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Article

Antidiabetogenic impact of Bitter Melon *(Momordica charantia)* and Black Cumin *(Nigella sativa)* on alloxan induced diabetic rabbit model

Kishor Kumar Roy[1*], Md. Rakibul Islam[1], Md. Bazlar Rashid[1], Md. Mahmudul Hasan[1], Misrat Masuma Parvez[1], Md. Anowarul Haque[2] and Boby Rani Saha[1]

[1]Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh
[2]Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh

*Corresponding author: Kishore Kumar Roy, MS in Physiology, Department of Physiology & Pharmacology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh. Phone: +8801774600533; E-mail: roykishore.dvm@gmail.com

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Abstract: The present study was undertaken to investigate the antidiabetic effect of the bitter melon and black cumin on alloxan induced diabetes in experimental rabbits. At 2 to 3 months of age, rabbits were assigned into five groups (A, B, C, D and E) and rabbits were included each group. Group A was kept as control, Group B was treated with alloxan intramuscularly at a dose of 75mg /kg body weight, Group C was treated with bitter melon at a dose of 150gm/kg body weight orally, Group D was treated with black cumin at a dose of 250mg/kg body weight orally, Group E was treated with combined at previous dose. After acclimatization, diabetes was induced in four groups of rabbits (B, C, D and E) by administering alloxan injection. There was significant ($p<0.05$) decreased in blood glucose level in all treated group C, D, E compared to the B group and lowest glucose was recorded in E group when treated with combined medicinal herbs and body weight was increased in all treated group C, D, E compared to the B group and highest was recorded in D group while treated with those. % of PCV level and Hbgm/dl concentration was the highest in group E which was treated with both black cumin and bitter melon compare to the A group. ESR was highest in group B treated with alloxan and lowest in group E. The present study demonstrated that combined treatment increased body weight and decreased glucose level without affecting health of rabbits.

Keywords: alloxan; diabetes; bitter melon; black cumin; rabbit

1. Introduction

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems; as well as the sexual organs, the skin, vision, hearing and taste (Bailey *et al.*, 1989). Diabetes mellitus is a wide spread disorder which has long been in the history of medicine. World Health Organization (WHO) estimates that 346 million people suffer from diabetes worldwide. Despite continuous introduction of the modern drugs, diabetes and its related complication is still a global medical issue. Before the advent of synthetic insulin and oral hypoglycemic drugs, the major form of treatment involved the use of plants (Wadkar *et al.*, 2008). Diabetes mellitus can be chemically or surgically induced in different animal species. Chemical induction of diabetes can be achieved by injecting uric acid, dial uric acid, dehydro ascorbic acid, quinoline and magnesium. However, the most commonly used means of chemical induction of diabetes has been either alloxan or streptozotocin, as their diabetogenic dose is usually 4 to 5 times less than their lethal dose. However Guinea pigs are totally insensitive to alloxan (Gordsky *et al.*,1982). Alloxan (mesoxal urea) was the first chemical used to induce experimental diabetes. It was found by

Leibig in mucus excreted during dysentery (Merck Index, 1976). The diabetogenicdose of alloxan vary considerably amongst species, age and metabolic state of the animal. Nephrotoxicity is also a side effect (Bonar, 1980). *Momordica charantia* (*M. charantia*), is a popular plant used for treating of diabetes related conditions. Fruits and leaves of most wild Momordica species are consumed as vegetables and have a similar bitter taste and almost identical medical uses. It has been used as a traditional antidiabetic remedy in eastern countries for many years. Bitter melon suppresses weight gain and has the potential to reduce adiposity (Chen *et al.,* 2003). Bitter melon may possess insulin-like properties, preserved pancreatic islet beta cells (Ahmed *et al.,* 1998). A recent study proved that bitter melon could upregulate the significance of glucose transporter 4 (GLUT-4), peroxisome proliferator-activated receptor γ (PPARγ) and phosphatidylinositol 3 kinase (PI3K) by augmenting the glucose uptake and homeostasis (Kumar *et al*., 2009). Bitter melon can also improve insulin sensitivity by increasing insulin-stimulated insulin receptor substrate-1 (IRS1) tyrosine phosphorylation in high-fat diet-fed mice/rats (Nerurkar *et al.,* 2008; Sridhar *et al.,* 2008). Black Cumin is used for the treatment of diabetes, recent researches record the activity of extraction of black cumin decreases the level of glucose in normal and in alloxan induced diabetic rabbits and decreases lipid profile. The objective of this study is to evaluate the alloxan induced diabetes occurred in experimental rabbits and investigate the combined effect of *Momordica charantia* (bitter melon) and in *Nigella sativa* (black cumin) alloxan induced diabetic rabbit and to study the effects of plants suspension on body weight, blood sugar, in experimental rabbits.

2. Materials and Methods

Twenty healthy white rabbit aged between 2 to 3 months and weighting between 1000 to1200g were collected from rabbit farm under the department of Animal Genetics and Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur and grouped into A, B, C, D, E and each group contained 4 rabbits. Group A was kept as normal control, group B was kept as positive control (alloxan administered). Group C was kept as treatment 1(alloxan+bitter melon), Group D treatment 2(alloxan+garlic) and Group E treatment 3 (alloxan+bittermelon+garlic). The rabbits were maintained for a period of two weeks to acclimatize them prior to experimental uses.Twenty rabbits were used to carry out this investigation. These rabbits were divided into five groups containing 4 rabbits in each group. The groups were designated and maintained as follows:

Group A: This group of rabbits served as normal rabbits. Body weights and blood glucose level were measured at the time when that of other groups was measured. This group was served as normal control group.

Group B: Alloxan hydrochloride injection was given at a dose rabbits of 75 mg/1000 to 1200gm (Puri and Prabhu, 2002) in intramuscular route to each rabbits to induce diabetes. The rabbit were fed normal diet and given water adlibitum from Day 1-15 on 15th day blood glucose level and the body weight were again measured to ensure diabetic condition. Then all the rabbit of this group were kept for 14 days without any treatment. During that period on Day 0, 7 & 14 the body weight and blood glucose level were measured. This group served as diabetic (positive) control group.

Group C: Alloxan hydrochloride was injected in all rabbits of this group at a dose rabbits of 75 mg/kg in intramuscular route. The rabbits were fed normal diet and given water ad libitum for 15 days. Then blood glucose level and body weight were measured on 15th day of Alloxan hydrochloride injection for confirming diabetic condition. After that suspension of bitter melon fed at a dose of 250gm, 3mL water/1000 to 1200gmb.w./day for 21 days. During treatment of bitter melon suspension body weight and blood glucose level were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 1 to find the effect of suspension of bitter melon as antidiabetic drug.

Group D: Alloxan hydrochloride was injected in all rabbit of this group at a dose rabbits of 75 mg/kg in intramuscular route. The rabbits were fed normal diet and given water ad libitum for I5days. Then blood glucose level and body weight were measured on 15th day of Alloxan hydrochloride injection for confirming diabetic condition. After that suspension of garlic were fed at a dose of 750 mg/kg body weight/day for 14 days. During treatment of suspension of black cumin seed body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 2 to find the effect of suspension of black cumin seed as antidiabetic drug.

Group E: Alloxan hydrochloride injection was given at a dose rabbits of 75 mg/1000 to 1200 gmin intramuscular route to each rabbits to induce diabetes. The rabbit were fed normal diet and given water ad libitum from Day 1-15 on 15th day blood glucose level and the body weight were again measured to ensure diabetic condition. After that suspension of bitter melon and garlic were fed at a previous dose for 14 days. During combined treatment of suspension of bitter melon and black cumin seed body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment

group 3 to find the combined effect of suspension of bitter melon and black cumin seed as antidiabetic drug. Fresh bitter melon and garlic are purchased from the local market at a reasonable price then these are measured separately by electronic balance and grinded with mortar and pestle than blended with blender machine. Finally, the extracts are mixed with 100 ml distilled water separately and stirred to make homogenous mixture and then filtered through silk cloth.

Body weight and fasting blood glucose level of each rabbits were measured after 18 hours of fasting before alloxan injection, 72 hours of after alloxan injection and Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment) of different treatment. Blood samples were collected from ear vein at Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment) for estimation of blood glucose levels. Estimation of blood glucose level was performed by Glucolab® Active monitor blood glucose system (strip method). Body weight was taken on day 0(pretreatment), 7, 14 and 21(during treatment). The data were analyzed with SPSS statistics 20.0 software. Probability $P<0.05$ was considered statistically significant. Data were analyzed using SPSS v.11 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Mean values were considered significantly different at $P<0.05$. Data are expressed as mean ± SEM.

3. Results and Discussion

The experiment was conducted to determine the efficacy of alloxan to induce diabetes in rabbits. Attempts were also made to study the antidiabetogenic efficacy of bitter melon fruits and black cumin seeds on blood glucose levels and body weights in alloxan induced diabetic rabbits and also to study the combined effect of bitter melon fruit and black cumin seed. To perform the experiment, twenty rabbits were randomly divided into five equal groups. Alloxan was injected (I/M) at the dose rate of 75mg/kg body weight to the groups of rabbits (B, C, D and E) for induction of diabetic syndrome. Group A rabbits were kept as non-diabetic (Normal) control without giving alloxan and any other treatment. Group B rabbits were kept as diabetic control without (giving any other treatment except alloxan). Next two groups of rabbits (C and D) were treated with suspension of bitter melon fruit at dose of 150 gm/kg and black cumin seed ata dose of 250mg/kg for consecutive 21 days respectively after 21 days of alloxan administration. All the control and treated rabbits were closely observed 21 days of treatment period.

3.1. Blood Glucose Level (mmol/L)

3.1.1. Alloxan induced diabetics and comparison with control

Blood glucose level of different groups of rabbits are presented in Table 1.The study revealed that glucose level was the highest in group B, which was treated with alloxan compared to the A group. This treatment significantly ($p\leq.0.05$) increased the blood glucose level in treated rabbits. The present results are agreed with other results of Lenzen, 2008; Tasaka *et al.,* 1988; West *et al.,* 1996 suggested that alloxan treatment increased the blood glucose level in treated rabbits compared to the control rabbits.

3.1.2. Alloxan induced diabetics and comparison with bitter melon fruit

Blood glucose level of different groups of rabbits are presented in Table 1.The study was revealed that glucose level was the lowest in group C, which was treated with bitter melon compare to the B group. The effect of fruit suspension at a dose of 250 gm/kg body weight in lowering blood sugar level showed statistically significant comparison with B group. We have evaluated the suspension of the unripe fruit of the *Momordica charantia* (bitter melon) was assessed for its antidiabetic activity in alloxan induced diabetic rabbits. The blood sugar levels were highly decreased with a treatment of high dose of extract. The blood sugar levels were almost decreased to the normal levels. The present results are agreed with other results of Sarkar *et al.,* 1996; Leatherdale *et al.,* 1981 who suggested that oral administration of an extract of *Momordica charantia* fruit at an appropriate dosage may be good alternative antidiabetic agent in alloxan induced diabetics.

3.1.3. Alloxan induced diabetics and comparison with black cumin seed

Blood glucose level of different groups of rabbits are presented in Table 1.The study revealed that glucose level was the low in group D, which was treated with black cumin compared to the B group. The effect of seed suspension at a dose of 250mg/kg body weight in lowering blood sugar level showed statistically significant comparison with B group. We have evaluated the suspension of the seed of the *Nigella sativa* (black cumin) was assessed for its antidiabetic activity in alloxan induced diabetic rabbits. The blood sugar levels were highly decreased with a treatment of high dose of extract. The blood sugar levels were almost decreased to the normal levels. The present results are agreed with other results (Al-Hader *et al.,* 1993; El-Dakhakhny *et al.,* 2002;

Kanter *et al.*, 2004).The results of this study indicated that a dose of 250 mg/ kg body weight of *Nigella sativa* might be a beneficial adjuvant to oral hypoglycemic agents in alloxan induced diabetes.

3.1.4. Alloxan induced diabetics and comparison between different groups of rabbits

The fall in the blood sugar was compared among the groups of animals. The study was revealed that blood glucose level was the lowest in group E compared to the C and D group, which was treated with bitter melon fruit and black cumin seed. The effect of this combined treatment significantly ($p \leq .0.05$) affected the blood glucose level.

Table 1. Effects of bitter melon fruit and black cumin seed suspension and combined treatment on blood glucose (mmol/L, mean ± SE) concentration in alloxan induced diabetic rabbits (n=4).

Group	Day 0 (Mean ± SE) mmol/L	Day 7 (Mean ± SE) mmol/L	Day 14 (Mean ± SE) mmol/L	Day 21 (Mean ± SE) mmol/L
A	$7.550^{b} \pm 0.44$	$7.725^{c} \pm 0.37$	$7.425^{d} \pm 0.25$	$7.875^{d} \pm 0.13$
B	$28.33^{a} \pm 0.69$	$27.00^{a} \pm 1.15$	$24.23^{a} \pm 0.60$	$19.02^{a} \pm 0.70$
C	$27.98^{a} \pm 0.73$	$23.45^{b} \pm 0.76$	$18.27^{bc} \pm 0.71$	$12.98^{b} \pm 0.45$
D	$28.98^{a} \pm 0.52$	$24.55^{b} \pm 0.34$	$19.52^{b} \pm 0.52$	$12.70^{b} \pm 0.44$
E	$28.65^{a} \pm 0.81$	$24.80^{b} \pm 0.58$	$16.83^{c} \pm 0.86$	$10.93^{c} \pm 0.29$

Values with the different superscripts in the same column are statistically significant (P<0.05). A, Control (without treatment); B, Alloxan induction (75mg); C, Bitter melon treatment (150gm); D, Black cumin treatment (250mg); E, combination of Bitter melon and Black cumin treatment.

3.2. Body weight (gm)

The percent increased in body weight gain in normal control rabbits (Group A, n=4) was 1130 gm. On the contrary, in diabetic control group (Group B, n=4), the percentage of body weight loss was 1000gm. The percent increased in body weight gain over 21 days in. Group C (n=4), following oral administration of suspension of bitter melon@ 150 gm/kg was 1080 gm. In Group D (n=4), following administration of black cumin seeds @ 250 mg/kg for 21 days the percentage of body weight gain was 1078 gm. In Group E (n=4), following administration of bitter melon and black cumin seeds @ previous doses for 21 days the percentage of body weight gain was 1131 gm comparison with B group which is treated with alloxan.

Table 2. Effects of bitter melon fruit and black cumin seed suspension and combined treatment on body weight (gm) in Alloxan induced diabetic rabbits (n=4).

Group	Day 0 (Mean ± SE) gm	Day 7 (Mean ± SE) gm	Day 14(Mean ± SE) gm	Day 21 (Mean ± SE) gm
A	1056.0 a ± 21.34	1078.0 ab ± 21.75	1083.0 a ± 26.89	1133.0 a ± 20.56
B	1025.0 a ± 32.27	1020.0 b ± 31.092	1010.0 b ± 29.72	1000.0 b ± 32.40
C	1081.0 a ± 11.97	1048.0 ab ± 20.56	1065.0 ab ± 21.02	1080.0 a ± 20.82
D	812.5 a ± 237.71	1044.0 ab ± 14.63	1060.0 ab ± 14.72	1078.0 a ± 17.50
E	1088.0 a ± 7.50	1095.0 a ± 11.90	1113.0 a ± 11.09	1131.0 a ± 5.15

Values with the different superscripts in the same column are statistically significant (P<0.05). A, Control (without treatment); B, Alloxan induction (75mg); C, Bitter melon treatment (150gm); D, Black cumin treatment (250mg); E, combination of Bitter melon and Black cumin treatment.

4. Conclusions

This experiment supports the traditional usage of the herbal preparation by Ayurvedic physicians for the control of diabetes has the potentiality to be used as an adjuvant in the treatment of diabetes but which requires further study. These findings also indicate the oral administration over 21 days of *Momordica charantia* (Karela) and *Nigella sativa* (Black cumin) seed suspension significantly lower the blood glucose levels. This herbal preparation also increases the body weight. *Momordica charantia*has the potentiality to be used as an adjuvant in the treatment of diabetes but which requires further study. Also *Nigella sativa* may be the beneficial adjuvant to oral hypoglycemic agents in diabetic patients. Moreover, combination of *Momordica charantia* and *Nigella sativa* will be used for the treatment of diabetic patients without any health hazzards. However, due to some short comings only one trial is performed in short term basis and modern equipments are also not available.

Before field application as the hypoglycemic agents in case of diabetic patients further trial on a large scale basis is needed and also to make the findings more accurate and effective further study is essential to see any adverse effect in relation to histopathology before making a definite conclusion.

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Conflict of interest
None to declare.

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