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Research Article

ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF YOUNG BAMBOO SHOOT MELOCANNA BACCIFERA AND CHAKHWI – TWO TRADITIONAL TRIBAL FOOD INGREDIENTS OF TRIPURA

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ABSTRACT

Diabetes was induced in Wistar rats with STZ. Animals were divided into four groups, while one normal control, one diabetic control and the remaining two groups were treated with bamboo shoot (Muia) and chakhwi - a prepared food ingredient. After 24 hours of Diabetes induction by STZ at dose of 65 mg/kgbw intraperitoneally , treatment was started for 14 days with sample Muia and Chakhwi on rats of group III and IV. The Experiment was carried out through few biochemical estimations such as serum cholesterol, urea, triglyceride and creatinine. Study showed a significant fall in blood glucose level and other supporting parameters with the treatment of Muia and Chakhwi. Further, Muia treated diabetic rats also showed marked improvement in body weight in compare to diabetic control. Treatment with Muia, and Chakhwi shows significant reduction in blood glucose level with preventing loss of body weight compare to diabetic control.

INTRODUCTION

Tripura is a small but beautiful hilly state. It is one of the states of North-Eastern part of India, commonly known as Seven Sisters State. Moreover, it is a land of tropical climate, generally hot and humid with maximum temperature near about 35^0C and minimum 11°C. In the month of October, temperature comes down and cool dry winter season begins from November and continues till February (Mazumdar, P).

As per census-2011, the population of Tripura stands at 36,73,917. Schedule Tribes (ST) population of Tripura is about 8,53,920 (30.95%), out of which about 6,79,720(79.59%) population are within the TTAADC(http//en.m.wikipedia….) Nutritional biodiversity among the Tribal people of Tripura is well known. Generally they use less amount of spices and least amount of oil. Maximum of them take dry fish in each recipe. In this part of research, we are concentrating on one prepared food ingredient *chakhwi* and one plant originated raw food ingredient- *muia*. Tribal people of Tripura mix *chakhwi* with almost all curry. *Muia* is also used daily & vigorously by the tribal people by allowing it as one of the major ingredient of almost all curry.

Nutrition has great deal for survival of society & for its people and exploration of the nutritional status is still unfolded in case of Tribal people of Tripura. That is why concentration is given on searching the antidiabetic effect of *Muia* and *Chakhwi*.

METHODS AND MATERIALS

The experiment was conducted in vivo in rats which was carried out in TMC (Tripura Medical College & Dr. B. R. Ambedkar Teaching Hospital), Hapania, Agartala, Tripura. The Ethical No. of TMC is 1006/GO/ac/06/CPCSEA. The experiment lasted for 16 days.

Methods of preparation of different tribal dishes along with food ingredients was come to know by conducting a survey, through an eventually prepared printed format, extensive interviews among the ten numbers aged personalities and housewives.

The food ingredient *Chakhwi* prepared as per standard procedure(Das, P.), as published by Tripura Tribal cultural Research Institute & Museum (Govt. of Tripura) was subjected for Antidiabetic activity. The raw ingredients i.e. tender shoot of bamboo (*Melocanna baccifera, family: Poaceae*) (known as

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Muia in *Kokborok*) was also subjected for methanolic extraction and then was studied for Antidiabetic activity.

Preparation of Extract of Muia-Sample-1

Very young stem of bamboo *(Melocanna baccifera)* i.e. *muia* was collected. Removing the outer shell and internodes, 2 kg of *muia* was pieced (1.5mm in length). Certain compounds may get denatured in sunlight, so it was dried under shade to avoid decomposition and followed by grinding into fine powder by electric grinder(BAJAJ, REX 500, India). After shed dry, it was 113.4 gm and soaked into 400 ml methanol for 7 days. The extract was filtered through cotton plug followed by vaccum suction. The filtrate was obtained. Further the filtrate was allowed to dry to get powder like substance.

Though *muia* is taken by Tribal people either raw or cooked preferably, then also the methanolic extract preferred to have ingredients as extracts in raw form could not be preserved due to fungal/bacterial decomposition & cooked extract contains some other ingredients from other vegetables/spices also.

Chakhwi was prepared in present study from ash of bamboo only. Therefore the results will reflect the comparative assessment in two different form of bamboo and bamboo shoots.

Preparation of Chakhwi-Sample -2

To prepare *chakhwi*, dry stem and shoots of bamboo (M. baccifera) was allowed to burn. The burnt ash was taken in a specially prepared basket called *cheyakhok* in *Kokborok* (The popular Tribal Language/dialect). The basket is hanged from a suitable support & a container is kept below it to collect the extract of ashes. The water is poured slowly on ash to bath the whole ash. This extract is collected in the container which is known as *chakhwi. Chakhwi* was also allowed to evaporate under very low flame. When the water portion was evaporated entirely, the whitish substance like powder was obtained from the bottom of the container and allowed to dry which is treated as sample 2.

Animals and Experimental Protocol

The antidiabetic activity by observing the hypoglycaemic effect was carried out in streptozotocin (STZ) induced diabetic rats by following standard method (Ghosh 2005, Pillai & Qudry 1995, Deb, L. et.al 2006). Albino wistar rats were selected for the experiment. After 48 hours, diabetic condition was confirmed by use of a glucometer (Dr. Morepen glucoone – BG-03) using glucose reagent strip[7].The animals display more than 200-300 gm% of blood glucose was selected for the study, as per the information obtained in references.

Dose and route of administration = 65 mg/kgbw Streptozotocin (STZ) in saline, (0.9% solution) intraperitonealy & Sample 150 mg/kgbw/p.o/day. The sample administrated as suspension in dist. water.

1. Albino rats of weighing between 150 to 250 gm were used.
2. The rats were randomly divided into four groups of six rats each marked and maintained on a standard fed and water adlibitum throughout the experiment.
3. Diabetes was induced in groups II, III & IV rats by injecting them with 65 mg/kg body weight Streptozotocin **(STZ)** in saline (0.9%), administered through the intraperitoneal route after fasting the animals for overnight (The rats were provided with 5% glucose solution for the next 24hrs to prevent hypoglycemic shock).
4. Animals in III groups and IV was selected for the treatment with sample drug 150 mg/kg bw after diabetic condition was confirmed with single dose and the treatment was continued for next 14 days. While group I rats served as the normal control and was treated with purified water. During this period animals in all groups had fed standard diet and water.
5. The body weights of the animals in all the groups were recorded daily throughout the duration of the experiment. Blood glucose levels was assessed in all the animal groups by using blood from the tail vein of the rats.
6. On 1st day of treatment and on 7th day blood samples (one time) was collected from overnight fasted rats from tail vein for estimation of blood glucose level.

Finally on 14th day blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for estimation of blood glucose level. Plasma was separated and subjected to biochemical estimation such as serum cholesterol, urea, triglyceride and creatinine. Biochemical parameters were assayed by using assay commercial kits (Erba Mannhein, India) and AU480 Chemistry analyzer (Beckman Coulter).

Biochemical Markers

Analysis of Blood Glucose level

The blood glucose level was analysed with glucometer using glucose reagent strip (Solanki and Bhavsar 2015)

Serum Urea

Serum urea was estimated by a method as per assessment kit (Take, Tiffany *et al*.)

Serum Cholesterol

Serum cholesterol was estimated using cholesterol reagent by a method as per assessment kit(Allain and Roeschlau 1974).

Serum Triglyceride

Serum triglyceride was estimated using triglyceride reagent by a method as per assessment kit(McGowan, Fossati. *et al*.)

Serum Creatinine

Serum creatinine was also estimated using creatinine reagent by a method cited in reference(Bowers, L.D. *et al*. 1980).

Statistical Analysis

The Values are expressed as Mean±SEM where n=6. Data obtained were analysed using the analysis of variance and Microsoft excel 2007. Copmarisons between different groups was carried out by unpaired two tailed Student's t test. Differences were considered statistically significant at ***$p<0.001$ or **$p<0.05$ when compared with 7th day vs 14th day blood glucose level of Diabetic control, Test groups, 1st day vs 14th day blood glucose level of Test groups. In control group, there is no significant difference in blood glucose level

when compared day vs day (paired two tailed student's t-tes). In diabetic control, there is no statistical significant difference when compared 1st vs 7th day and 1st vs 14th day blood glucose level. ($p>0.05$). *Chakhwi* significantly reduces blood glucose when compared day vs day. Differences of blood glucose level were considered extremely significant at ***$p<0.001$ or **$p<0.05$ when compared with Normal control vs Diabetic control, normal control vs Test groups, on 1st day, 7th day and 14th day, Diabetic vs *Muia* on 14th day, Diabetic vs *Chakhwi* on 14th day. But there was no significance difference when compared with Diabetic vs test groups on 1st day, 7th day, test group vs test group on 1st, 7th and 14th day.

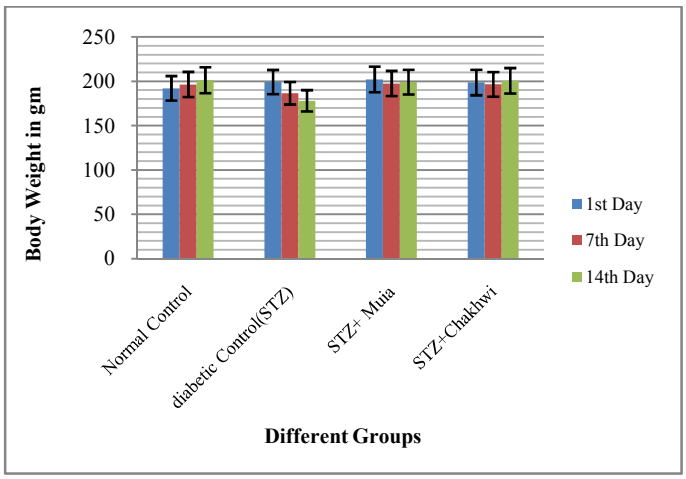
RESULTS

STZ injected Wistar rats had produced cardinal sign of diabetes i.e. change in body weigt is shown on Table 1 (Fig 1). Chronic treatment with *Muia* in diabetes prevented weight loss as compared to diabetic control rats. There was significant reduction seen in blood glucose level of *Muia* treated diabetic animals as compared to DC rats (Table 2, Fig 2) Blood glucose level was significantly inceased in diabetic animals(454 ± 37.888) on 14th day while methanolic extract of *Muia* treated animals showed significant reduction in blood glucose level(270.33± 20.57). *Chakhwi* reduced the blood sugar level on 14th day very significantly (219.167±27.18.)

Results of biochemical parameters are also shown in Table 3 (Fig 3 and Fig 4). Serum cholesterol level increased about 3 fold in diabetic control. *Muia* treated group showed a highly significant effect in controlling serum cholesterol level in diabetic animals. *Muia* also decreases the level of creatinine and triglyceride in diabetic animals significantly. Treatment with *Chakhwi* showed a significant reduction of serum urea and creatinine level.

Table 1 Effect of STZ, *Muia and Chakhwi* on body weight on diabetic rats.

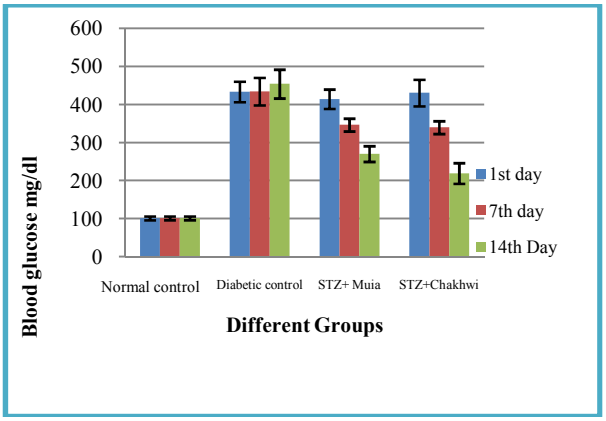
Sl.No.	Group	Body Weight in gm		
		1st Day	7th Day	14th Day
1.	Normal Control (Mean ± SEM)	192.08 ± 13.772	196.42 ± 14.111	201.17 ± 14.52
2.	Diabetic Control (Mean ± SEM)	199.08 ± 13.619	186.5 ± 12.698	178 ± 11.942
3.	STZ + *Muia* (Mean ± SEM)	202 ± 14.445	197.41 ± 14.187	199.08 ±13.939
4.	STZ + *Chakhwi* (Mean ± SEM)	198.6 ± 14.423	196.5 ± 13.872	200.5 ± 14.386



Error bars in the graph represent the mean ± standard error
Fig 1 Body weight of Rats of different groups on 1st, 7th and 14th Day

Table 2 Blood glucose level

Sl.No.	Groups	Blood glucose level (mg/dl)		
		1st Day	7th Day	14th Day
1	Group-I: (Normal control) Mean ± SEM	101.33 ± 5.11	101.33 ± 4.904	101.33 ± 4.773
2	Group-II: (Diabetic Control) Mean ± SEM	433.5 ± 26.83	434.33 ± 36.126	454.0 ± 37.888
3	Group-III: (*Muia)* Mean ± SEM	414.17 ± 25.51	346.33 ± 16.750	270.33 ± 20.570
4	Group-IV: (*Chakhwi)* Mean ± SEM	430.5 ± 35.12	339.67 ± 16.750	219.167 ± 27.183



Error bars in the graph represent the mean ± standard error
Fig 2 Blood glucose of Rats of different groups on 1st, 7th and 14th day

Table 3 Biochemical parameters of diabetic rats after 14 days.

Sl. No.	Groups	Cholesterol (mg/dl)	Urea (mg/dl)	Triglyceride (mg/dl)	Creatinine (mg/dl)
1.	Group-I: (Normal control) Mean ± SEM	49.252 ± 5.496	27.47 ± 2.879	89.177 ± 6.219	0.545 ± 0.0377
2.	Group-II: (Diabetic C.) Mean ± SEM	138.49 ± 13.426	64.59 ± 5.773	318.973 ± 24.561	1.251 ± 0.153
3.	Group-III: (*Muia)* Mean ± SEM	68.90 ± 4.555	60.467 ± 3.877	182.867 ± 17.316	0.91 ± 0.184
4.	Group-IV (*Chakhwi*) Mean ± SEM	114.45 ± 4.531	34.735 ± 3.319	278.832 ± 31.206	0.64 ± 0.043

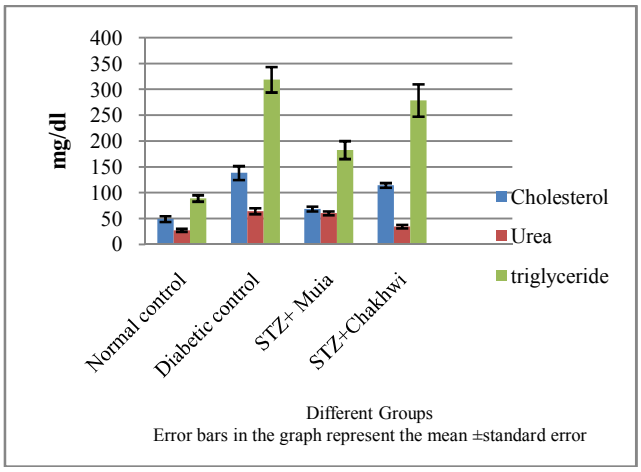
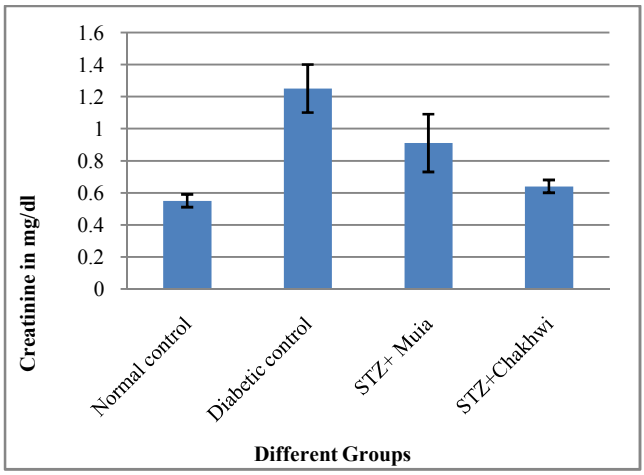


Fig 3 Biochemical data in Diabetic & normal Rats



Error bars in the graph represent the mean ± standard error
Fig 4 Creatinine level in Diabetic and Normal Rats

DISCUSSION

Streptozotocin causes direct DNA damage to the pancreatic islets of beta cells, which leads to hyperglycaemic state(Deb, Dutta. *et al*. 2006)

STZ causes a massive reduction in insulin release by the destruction of the beta cells of Islets of Langerhans of Pancreas and induces hyperglycaemia. *Muia* and *chakhwi* reverse this effect may be by increasing either the pancreatic secretion of insulin from beta cells or its release from bound form(Prince *et al* 1998)[13] . In our present study, we have observed a significant increase in the fasting blood glucose in STZ treated groups, *Muia* and *Chakhwi* can significantly reverse hyperglycaemic activity that confirmed hypoglycaemic potential of *Muia* and *Chakhwi.* The antidiabetic activity was assessed by monitoring fasting blood glucose levels and change in the body weight at 1st day, 7th day and 14th day. If the treatment is continued for another 14 days blood glucose level may come to normal in diabetic animals.

Other supporting data such as serum cholesterol, serum creatinine, serum urea and serum triglycerides was performed to substantiate the anti-diabetic action of the test samples. The loss of body weight was noted in STZ induced diabetic rats, the recovery of body weight were observed in increasing order with samples after 7th day. We have observed that the lipid profiles(cholesterol and triglycerides) level was increased significantly and showed a decreasing tendency in comparison with the diabetic control animals. In case of decreasing trend of cholesterol by *Muia* is considered more active when compared to *Chakhwi*. These tendencies were advantageous to the diabetic conditions where these could put on Hallmark to the atherosclerosis and other delayed diabetic complications arising due to alteration of fat metabolism. The problem of hypercholesterolemia is more common in patients with diabetic mellitus. This will increase the risk of premature coronary heart disease. Lipid peroxide mediated tissue damage has been observed in the development of both type of Diabetes i.e. Type I and type II diabetes. It has been observed that insulin secretion is closely related to lipoxygenase derived peroxides(Prince *et al* 2004). Increased concentration of lipid peroxide in the liver can result in decreased activity of cytochrome P_{450} which may affect the drug metabolism activity in chronic diabetes (Deb, Bhattacharjee . *et al*.).

In our study, blood urea and creatinine levels also increased significantly in STZ induced animals and also showed a declining trend in test groups. It is fact that the kidney function is disturbed in diabetic conditions leading to elevated level of blood urea and creatinine. The test sample Chakhwi may have almost normalized the kidney function as indicated by the reversal of serum urea and creatinine. In case of decreasing trend of urea and creatinine by the *chakhwi* is considered extremely significant when compared to STZ and *Muia* too. It is clear from the Table that both the Tribal food ingredients have the antidiabetic activity but *Chakhwi* has shown maximum antidiabetic efficacy as compared to *Muia*. *Muia* has showed its activity by reducing the cholesterol level significantly.

CONCLUSION

The diabetes mellitus is a disease that can be called as silent killer. To control such disorder, with other than insulin, could be possibly achieved through the plant sources. It can be concluded that *Chakhwi* and *muia* could be potent antidiabetic component for the next generation. Further intensive studies on search of active ingredients may confirm about the responsible ingredients incase of *Chakhwi* showing the antidiabetic activity.

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