Research paper

Study of the hypoglycemic effect of *Tamarindus indica* Linn. seeds on non-diabetic and diabetic model rats.

Abbreviation:

STZ = Streptozotocin
BW = Body weight
GOD-POD = Glucose-Oxidase and Peroxidase
GI = Gastro intestine
SPSS = Statistical Package for Social Sciences
SEM = Standard error of mean
SD = Standard Deviation
OGTT = Oral glucose tolerance test
DM = Diabetes Mellitus
i.p = intraperitonial

ABSTRACT

Aim: The present study was undertaken to evaluate the antidiabetic effects of the *Tamarindus indica* Linn seed in normal (non-diabetic), type-I and type-II model rats and to investigate their effect on gastrointestinal motility and intestinal glucose absorption.

Methodology: *T. indica* seed powder was used at a dose of 1.25g/kg bw/10 ml water. Male Long-Evans rats (160-210g body weight) were used for the experiment. Experiments were done in non-diabetic and streptozotocin-induced diabetic model rats with a single feeding in different prandial states and blood was collected. An intestinal perfusion technique was used to study the effects of *T. indica* seed powder on intestinal glucose absorption in normal and type-II model rats. Gut motility was evaluated using barium sulfate milk. Glucose was measured by Glucose oxidase-peroxidase (GOD-POD) method.

Result: The screening results showed that *T. indica* seed powder had no effect on fasting or postprandial serum glucose level of normal and type-I diabetic rat. The seed powder also showed no hypoglycemic effect in the fasting state and no antihyperglycemic effect in type-II model rats when fed simultaneously with oral glucose load, but it exhibited significant antihyperglycemic effect when the seed

powder was fed 30 minutes prior to the glucose load at 105 minutes ($p<0.03$). Glibenclamide significantly lowered postprandial serum glucose levels of non-diabetic and type-II diabetic model rats ($p<0.02$-0.001). *T. indica* exerted inhibition on glucose absorption in type-II rats during the whole perfusion period when compared with control. On the other hand, *T. indica* seed powder significantly inhibited the gastrointestinal motility in type-II rats.

Conclusion: The present data suggest that *T. indica* possesses antihyperglycemic properties in type-II rats which are at least partly due to its inhibitory effect on intestinal glucose absorption. This effect cannot be attributed to the acceleration of intestinal transit.

KEY WORDS: Anti-hyperglycemic, *Tamarindus indica,* streptozotocin, type-I diabetes, type-II diabetes, gastro intestine.

1. INTRODUCTION

Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin. It is the most prevalent chronic disease in the world affecting a large population and its prevalence is about 6.8% [1].

Hyperglycemia and hyperlipidemia are two important characteristic of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus [2]. Though pharmaceutical drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications [3, 4]. In recent years, there has been renewed interest in plant medicine [5, 6, 7] for the treatment against different diseases as herbal drugs are generally out of toxic effect [8, 9] reported from research work conducted on experimental model animal. Although in human, whether there is any toxic effect are not investigated but anti-diabetic effect of various plants having “folk medicine reputation” have been screened [10].

Tamarindus indica Linn. (*family*- Caesalpiniaceae [Fabaceae]), locally known as Tetul tree, is found throughout the South Asian region and some portions of Africa. It is a large ample, evergreen tree, 12-18 m high with round bushy crown and comparatively smaller bole. Phytochemical investigation revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides, L-(-)mallic acid, tartaric acid, the mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid [11, 12]. The fruit pulp contains large quantities (16-18%) of tartaric, citric, malic and acetic acids, potassium tartrate, invert sugar, gum and pectin. It also contains traces of oxalic acid. Seed testa contains a fixed oil. Seeds cotyledons contain albuminoids, fat and carbohydrates [13, 14]. Traditional healers claim that the seed of the plant possess antidiabetic properties. Scientific reports also support the hypoglycemic activity of this plant [15, 16, 17, 18]. However, no published report supports the underlying mechanism of the hypoglycemic effect of *T. indica* seed powder in normal, STZ induced type-I and type-II diabetic model rats.

The purpose of this work was to explore the possible hypoglycemic / anti-hyperglycemic activity of *T. indica* seed extract in type-I and type-II diabetic rats as well as to investigate the possible mode of action beyond this activity.

2. MATERIALS AND METHODS

2.1. Plant materials and preparation of test sample

Tamarindus indica Linn. seeds were collected from Kushtia, a district of Bangladesh. The plant was identified by the Bangladesh National Herbarium (voucher specimen no. DACB-38203). The seeds were collected from the fresh fruit pulp and were washed carefully and dried for two days at 40^{o}C in an oven. Then the seeds were crushed in an electric grinder to make fine powder. Afterwards the powder was stored immediately in the refrigerator at -20^{0}C and kept in the same temperature up to end of the experiment.

2.2. Experimental animals

The study was conducted on adult male Long-Evans rats (weighing 160-210g) bred at the BIRDEM animal house maintained at a constant room temperature of 22±5^{0}C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure

when animals were used after 12hrs fasting. The rats had no access to food during the whole period of blood sampling. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

2.3. Induction of diabetes in rats

Diabetes stimulating type-I was induced by a single intra-peritoneal injection of streptozotocin (STZ, Upjohn Company, Kalamazoo, MI USA) dissolved in 0.1 M citrate buffer, pH 4.5, at a dose of 65mg/kg body weight to adult rats [19]. On the 7^{th} day rats (fasting blood glucose ≥18mmol/l) were taken for carrying out the experiments.

Type-II diabetes was induced by a single intra-peritoneal injection of STZ at a dose of 90mg/ kg body weight, in citrate buffer to the 48 hours old pups as described by Bonner *et al.* [19]. Experiments were carried out 3 months after STZ injection an oral glucose (2.5g/kg bw) tolerance test was performed to check the blood glucose levels of the rats. Rats having blood glucose level 8-11 mmol/l at fasting condition were taken to carry out the experiments.

A total number of 250 rats were used to carry out the experiments, which include normal, type-I and type-II diabetic model rats. The animals were divided into 3 groups: Gr 1- water control group; Gr 2- glibenclamide treated positive control group in case of type-II diabetic model rats and inulin treated in case of type-I; Gr-3 *T. indica* seed powder treated group. Number of rats were 6-8 rats in each group.

2.4. Acute effect on fasting and postprandial glucose level

2.4.1. Fasting condition

The powder (1.25g/kg bw/10ml) was fed to overnight fasting (12hrs) rats and blood samples were drawn at 0, 60 and 120 minutes [20]. The positive control group received glibenclamide (5mg/kgbw) for normal and type-II rats and insulin (10µl/rat) for type-I rats whereas the control group received only water (10ml/kg bw) [20]. Blood samples were collected by amputation of the tail tip under mild ethar anesthesia. The rats were kept unfed throughout the experimental period.

2.4.2. Postprandial condition

T.indica seed powder (1.25 g/kg bw), with or without glucose (2.5g/10ml/kg bw), were fed to overnight fasted (12hrs) rats and blood samples were drawn at 0, 30, and 75 min when fed simultaneously with

glucose and at 0, 60 and 105 min when fed 30 min prior to glucose load. Both positive control and water control rats were fed with glucose solution (2.5g/10ml/kg bw) and water (10ml/kg bw) following glucose load [21].

2.5. Effect of T. indica seed powder on intestinal glucose absorption

An intestinal perfusion technique [22] was used to study the effects of *T.indica* seed powder on intestinal absorption of glucose in nondiabetic and type 2 diabetic rats fasted for 36 hours and anesthetized with sodium pentobarbital (50 mg/kg). The seed powder were added to a kreb's solution (g/L 1.02 $CaCl_2$, 7.37 NaCl, 0.20 KCl, 0.065$NaH_2PO_4.6H_2O$, 0.6 $NaHCO_3$, pH 7.4), supplemented with glucose (54.0 g/L) and perfused at a perfusion rate of 0.5 mL/min for 30 min through the duodenum. The perfusate was collected from a catheter set at 40 cm. *T. indica* seed powder were added to kreb's solution to a final conc. of 25 mg/mL so that the amount of seed powder in the perfused intestine is equivalent to the dose of 1.25 g/kg. The control group was perfused only with kreb's buffer supplemented with glucose. The results were expressed as percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

2.6. Gastrointestinal (GI) motility test:

Gastrointestinal motility was evaluated by using barium sulfate ($BaSO_4$) milk method [23].

The experiment was carried out by the method previously described by Chatterjee (1993). Distilled water at a dose of 10ml/kg bw was fed with smooth metallic tube to control rats and *T indica* powder (1.25g/kg bw/ 10ml) was fed to treated group. Baso4 milk was prepared by adding $Baso_4$ as 10% w/v in 0.5% CMC suspension. The milk was given to rats after 1 hour of adminstration of the test material. The rats were sacrificed 15 minutes after the adminstration of the milk. Before sacrificing the rats were anesthetized with di-ethyl ether. Then the abdominal part was opened and the intestinal part from pylorous to the ileoceccal junction as a total length of GI was taken into a petridish filled with distilled water for washing. It was then soaked by tissue paper to make it dry and taken into a white paper marked with 100 cm scale for measurement. The total gastrointestinal tract was measured first. Then the length traversed by $Baso_4$ was measured (white color). This length traversed by $Baso_4$ was expressed as a percentage of the total length of small intestine and the result of the test group was compared with that of control group (Chatterjee1993).

2.7. Biochemical analysis*:*

Serum glucose was measured on the same day by glucose-oxidase method (GOD-POD) using a commercial kit (Boehringer-Mannhein, GmbH, Germany) (Sera Pak, USA).

2.8 Statistical analysis:

Data from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for Windows version 12 (SPSS Inc, Chicago Illinois, USA). Values were expressed as mean±SD (Standard Deviation), Analysis of variance (ANOVA, Bonferroni Post Hock Test) and independent sample 't'-test were done as the test of significance $p \leq 0.05$ was considered as the minimal level of statistical significance.

3. RESULTS

3.1. Acute effect of Tamarindus indica seed powder on blood glucose level of normal (non-diabetic), type- I and type- II diabetic model rats

Blood glucose level was analyzed at the fasting level and the results showed that the seed powder had no significant effect on the fasting state of normal rats but lowered the serum glucose level 2.43% at 60 min and 7.68% at 120 min. As expected glibenclamide, the positive control, lowered serum glucose level significantly both at 60 minutes (p=0.000) and at 120 minutes (p=0.000) compared with water control and powder treated groups (Table 1).

Table 1: Effect of *T .indica* seed powder on fasting serum glucose level (M±SD) of normal, type- I and type- II diabetic model rats:

Group	Min 0 (mmol/l)	Min 60 (mmol/l)	Min 120 (mmol/l)
Normal rat			
Water control(n =6)	8.05±0.51	7.63±0.49	6.99±0.73
Glibenclamide(n =6)	8.39±0.76	5.67±0.88*	4.83±0.48*
***T. indica* seed powder (n =8)**	7.82±0.68	7.63±0.41	7.22±0.61
Type I diabetic model rat			

Water control **(n = 6)**	22.04±1.28	20.53±3.09	19.40±3.92
Insulin **(n =6)**	21.49±1.58	4.30±1.87*	2.32±0.37*
T. indica **seed powder (n =6)**	21.38±1.51	20.84±1.90	19.51±2.66
Type-II diabetic model rat			
Water control(n =6)	8.35±1.57	8.87±2.50	10.53±3.88
Glibenclamide(n =6)	8.55±1.55	8.01±1.91	7.42±1.56*
T. indica **seed powder (n =8)**	9.10±1.12	9.93±2.37	9.57±1.94

Results are expressed as mean ±standard deviation (M±SD). One-way ANOVA (Bonferroni test) was done for comparing the different group and for the test of significance. *P= 0.000 when compared with water control; n=number of rats.

The oral glucose tolerance test (OGTT) was performed and the results showed that powder had glucose lowering effect but non-significantly and the glibenclamide treated group showed a significant fall in serum glucose level at 75 minutes (p=0.000) (Table 2).

Table 2: Effect of *T .indica* seed powder on serum glucose level (M±SD) of normal, type-I and type-II diabetic model rats when seed powder was fed simultaneously with glucose load:

Group	Min 0 (mmol/l)	Min 30 (mmol/l)	Min 75 (mmol/l)	iobv
Normal rat				
Water control(n = 7)	6.38±0.51	8.36±0.68	7.79±0.75	3.39±1.71
Glibenclamide**(n = 6)**	5.84±0.66	7.27±0.38	5.55±0.78*	1.14±2.18
T. indica **seed powder (n = 7)**	6.15±1.21	8.64±0.90	7.92±0.63	4.26±3.28
Type-I diabetic model rat				
Water control(n = 7)	20.90±3.33	26.90±2.62	25.25±3.79	10.35±7.06

***Insulin*(n = 6)**	21.86±2.16	18.38±6.48*	9.15±4.77**	-16.19±9.95
***T. indica* seed Powder (n = 6)**	20.88±1.80	28.67±2.56	25.63±3.23	12.53±6.35
Type-II diabetic model rat				
Water control(n = 6)	7.64±1.42	17.10±4.24	17.36±3.42	19.17±8.80
***Glibenclamide*(n = 6)**	9.01±1.40	16.25±0.98	12.72±0.90*	10.95±3.01
***T. indica* seed powder (n = 7)**	8.21±2.10	16.97±1.86	15.49±2.40	16.03±6.24

Results are expressed as mean ±standard deviation (M±SD). One-way ANOVA (Bonferroni test) was done for comparing the different group and for the test of significance. *P= 0.000 when compared when compared with water control; Iobv =Sum of the increments over the basal value. n=number of rats.

When fed 30 min before to glucose load, it was found that glibenclamide treated group showed significant blood glucose lowering effect at 60 minutes (p = 0.001) and 105 minutes (p = 0.000) normal and type-I diabetic model rats in comparison to water control and powder treated group (Table 3). T. indica seed power showed significant (p = 0.003) blood glucose lowering effect at 105 min. on type-II diabetic model rats in Comparison to water control and glibenclamide treated group (Table 3; Fig. 1).

Table 3: Effect of *T .indica* seed powder on serum glucose level (M±SD) of normal, type-I and type-II diabetic model rats when seed powder was fed 30 minutes before to glucose load:

Group	Min 0 (mmol/l)	Min 60 (mmol/l)	Min 105 (mmol/l)	iobv
Normal rat				
Water control (n =6)	6.64±1.09	9.05±0.61	8.51±0.70	3.27±2.86
Glibenclamide (n =6)	6.85±1.23	5.37±0.93*	3.83±0.48**	-4.49±1.90
T.indica* seed *Powder (n=8)	6.87±0.82	8.00±0.78	8.30±0.63	2.55±1.85
Type-I diabetic model rat				
Water control(n = 6)	24.26±2.03	31.71±3.62	30.78±1.20	9.97±6.66

***Insulin*(n = 7)**	22.99±2.09	11.47±5.84 *	8.28±3.62*	-26.22±6.06
T. indica* seed *Powder* *(n = 6)	23.79±2.41	32.12±4.06	28.08±2.42	12.62±8.42
Type-II diabetic model rat				
Water control (n = 6)	7.96±1.79	19.65±4.36	19.30±4.27	23.01±9.99
***Glibenclamide* (n = 6)**	9.05±1.78	18.99±5.37	16.09±4.68	19.98±8.91
***T. indica* seed Powder (n = 6)**	9.59±1.72	17.10±2.31	14.65±2.82*	12.57±2.84*

Results are expressed as mean ±standard deviation (M±SD). One way ANOVA (Bonferroni test) was done for comparing the different group and for the test of significance. **P= 0.000.and. *p= 0.001.+ when compared when compared with water control; Iobv =Sum of the increments over the basal value. n=number of rats.

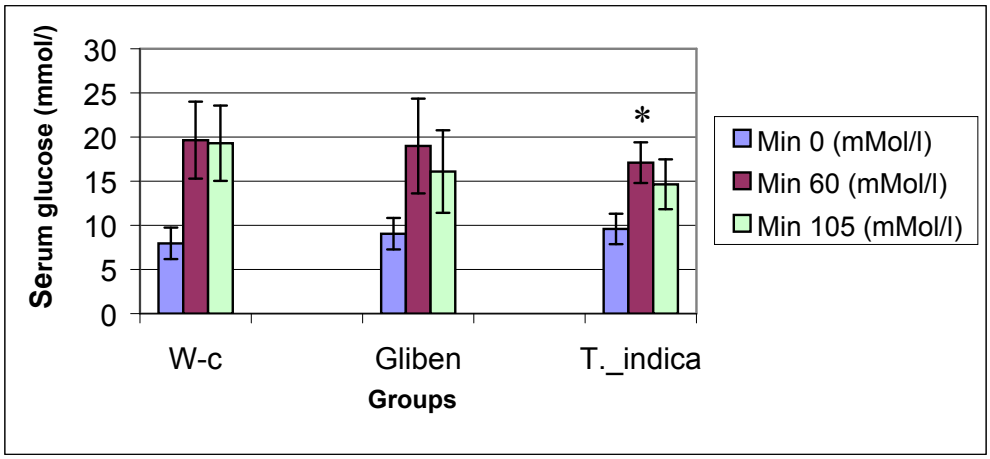

Figure 1: Acute effect of T. indica seed powder on serum glucose level on Type II diabetic model rats at 105 min. when feed at 30 min. before to glucose load. W-c=Water control.

3.2. Effect of Tamarindus indica seed powder on gastrointestinal motility

The length of GI traversed by $BaSO_4$ milk in powder treated group was lower than the water treated group. The inhibition in motility was not statistically significant in treated group of normal rats (Table 4).There was decreased percentage of length traversed by BaSO4 with seed powder on type-II

model rats in comparison to water control group. *T. indica* seed powder showed significant inhibitory effect on gastrointestinal motility (p=0.02).

Table 4: Effect of *Tamarindus indica* Linn seeds powder on gastrointestinal motility test by $Baso_4$ milk of normal and type-II diabetic model rats:

Group	GI total length (cm)	Length traversed by $BaSO_4$ (cm)	% of Length traversed by $BaSO_4$
Normal rat			
Control (n=6)	118.33±9.83	56.33±6.18	47.93±7.04
***T indica* Seed Powder (n=6)**	123.83±6.11	47.67±7.86	40.77±9.77
Type-II diabetic model rat			
Control (n=5)	121.00±5.47	58.00±6.78	47.89±4.88
***T indica* Seed Powder (n=5)**	121.00±9.61	48.00±8.09	39.46±4.31*

GI= Gastro Intestine. Data are presented as mean ±standard deviation (M±SD) and Group was Compared by using independent samples't' test.*p =0.02. n=number of rats.

3.3. Effect of T indica seed powder on upper intestinal glucose absorption

It is evident that the upper intestinal glucose absorption was almost constant during 30 minutes of perfusion with glucose. When the seed powder solution was supplemented with the glucose solution, it showed no effect on intestinal glucose absorption in normal rats (Figure 2).

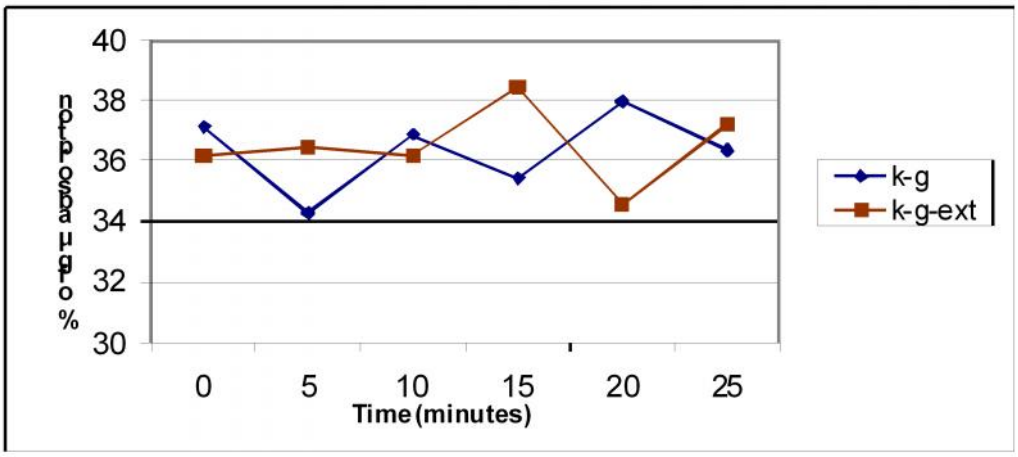

Figure 2: Effect of the *T. indica* seed powder on upper intestinal glucose absorption on normal rats.

Results are presented as mean± SD (n=5). Rats were fasted for 36 hours and intestine was perfused with glucose solution (54 g/l) with or without *T. Indica* seed powder (25mg/ml); glu= glucose, K-g= Kreb's solution, K-g-ext= Kreb's solution + glucose +seed powder juice.

In case of type-II model rats, intestinal glucose absorption was nearly constant during the 30 min of perfusion with glucose. There was a decrease in glucose absorption with glucose solution when supplemented with seed powder solution (Figure 3).

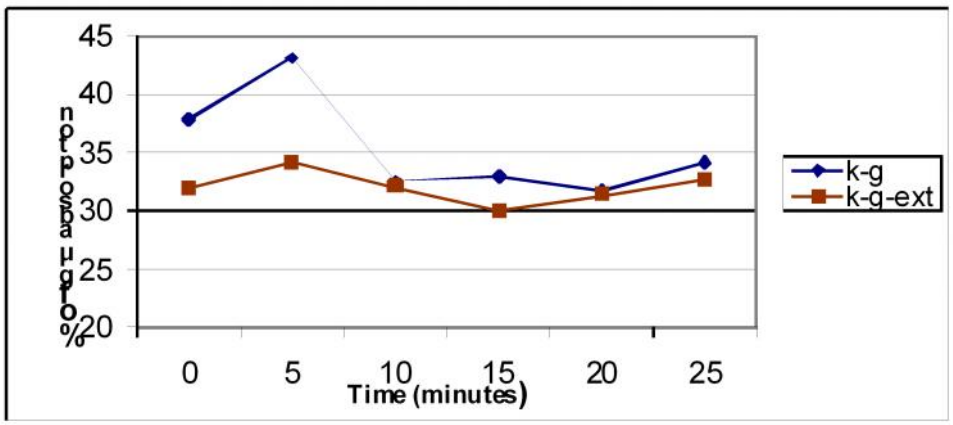

Figure 3: Effect of *T. indica* seed powder on upper intestinal glucose absorption on type-II diabetic rats.

Results are presented as mean± SD (n=6). Rats were fasted for 36 hours and intestine was perfused with glucose solution (54 g/l) with or without *T. Indica* seed powder (25mg/ml); glu= glucose, K-g= Kreb's solution, K-g-ext= Kreb's solution + glucose + Extract.

It denotes that *T indica* seed powder strongly affected the amount of absorbed glucose throughout the notable period of experiment in type-II rats. Figure 3 depicts the gradual fall in glucose absorption during the whole perfusion period in type-II rats compared to Krebs solution. Therefore, the obtained results suggest that seed powder of the *T. indica* delays glucose absorption in the upper part of the gastrointestinal tract.

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4. DISCUSSION

Synthetic drugs such as Sulphonylureas and Biguanides are valuable in the treatment of DM, but their uses are restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects [24]. Moreover, these therapies only partially compensate for metabolic derangements seen in diabetes and do not necessarily correct the fundamental biochemical lesion [25]. As the incidence of diabetes is rising relatively around the world, there is an urgent need to expand the range of effective palliatives available to patients.

The present study has been undertaken to screen the hypoglycemic and anti-hyperglycemic activity of *Tamarindus indica* seed powder in nondiabetic, type-I and type-II diabetic model rats. The experimental approach that has been followed, in addition to screening the hypo-/antihyperglycemic activity, gives an approximate idea about the mechanism of action of the plant by analyzing the model, prandial states and timing of hypoglycemic activity. Moreover, the study was also extended to explore the possible mechanism of action by elucidating the effect of the plant on gut motility and intestinal glucose absorption.

Our results demonstrate that *T. indica* seed powder had no effect in the fasting state of nondiabetic, type-I or type-II rats. At the post prandial state when the seed powder was administered simultaneously with oral glucose load, no significant reduction was noticed with a single feeding in any group of rats. On the contrary, when *T. indica* seed powder was administered half an hour before oral glucose load in type-II rats, the seed powder caused a significant attenuation in the rise of blood glucose at 105 minutes compared to the control groups (glucose M±SD, mmol/l. 14.65±2.82 in the treated group Vs 19.30±4.27 in the control group, $p<0.03$). The antihyperglycemic effect of *T. indica* seed in STZ induced diabetic rats have been found by other investigators [17].

T. indica seed powder was effective in type-II diabetic model rats when fed 30 minutes before glucose load. This effect might be due to a systemic action, i.e. as a result of the stimulation of Beta cells and improving the insulin secretory capacity or enhancement of insulin action by the extract. This effect could not be confirmed by our study since serum insulin level after a single feeding was not determined. It has been claimed that the chronic aqueous extract of *T indica* improves glycemic status [17, 26].

The inhibition of intestinal glucose absorption may contribute to the reduction of postprandial glucose level. One of the objectives of the present study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the gut. Since the antihyperglycemic effect of *T. indica* was found in type II rats, therefore, this gut perfusion experiment was investigated in normal and type-II rats. where *T. indica* seed powder showed strong inhibition of glucose absorption. This result strongly suggests that the antihyperglycemic effect of *T. indica* as previously reported [26, 27] may be due to, at least in part to the retardation of glucose absorption in the small intestine. *T. indica* is rich in pectin's and dietary fibers such as cellulose, xylose and mucilage [16] and the presence of such substances in the powder of *T. indica* may be responsible for the observed effect. Similar results have been reported by some other scientists [16, 27]. Moreover, *T. indica* seed powder also inhibited the $BaSO_4$ induced gastrointestinal motility in Type-II rats. This result suggests that the decrease of glucose absorption by *T. indica* seed powder is not achieved by an enhanced intestinal motility. It is now well established that diabetes mellitus is not a single desease entity, but a heterogenous group of disorders with a striking diversity of etiopathogenetic mechanisms as well as clinical manifestations [28]. It is also established that the basic pathophysiology of Type-I and Type-II diabetes is quite different. In type-II diabetes, there are multiple abnormalities in diverse tissues. So, a plant material can show glucose lowering effect in diverse way. It may not be active in type-I diabetes, but may be active in type-II diabetes, which we found in our study.

5. CONCLUSION

Based on the results of this study, it may be concluded that *T. indica* seed powder possesses significant antihyperglycemic activity in type II diabetic model rats but not in type I and this is partly due to inhibition of intestinal glucose absorption.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHOR'S CONTRIBUTION

Anzana Parvin, designed the proposal and protocol, performed the experiments and analysis; Md. Morshedul Alam wrote the first draft of manuscript; Md. Anwarul Haque, Amrita Bhoumik and Liaquate Ali managed the study analysis; Begum Rokeya designed the protocol, performed statistical analysis, managed the experiment. All authors approved the manuscript.

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