Comparative study of antihyperglycemic and antihyperlipidemic activities of aqueous extract of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves in normal and alloxan diabetic rats

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ABSTRACT

Effective and safer hypoglycemic agents from herbal plants are being considered as area of research as the synthetic oral anti-diabetic agents have several serious adverse effects. The aim of this research was to investigate the single and combined effect of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves on blood glucose and cholesterol in diabetic rats. In this study 60 rats were divided into 5 groups and containing 12 individuals as follows: normal control (A), diabetic control (B), Alloxan with BG treated (C), Alloxan and TK treated (D) and Group E was Alloxan, BG and TK treated. Alloxan was injected at the dose of 100mg/kg body weight intra-peritoneal to each rat to induce diabetes in groups B, C, D and E. Aqueous extract of BG and TK were fed at a dose of 300mg/kg and 500 mg/kg body weight for 21 days in group C and D, respectively and combined in group E. The body weight, blood cholesterol (mg/dl) and glucose (mg/dl) level were measured on Day 0, 7, 14 and 21. The blood sugar and cholesterol level were reduced in Group C from 178.5±11.55 mg/dl to 120.45±1.98 mg/dl and 209.11±5.24 mg/dl to 126.66±6.58 mg/dl respectively and in group D, they reduced from 150.5±1.6 to 140.17±2.49 mg/dl and 212.43±6.2 to 129.77±5.94 mg/dl respectively. But the reduction of blood sugar level and blood cholesterol level were significant in Group E from 156±4.11 mg/dl to 110.21±3.98 mg/dl and 217.17±4.34 mg/dl to 114.81±4.18 mg/dl, respectively. From these findings it concluded that the combination of BG and TK can be used as anti-hyperglycemic and anti-hypercholesterolemic agent.

Keywords: Anti-diabetic, anti-hyperglycemic, anti-hyperlipidemic

INTRODUCTION

Diabetes mellitus is a worldwide problem due to the high rates of morbidity and mortality that are connected with the condition. It may have immediate or prolonged damaging effects (Lotti & Maggi, 2023). Modern medical practices and innovative scientific discoveries have completely altered the treatment regimen. The use of glucose-lowering medications does, however, come with a number of side effects, including chronic vascular disease, renal dysfunction, liver disease, and several skin issues. These issues have prompted to consider alternate diabetic treatments with minimal or no adverse effects (Singh et al., 2022). Today, medical professionals and the public in general in both developed and developing countries alike use natural herbs and their preparations to cure disorders on the assumption that organic products are safe for body (Thikekar et al., 2021). These natural remedies encompass organic substances including flavonoids, terpenoids, glycosides, and alkaloids that exhibit a variety of anti-diabetic actions (Singh et al., 2022).

Momordica charantia (M. charantia) is a plant that has recently received the greatest interest in recent years due to its many biological benefits, which include anti-diabetic, anti-obesity, anti-inflammatory, and anti-cancer capabilities and many more (Bora et al., 2023). It also goes by

the names bitter melon or Karela in Bengali and has a wide range of pharmacological effects. Thiamine, beta-carotene, folate, riboflavin, calcium, iron, phosphorus, manganese, potassium, magnesium, zinc, and dietary fiber are just a few of the micronutrients that the bitter gourd is rich in. Regular consumption of bitter gourd juice improves physical stamina and lessens chronic fatigue. This bitter gourd's beta-carotene concentration helps to treat eye disorders and enhances vision (Mukherje & Karati, 2023). Studies have also revealed that M. charantia contains numerous phytochemicals that have hypoglycemic properties, suggesting that the plant may be useful in the treatment and control of diabetes mellitus (Oyelere et al., 2022).

Similarly, fresh leaves and branch tips from Coccinia indica (C. indica) are frequently used in Ayurvedic, Siddha, and Unani traditional medical systems throughout Asia. The common applications of this plant's many parts in folk medicine included anti-diabetic, antibacterial, anti-inflammatory, antioxidant, antimalarial, antidyslipidemic, anticancer, analgesic, antipyretic, antitussive, antinociceptive, hepatoprotective, anti-obesity, and neuroprotective functions (Padma R & Vinoth, 2022). In rats with diabetes caused by alloxan, Coccinia indica (Telakucha) leaf extract significantly reduced blood sugar and total cholesterol levels while also having regulatory effects on total erythrocyte count (TEC), total leukocyte count (TLC), and hemoglobin content (Hb) (Sarkar et al., 2020).

As both M. charantia and C. indica exert anti-diabetic properties, this study was designed to investigate the comparative efficacy of these two plants to fight against diabetes mellitus in rats' model.

MATERIAL AND METHODS

This research work was conducted in the Laboratory of Anatomy, Department of Anatomy, Histology and Physiology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka for a period of 12 months from 2021 to 2022 to evaluate the single and combined efficacy of Momordica charantia (bitter gourd) and Coccinia indica (Telakucha) leaves on alloxan induced diabetic rats.

Collection and acclimatization of rats

Total 60 mixed male albino rats (aged 2-3 months) and weighing (70 to 100g) were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. For five experimental trials, all the rats were divided into 5 groups each containing 12 rats. Each group of rats was housed at serene bottomed wire cages arranged in rows and kept in the animal house of this department. The animals were fed with pellet at a recommended dose of 100 g/kg body weight. Drinking water was supplied ad libitum. The rats were reared in this condition for a period of two weeks to acclimatize them prior to experimental uses.

Induction of diabetes

Diabetes mellitus was induced by injecting alloxan through intraperitoneal route which increases the blood glucose level and at the same time decreased body weight. Single dose of alloxan administered intraperitoneal @100 mg/kg body weight (Junod et al., 1996). In this experiment, polyuria, polydipsia, and polyphagia after 24 hours of alloxan injection were

observed. Rats with serum glucose level ranging between 150mg/dl or above considered as hyperglycemic. At the same time the rats with cholesterol level above 200 mg/dl were considered as hypercholesterolemic (Reeves et al., 1993).

Experimental design

In this study, a total of 60 rats (12 normal rats and 48 alloxan induced diabetic rats) were used for each trial. The rats were divided into 5 groups each containing 12 individuals as follows:
Group A: Normal control
Group B: Diabetic control
Group C: Alloxan+ BG treated
Group D: Alloxan+ TK treated
Group E: Alloxan+ BG +TK treated

After 18 hours of starvation, body weights and blood glucose level were measured after acclimatization of rats. Then alloxan was injected at a dose of 100 mg/kg body weight in intraperitoneal route to each rat to induce diabetes in groups B, C, D and E. All the group of rats was reared under normal diet and water ad libitum from Day 1-10, on 10th day blood glucose level, blood cholesterol levels and body weights were measured for the first time to ensure diabetic induction as well as hypercholesterolemia. Then rats of all groups were kept for more 21 days for the treatment of hyperglycemia and hypercholesterolemia. During that period on day 0,7,14 and 21st the body weight, blood cholesterol, blood glucose level were measured. Aqueous extract of BG and TK leaves extract were fed at a dose of 300 mg/kg and 500 mg/kg body weight daily for 21 days in groups C and D respectively, and combined dose of both BG and TK in group E.

Preparation of TK Leaf Extract

TK leaves were collected from Sher-e-Bangla Agricultural University campus and dried by using freeze dry method and powdered with the help of mortar and pastel. From the powder 10% aqueous solution of TK leaf extract was prepared (Singh, et al., 2011)

Preparation of bitter melon

According to the methods of Chen and Li, (2005) unripe bitter melon fresh fruit was cut open and the seeds were removed. The extracted juice from the edible portion was frozen and completely lyophilized by continuous freeze-drying operation for 72hrs. The powder was kept in airtight containers at -700C until used.

Determination of Blood Glucose

Blood samples were collected from tail vein on 0, 7, 14 and 21st day of experiment and blood glucose was determined by using glucose oxidase-peroxidase reactive strips and a glucometer (UNI-CHECK®,Visgeneer, Taiwan). The tail was disinfected by rubbing a cotton ball soaked in Hexisol® Solution. A small amount of blood was drawn from tail vein of the rats by venipuncture with help of 13 insulin syringe and needle. At the same time the glucometer was started with a single press. Before using the test strip a new coding chip was inserted by the side of the monitor. After the monitor showed the code number the strip was inserted into the designated slot. A drop of blood was then dropped on the test zone of the strip. The result was

shown on monitor within 5 seconds of dropping the blood on the zone of the strip in mg/dl.

Determination of Total Cholesterol

Blood samples were collected from tail vein on 42nd day of experiment and blood cholesterol was determined by using a blood testing meter (EasyMate® GCU, Bioptik Technology Inc., Taiwan). The tail was disinfected by rubbing a cotton ball soaked in Hexisol® Solution. A small amount of blood was drawn from tail vein of the rats by venipuncture with help of insulin syringe and needle. At the same time the EasyMate® GCU blood testing meter was started with a single press. Before using the test strip a new coding chip was inserted by the side of the monitor. After the monitor showed the code number the strip was inserted into the designated slot. A drop of blood was then dropped on the test zone of the strip. The result was shown on monitor within 5 seconds of dropping the blood on the zone of the strip in mg/dl.

Statistical analysis

Changes in body weight, blood glucose level and blood cholesterol level of rats were compared statistically by means of one-way analysis of variance (ANOVA) test. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first comparative study of anti-hyperglycemic and anti-hyperlipidemic activities of aqueous extract of Momordica charantia and Coccinia indica leaves in normal and alloxan diabetic rat. Changes in blood glucose level of rats were summarized in the Table 1 describing that treatment of diabetic rats with BG, TK & combined treatment induced a significant decrease in fasting blood glucose levels compare with diabetic untreated group. At the day of 21, the blood sugar level was reduced in Group C from 178.5±11.55 mg/dl to 120.45±1.98 mg/dl. Likewise, in group D the blood sugar level was reduced from 150.5±1.6 mg/dl to 140.17±2.49 mg/dl. But the reduction of blood sugar level was prominent in Group E from 156±4.11 mg/dl to 110.21±3.98 mg/dl.

Table 1 Descriptive statistics of mean values of average blood sugar level (mg/dl) with standard deviation in different rat groups

Grs	Day 0	Day 7	Day 14	Day 21
	ABSL (mg/dl)	ABSL (mg/dl)	ABSL (mg/dl)	ABSL (mg/dl)
A	92.5 ±2.31	94.4 ±3.25	96.35 ± 2.86	95.10± 3.25
B	178±12.51	211.63±8.47	235.5± 6.50	255.48 ±4.14
C	178.5±11.55	160.5±5.01	129.37±6.35	120.45±1.98
D	150.5±1.65	149.5±5.01	145.33±6.35	140.17±2.49
E	156 ±4.11	139.5±3.78	127.17±1.54	110.21±3.98

Legends:
Grs: Group, Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG + TK treated; ABSL: Average Blood Sugar Level

Table 2 Descriptive statistics of mean values of blood cholesterol level (mg/dl) with standard deviation in different rat groups

Grs	Day 0 ABCL (mg/dl)	Day 7 ABCL (mg/dl)	Day 14 ABCL (mg/dl)	Day 21 ABCL (mg/ dl)
A	120.35±5.64	119.31±5.45	110.39±4.57	117.65±6.55
B	205.12±5.34	224.34±6.33	232.13±6.57	240.76±5.78
C	209.11±5.24	180.51±5.78	155.44±6.34	126.66±6.58
D	212.43±6.21	187.12±5.89	156.32±5.98	129.77±5.94
E	217.17±4.34	177.77±4.36	144.25±4.44	114.81±4.18

Legends:
Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG +TK treated; ABCL: Average Blood Cholesterol Level

Table 3 Descriptive statistics of mean values of body weight (gm) with standard deviation in different rat groups

Grs	Day 0 ABW (gm)	Day 7 ABW (gm)	Day 14 ABW (gm)	Day 21 ABW (gm)
A	302.6±5.60	300.5 ±8.42	303.33± 7.25	300.55 ±6.0
B	265.83± 8.11	236.5±8.85	221.5±7.75	215.56 ± 7.42
C	256.65±6.31	255.58±6.9	276.67±4.63	287.19±3.11
D	256.67±6.35	267.33±5.5	277.67±4.63	288.17±2.56
E	304.78 ±2.79	307.44 ±2.31	309.10 ±3.57	310.45±5.10

Legends:
Grs: Group, Group A: Normal Control; Group B: Diabetic control; Group C: Alloxan + BG treated; Group D: Alloxan +TK treated; Group E: Alloxan + BG + TK treated; ABW: Average body weight

Total cholesterol was significantly increased in alloxan induced diabetic rats, these figures were significantly decrease after BG, TK and combined treatment (Table 2).The cholesterol level was reduced in Group C from 209.11±5.24 mg/dl to 126.66±6.58 mg/dl and from 212.43±6.2 mg/dl to 129.77±5.94 mg/dl in group D after 21 days of treatment. But the reduction of blood cholesterol level was remarkable in Group E from 217.17±4.34 mg/dl to 114.81±4.18 mg/dl.

Treatment of diabetic rats with BG, TK & combined treatment induced a significant increase in body weight compare with diabetic untreated group (Table 3). After the course of 21 days of treatment with BG, TK and combination of BG & TK, the average body weight was elevated in Group C and D from 255.58±6.9 gm to 287.19±3.11 gm and 267.33±5.5 gm to 288.17±2.56 gm respectively. However, a significant increase in body weight gain was observed in group E compared with other groups from 304.78 ±2.79 gm to 310.45±5.10 gm after exposure of both BG & TK for 21 days in alloxan induced diabetic rats.

Diabetes mellitus is probably the fastest growing metabolic disease in the world. As the knowledge of multifactorial nature of this disease increases so does the need for more challenging and appropriate therapies (King et al., 1998; Dans et al., 2007). Alloxan is known for selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (Fernandes et al., 2007). Generalized increase in the level of blood glucose during diabetes have been consistently reported both in animal models and humans especially those suffering from insulin dependent diabetes mellitus (Mathew et al., 1973; Lorenzati et al., 2010).

In the present study we found that, both BG and TK extract reduced the blood glucose, and cholesterol in diabetic rats. Regarding serum glucose level, treatment of diabetic rats with bitter melon caused significant decreases in fasting and post- prandial serum glucose levels as compared to the diabetic untreated group. These results are in accordance with the previous findings (Shibib et al., 1993; Chaturvedi et al., 2004; Fernandes et al., 2007; Yuan et al., 2008; Balaraman et al., 2010; Jayasuriya et al., 2000). The present finding disagrees with the finding of Dans et al., 2007 who reported that BG had no significant hypoglycemic effect in alloxan diabetic rats. The present results elucidated a significant increase of total cholesterol concentrations in the serum of diabetic control rats as compared to normal control group. These results are in agreement with the findings that described hypo-cholesterolmic effects of BG (Newairy et al., 2002).

CONCLUSION

In conclusion, the present study reinforces our previous findings (Akhter et al., 2018a; 2018b) that BG and TK had a significant effect in reducing blood glucose as well as blood cholesterol and they may be considered as better therapeutic options for diabetes mellitus if administered together.

CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

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