferes with purinergic signaling. This consequent increase in the ectonucleotidases activities reflects an increased degradation of ATP, ADP, and AMP resulting in an increment in the adenosine formation. In this sense, it can be suggested that the supplementation of jute leaf may have an antiaggregant effect similar to the report for red wine, limiting the bioavailability of ADP, the main agonist to platelet aggregation (Anfossi *et al*., 2002). Moreover, it also promotes the production of adenosine, an antiaggregant and vasodilating agent, contributing to the control of hemostasis in diabetic state (Birk *et al*., 2002).

Based on these findings, it could be suggested that jute leaf may be able to preserve adenosine levels in the circulation, which act upon platelet adenosine receptors and can inhibit platelet aggregation and promote vasodilatation, exerting an important protective role in the prevention of the development and progression of vascular complications caused by the hyperglycemic state. In fact, studies have shown that polyphenolic compounds present in some plant foods can inhibit the process of thrombus formation (Dohadwala, and Vita, 2009; Gresele *et al*., 2011).

Thus, these results support the hypothesis that one of the ways by which jute exerts its cardioprotective actions may be mediated by an increase in the adenosine levels and an amplification of the effect of this nucleoside via adenosine receptors, since this vegetable has demonstrated the ability to reduce ADA activity.

CONCLUSION

Dietary supplementation of jute leaf in type 2 diabetic rat showed antihyperglycemic and hypolipidemic properties of this vegetable by enhancing glucose homeostasis via delaying carbohydrate digestion, protecting the tissue damage, improving blood lipid profile and membrane integrity and suppressing oxidative stress. Furthermore, they could interfere with the ectoenzymes´ activities in the platelet of diabetic rats thereby contributing to the prevention of platelet abnormality and consequently vascular complications in diabetic state. In addition, this study provides a biochemical rationale for clinical studies. Further studies in animal models and human volunteers need to be done to substantiate these findings. Jute leaf may therefore represent a potential functional food for the prevention and management of type-2 diabetes and its attendant complications.

REFERENCES

Ademiluyi A.O and Oboh G. (2013). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin-I-converting enzyme) in vitro. *Experimental Toxicology and Pathology*; **65**(3):305-309.

Anfossi G., Russo I., Massucco P., Mattiello L., Cavalot F., Balbo A., (2002). Adenosine increases human platelet levels of cGMP through nitric oxide: Possible role in its antiag-gregating effect. *Thrombosis Research*; **105**(1): 71–78.

Apostolidis E., Kwon Y.I. and Shetty K. (2007). Inhibitory potential of herb, fruit, and fungal enriched cheese against key enzymes linked to Type-2 diabetes and hypertension. *Innovative Food Science and Emerging Technology*; **8**: 46 - 54.

Bahadoran Z., Golzarand M., Mirmiran P., Saadati N and Azizi F. (2013). The association of dietary phytochemical index and cardio-metabolic risk factors inadults: Tehran lipid and glucose study. *Journal of Human Nutrition and Diet*. In print.

Balamurugan R. and Ignacimuthu S. (2011). Antidiabetic and Hypolipidemic effect of methanol extract of Lippia nodifloraL. in streptozotocin induced diabetic rats. *Asia Pacific Journal of Tropical Biomedicine*; S30-S36.

Birk A.V., Broekman M.J., Gladek E.M., Robertson H.D., Drosopoulos J.H., Marcus A.J., (2002). Role of extracellular ATP metabolism in regulation of platelet reactivity. *Journal of Laboratory and Clinical Medicine*; **140**(3): 166–175.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quatities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**:248-54.

Chan K., Delfert D. and Junger, D. (1986). A direct colorimetric assay for Ca2?-ATPase activity. *Analytical Biochemistry*; **157**: 375–378.

Chen X., Zheng Y., Shen Y. and Voglibose (Basen, AO-128). (2006). One of the most important α-glucosidase inhibitors. *Current. Medical. Chemistry*; **13**: 109-116.

Cushman D.W. and Cheung H.S. (1971). Spectrophotometric assay and properties of the Angiotensin I-converting enzyme of rabbit lung. *Biochemical Pharmacology*; **20**: 1637 -1648.

Dohadwala M.M., and Vita J.A. (2009). Grapes and cardiovascular disease. *Journal of Nutrition*; **139**(9): 1788–1793.

Giusti G. (1974). Adenosine deaminase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. 3rd ed. *Academic Press, New York*, pp; 1092–1099.

Gresele P., Cerletti C., Guglielmini G., Pignatelli P., de Gaetano G., and Violi F. (2011). Effects of resveratrol and other wine polyphenols on vascular function: *An update.Journal of Nutritional Biochemistry;* **22**(3): 201–211.

Oboh G., Ademiluyi A.O., Akinyemi A.J., Henle T., Saliu J.A., and Schwarzenbolz U. (2012). Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin-I-converting enzyme) in vitro. *Journal of Functional Foods;* **4**(2): 403-574.

Ohkawa H., Ohishi N. and Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*; **95**: 351–8.
Puntel R.L., Nogueira C.W., and Rocha J.B.T. (2005) Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in Rat Brain In vitro. *Neurochemical Research*; **30**: 225–235.

Rosholt M.N., King P.A. and Horton E.S. (1994). High-fat diet reduces glucose transporter responses to both insulin and exercise. *American Journal of Physiology* **266**: R95-R101.

Saliu J.A., Ademiluyi A.O., Akinyemi A.J. and Oboh G. (2012). *In vitro* antidiabetes and antihypertension properties of phenolic extracts from Bitter leaf (*Vernonia amygdalina* Del.). *Journal of Food Biochemistry*; **36** (5): 569 - 576.

Santaguida P.L., Balion C. and Hunt D. (2008). Diagnosis, prognosis, and treatment of impaired glucose tolerance and impaired fasting glucose. *Evid Rep Technol Assess*; **12**: 1–11.

Shankaraiah P. and Reddy Y.N. (2011). α-amylase Expressions in Indian Type-2 Diabetic Patients. *Journal of Medical Sciences,* **11***:* 280-284.

Srinivasan K., Viswanad B., Asrat L., Kaul C. L., and Ramarao P. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening.*Pharmacological Research*; **52**: 313–320.

Tietz, N.W. (1982). Fundamentals of clinical chemistry. W.B. Saunders Company, Philadelphia. pp. 562 – 698.

Worthington, V. (1993). Alpha amylase, In: Worthington K. and Worthington V (Eds),Worthington enzyme manual, Worthington Biochemical Corp., Freehold, NJ, pp. 36–41.

Zar J.H. (1984). Biostatistical Analysis. Prentice-Hall Inc., Upper Saddle River, New Jersey.

Zhang L., Yang J., Chen X., Zan K. , Wen X., Chen H., Wang Q. and Lai M. (2010). Antidiabetic and antioxidant effects of extracts fromPotentilla discolor Bunge on diabetic rats induced by high fat diet and streptozotocin. Journal of Ethnopharmacology; **132**: 518–524.