IJPSR (2016), Vol. 7, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 August, 2015; received in revised form, 06 December, 2015; accepted, 16 January, 2016; published 01 February, 2016

ANTIDIABETIC ACTIVITY OF *PIPER BETLE* IN ALLOXAN INDUCED TYPE 1 DIABETIC MODEL RATS

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Keywords:

Diabetes mellitus, Antidiabetic, *Piper betle*, Blood glucose, Triglycerides, Cholesterol.

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ABSTRACT: The aim of this study was to investigate the potential antidiabetic activity of *Piper betle* in alloxen induced diabetics. Rats were divided into 6 groups and *Piper betle* was administered containing 50, 100 and 200 mg/kgbwt powder, respectively in 1ml water orally in group A, B and C rats. Metformin (150 mg/kgbwt) used as a reference standard drug. Blood glucose (BG), triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated from the serum by using standard kits. *Piper betle* juice had shown significant lowered the blood glucose levels in all groups. In addition, body weight, organ (liver, kidney, heart and pancreas) weight, food intake, water intake were also examined in all treated groups and compared against diabetic control group. After 22 days daily administration of *Piper betle*, diabetic treated rats showed improvement in body weight, water intake as compared to diabetic control rats. In alloxan induced diabetic rats the maximum reduction in BG, TG, TC, HDL, LDL, SGOT and SGPT were observed at a dose level of 100 mg/kgbwt. The result of this study demonstrates the potentiality of *Piper betle* juice as a source of an antidiabetic action that could be harness for use in the health care delivery process.

INTRODUCTION: Type 1 Diabetes (insulin dependent) introduces when the insulin-producing β-cell in the pancreas has been destroyed and the body unable to produce sufficient insulin which causes the increase of glucose levels in the blood [1]. Glucose is a sugar molecule that the body produces during the digestion of carbohydrates and its levels are controlled by the hormone insulin. Insulin is made and stored in the pancreas and helps glucose to enter into the cells where it is used as fuel by the body.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(2).675-80

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).675-80

Without insulin, the body's cells cannot absorb glucose from the blood and glucose level increases in the blood, leaving the body's cells and tissues starved for energy[1]. Long-term complications from high blood sugar can develop heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor circulation of limbs leading to amputations [2].

The management of diabetes is a global health care problem until now and successful treatment is not yet discovered [3]. Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. But these are observed to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea [4]. It is currently affecting around 143 million people [5] and the number of those affected is

increasing day by day, by 2030 it is predicted to reach 366 million populations worldwide [6].

Medicinal plants are used as an important therapeutic aid for alleviating ailments of humankind. There have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent over the last 2500 years. These traditions are still flourishing, since; almost 80% of the people in the developing countries depend on these systems of medicine for their primary health care needs [7]. These plants hold substances that can be used for therapeutic purposes, of which are precursors for the synthesis of drugs [8].

Piper betle Linn. (Local name 'Paan') Piperaceae, a dioecious, annual creeper, climbing by many small adventitious rootless, grows to a height of approximately one metre, generally grown in hotter and damper parts of the country [9, 10]. It is generally found in damp forests and is propagated in India, Bangladesh, Vietnam and China. Plant part used is mainly leaves and roots. Oral administration of the water extract from the whole plant of *Piper betle* significantly lowered the plasma glucose levels in healthy rats. The water extract of *Piper betle* treatments lead to significant lowering of blood sugar level and reduction in serum lipids. It also has insulinomimetic activity[11].

In this present study, the effects of *Piper betle* juice on blood glucose levels were evaluated in hyperglycemic and assessed by evaluating the comparative hypolipidemic total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triglyceride (TG)) and hepatoprotective serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)) activities in alloxan-induced diabetic rats and compared with those of metformin.

MATERIALS AND METHODS:

Plant extract and test animal: Fresh *Piper betle* plants were collected from medicinal plant garden. The plant parts were shade dried completely in room and the dried plants were powdered with an electric grinder into fine powder and used for making juice. The authenticity of the *Piper betle* was identified by the department of Botany, Rajshahi University, Rajshahi. A total number of 23 male long evan rat (140-250 g) were used in the experiment. Rats were obtained from the International Centre for Diarrhoeal Diseases Research, Bangladesh. Animals were maintained under standard and ambient temperature under lights for twelve hours followed by 12 h of darkness environmental conditions having proper ventilation in the room, were fed with a standard pellet diet and water.

Induction of diabetes and experimental procedure: Male long evan rats were divided into 6 groups and allowed to fast overnight and injected single intraperitoneal injection with freshly prepared alloxan monohydrate (120 mg/kgbwt) in saline water to made diabetic by alloxan monohydrate and served as diabetic control, standard and treatment groups respectively. Rats exhibited in plasma glucose levels >10mmol/dl, 5day after administration of alloxan were included in the study and selected for drug treatments. Treatment for diabetes (*Piper betle* extract 50,100 and 200 mg/kgbwt) was started from 6 th day of alloxan administration. Group A, B and C served as sample group were fed a sample (*C. asiatica*) containing 50mg/kgbwt, 100mg/kgbwt and 200mg/kgbwt powder respectively in 1 ml water orally (once daily), starting from the 6th day of alloxan administration, one diabetic control group (positive control group) which did not receive metformin or sample, one normal group (negative control group) which received only distilled water, and standard drug group (SD group) which received metformin as standerd drug in 1 ml water orally.

The whole experiment was continued for 22 days. Sample collection and estimation of biochemical parameter: Rats were sacrificed by ether anesthesia and about 3-5 ml of blood sample was collected directly from artery by syringes. Blood was collected in fresh centrifuge tube and centrifuged at 4000 rpm for 10 min and the serum was preserved to examine TG, TC, SGPT, SGOT, HDL and LDL level by semi-auto analyzer (Humalyzer 3000, Human, Germany) using wet reagent diagnostic kits according to manufacturer's protocol. In this present study, the effects of *Piper betle* juice on blood glucose levels were evaluated

in hyperglycemic and assessed by evaluating the comparative hypolipidemic (total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triglyceride (TG)) and hepatoprotective (serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)) activities in alloxan-induced diabetic rats and compared with those of metformin.

Statistical Analysis:
Data from the experiments were analyzed using SPSS software for windows version 10. All results were expressed as the mean ± Standard Deviation. One-way analysis of variance (ANOVA) used and paired or unpaired t-test was done to see any difference between groups.

RESULT AND DISCUSSION:

Effect of *Piper betle* on body weight (bwt) gain, food intake and water intake: In the course of investigation it was observed that, there is slightly increasing tendency of body weight in *Piper betle* juice of group A and group B compared with Standard drug (SD) administered group at final day (22th day) as compared with initial day. Very significant weight loss, approaching 20%, occurred 48 h after administration of alloxan (120 mg/kgbwt). The final body weight of diabetic control group was found lower than that of normal group (**Table 1**). Among the treatment, group A gain highest body weight (111%).Where as group B and C gain 106% and 95%, respectively. However, there was no significant difference between *Piper betle* and SD treatment group.

It was observed that body weight was found to significantly relate with food intake in all groups of rats. Among the three treatment groups the final (22nd day) body weight of group A increase 111% as food intake of this group was better when compared to diabetic control group. Moreover, improvement of body weight of treated animal further supports the antidiabetogenic effect of *Piper betle* as diabetic condition is associated with loss of body weight.

TABLE 1: EFFECT OF *PIPER BETLE* ON BODY WEIGHT (BWT) AND GROWTH PARAMETER OF ALLOXAN INDUCED TYPE 1 DIABETIC RATS FOR 22 DAYS

Experimental Groups	bwt initial day (g)	bwt-8th day (g)	bwt-15th day (g)	bwt-22nd day (g)	Food Intake (g/kg bwt)	Water Intake (ml/kg bwt)	Final Body Weight (g)
Normal	106.0±4.32 (100%)	149.75±14.17 (140%)	163.75±24.55 (153%)	163.75±24.5 (153%)	59.80±1.04^{b}	194.71±3.36^{c}	145.81±27.34^{b}
Diabetic control	191.75±38.96 (100%)	195.25±37.52 (102%)	183.0±32.01 (95%)	183.0±32.01 (95%)	50.47±8.06^{b}	198.45±1.98^{c}	188.25±6.22^{b}
Diabetic+ Group A	158.75±17.2 (100%)	156.5±17.44 (98%)	169.25±12.47 (106%)	176.75±11.3 (111%)	60.10±1.06^{b}	196.02±2.62^{c}	165.31±9.43^{b}
Diabetic+ Group B	152.25±28.19 (100%)	150.75±30.13 (98%)	160.25±21.74 (105%)	162.50±19.36 (106%)	57.26±2.56^{b}	195.04±3.35^{c}	156.43±5.80^{b}
Diabetic+ Group C	164.0±22.74 (100%)	157.75±23.44 (95%)	157.75±23.44 (95%)	155.25±14.7 (94%)	60.47±0.15^{b}	193.57±3.45^{c}	158.68±3.73^{b}
Standard drug (SD)	184.0±43.71 (100%)	186.0±48.28 (101%)	174.33±47.98 (94%)	163.75±24.5 (88%)	54.48±4.92^{b}	194.57±4.28^{c}	177.02±10.20^{b}

Each value is the mean ± S.D. for 4 rats. A, b, c value with superscript letter means significant among the group at $p<0.05$. NS Not significant. Group A = 50 mg/kgbwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt.

Effect of *Piper betle* on plasma glucose level, lipid profile, SGPT, SGOT and organ weight: Significant reduction in plasma glucose level in the A. B, C and SD administered groups were observed with different extent (**Fig.1**). As much as 36.1% reduction in plasma glucose level was observed in group A where only 16.6 % and 23.76% decrease in plasma glucose level could be noticed in group B and C, respectively. A significant reduction in TG, TC, HDL and LDL levels were noticed in all groups those administered *Piper betle* and SD than to diabetic control (**Table 2**).

It was found that, group C performed better, which decreased the TG level as much as 48.89% as compared to diabetic control group 95.65%. A substantial decrease in the plasma TC level 30.01% was found in group A, as compared to diabetic control group 42.85%. Increased level of HDL as

much as 106% as compared to diabetic control group 51.61% and decreased LDL level as much as 65.67% as compared to diabetic control group 191.36% were also found in group A. The values of SGOT and SGPT were shown to be reducing in all *Piper betle* and SD administered group which was increased by injection of Alloxan for inducing diabetes (**Table 2**). Both SGOT and SGPT level were found to significantly lower in group C 46.02% and group B 50.40%, respectively than the control group 85.41% and 101.61%. Our results indicate significant reduction of both SGOT and SGPT value was evidenced under treatment of *Piper betle* which signifies their corrective role in liver function of the diabetic rats. In this investigation the heart, liver, kidney and pancreas weight were shown to be significantly improved in all *Piper betle* and SD administered groups as compared to diabetic control group (**Table 3**).

TABLE 2: EFFECT OF *PIPER BETLE* ON PLASMA TRIGLYCERIDE, TOTAL CHOLESTEROL, HIGH DENSITY LIPOPROTEIN AND LOW DENSITY LIPOPROTEIN CONCENTRATION IN ALLOXAN-INDUCED TYPE 1 DIABETIC RATS

Experimental Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	SGOT (mg/dl)	SGPT (mg/dl)
Normal	69.92±9.2^{b}	69.92±9.2 ^{a}	47.7±7.7 ^{b}	22.2±4.1 ^{a}	83.23±4.7 ^{b}	32.4±2.7 ^{a}
Diabetic control	93.62±17.2^{b}	88.65±5.7^{a}	25.8±2.9 ^{a}	40.5±5.1 ^{a}	101.7±7.9 ^{b}	62.5±0.62 ^{b}
Diabetic+ Group A	57.22±2.67^{b}	62.06±7.1^{a}	53.3±6.6^{b}	13.9±2.7^{a}	68.45±6.8^{a}	37.5±12.8^{a}
Diabetic+ Group B	51.40±2.12^{b}	65.32±4.5^{a}	48.5±8.3^{b}	21.3±1.6^{a}	61.1±1.5^{a}	31.0±6.20^{a}
Diabetic+ Group C	47.85±5.04^{b}	69.52±7.3^{a}	46.2±4.5^{b}	17.9±5.9^{a}	54.85±2.4^{a}	35±5.80^{a}
Standard drug (SD)	69.56±12.0^{b}	69.56±12.0^{a}	51.9±4.3^{b}	20.3±1.4^{a}	92±4.6^{b}	32±2.70^{a}

Each value is the mean ± S.D. for 4 rats. a, b value with superscript letter in the same column significantly different among the group at p<0.05.NS Not significant. Group A = 50 mg/kg bwt, Group B = 100 mg/kg bwt, Group C = 200 mg/kg bwt, Standard drug (SD) = 150 mg/kg bwt. TG = Triglyceride, TC = Total cholesterol, HDL = High density lipoprotein, LDL = Low density lipoprotein.

TABLE 3: EFFECT OF *PIPER BETLE* ON VARIOUS ORGAN WEIGHT (G) IN ALLOXAN INDUCED DIABETIC TYPE 1 RATS

Experimental Groups	Heart wt (gm)	Liver wt (gm)	Kidney wt (gm)	Pancreas wt (gm)
Normal	0.51±3.10^{c}	5.31±0.80^{c}	1.25±.12 ^{b}	0.34±2.16 ^{b}
Diabetic control	0.57±0.22^{c}	5.23±1.30 ^{c}	1.45±.23 ^{b}	0.22±9.93 ^{b}
Diabetic+ Group A	0.64±0.44^{c}	4.39±0.93 ^{c}	1.31±2.64^{b}	0.26±.11 ^{b}
Diabetic+ Group B	0.53±0.17 ^{c}	5.08±0.26 ^{c}	1.317±6.23^{b}	0.26±.10 ^{b}
Diabetic+ Group C	0.43±7.5 ^{c}	4.46±0.65 ^{c}	1.2±8.67^{b}	0.27±8.9 ^{b}
Standard drug (SD)	0.54±0.13^{c}	6.50±5.36^{c}	1.5±.26^{b}	0.29±6.39 ^{b}

Each value is the mean ± S.D. for 4 rats. a, b, c value with superscript letter in the same column significantly different among the group at p<0.05.Group A = 50 mg/kg bwt, Group B = 100 mg/kgbwt, Group C = 200 mg/kgbwt, Standard drug (SD) = 150 mg/kgbwt

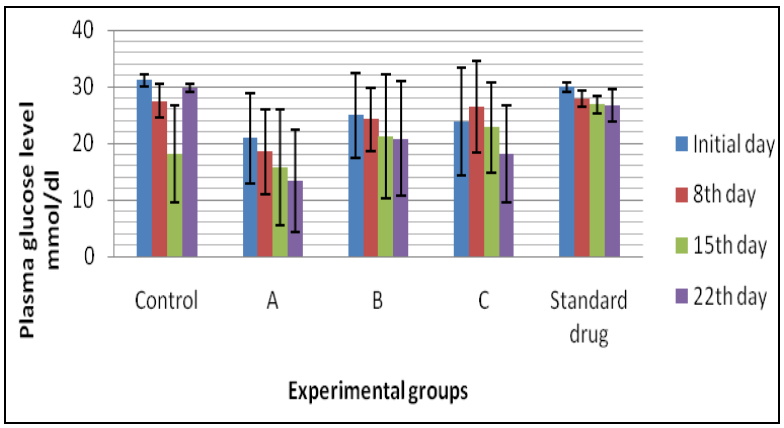

FIG.1: HYPOGLYCEMIC EFFECT OF *PIPER BETLE* IN ALLOXAN INDUCED TYPE 1 RATS BY ORAL ADMINISTRATION FOR 22 DAYS.

Human diabetes and experimental diabetic animal models show high oxidative stress due to persistent and chronic hyperglycemia which may cause depletion of the antioxidant defense system and proceed to increase de novo free radical formation[12]. Furthermore, high glucose contents can simply inactivate antioxidant enzymes [13]. The phenolic compounds of *Piper betle* inhibit the generation of free radicals that help to mediate antidiabetic effects [14].

The aqueous extract of betel leaves investigated as a hypoglycaemic activity when tested in fasted normoglycaemic rat [15]. In glucose tolerance test, betel leaves markedly decreased blood glucose level, glycosylated haemoglobin and decreased activities of liver glucose-6-phosphatase and fructose-1, 6- bisphosphatase, whereas liver hexokinase increased in Streptozocin (STZ) diabetic rats compared with untreated diabetic rats. The ability of this lowering blood glucose level of Streptozocin (STZ) induced diabetic rat provides an idea that the extracts have the insulinomimetic activity [11]. Research conducted on *Piper betle* in Sri Lanka showed that both of hot water extracts (HWE) and cold ethanolic extract (CEE) of *Piper betle* leaves have hypoglycemic activity [16].

In glucose tolerance test revealed that HWE, CEE and tolbutamide lowered the blood glucose level of rats with STZ induced diabetes treated with a dose (50 mg/kg). This research reported that *Piper betle* extracts have insulin omimetic activity (lowering blood glucose level) [17]. Many works have observed elevation in transaminase activity (SGOT and SGPT) in liver and serum in diabetic rat [18]. The restoration of SGOT and SGPT to their respective normal level after treatment with *Piper betle* strengthens the antidiabetogenic effect in this plant. Moreover, SGOT and SGPT level also act as indicators of liver function and restoration of normal level of these parameters indicate normal function of liver [19].

The present results are complementary with the findings of other workers [20] as *Piper betle* juice treated rats showed improvement in organ (heart, liver, kidney and pancreas) weight as compared to diabetic control rats. *Piper betle* extracts are free from unacceptable side effects even following chronic administration. There were no overt signs of toxicity, hepatotoxicity or renotoxicity [11]. In conclusion, our results demonstrate the antidiabetic activity of Piper betle leaves grown in Bangladesh show its therapeutic potential to be used as a cost effective safe herbal antidiabetic agent.

CONCLUSION: Diabetes mellitus is becoming a health-care problem worldwide, with the raise in disease prevalence being all the more worrying as it not only affects the developed world but also developing nations with fewer resources to cope with yet another major disease burden**.** Current therapies used for diabetics have side effects, so the current shift to the use of herbal preparations may be more effective, relatively low cost, less side effect and low toxicity. The present data indicates that *Piper betle* juice possesses potential as an antidiabetic action.

ACKNOWLEDGEMENT: The author would like to thank to the department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh for supporting the technical assistance.

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How to cite this article:
Mst. Khatun M, Md. Sapon A, Md. Hossain S and Md. Islam R: Antidiabetic Activity of *Piper Betle* in Alloxan Induced Type 1 Diabetic Model Rats. Int J Pharm Sci Res 2016; 7(2): 675-80.doi: 10.13040/IJPSR.0975-8232.7(2).675-80.