ROLE OF MOMORDICA CHARANTIA L. AS HERBAL MEDICINE TO CURE HYPERGLYCEMIA IN VIVO ON INDUCED DIABETIC MODEL ANIMALS

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Abstract

The present study was aimed to explore antidiabetic potential of wild fruit of Momordica charantia L. (Family: Cucurbitaceae) from local germplasm of District Bhimber Communities, Azad Kashmir. The purpose was to evaluate the herbal recipe of food folklores of the remote rural area, where majority population relies on herbal therapeutics. Ethnomedicinal knowledge was collected through Rapid Appraisal Approach (RAA) along with structured and semi-structured interviews with local people and herbalist. Pharmacological analysis was conducted in the laboratory using Rabbits as model organisms, diabetics were induced by use of alloxan. The antihyperglycemic effect of ethanol extract at 1mg/kg and 3mg/kg is studied in normal, glucose loaded hyperglycemic and alloxan induced Type2 diabetic rats by oral dose administration for 7, 15 and 30 days. The blood glucose level of normal control and treatment groups were monitored by using Star glucometer. This research explored that considerable reduction in sugar level was observed on 7th and 15th days samplings in both treatments (T1 group, with 1g dose has 224±12 value and T2 group with 3g dose has 149±1.4 value) in comparison with control group which showed 542±6 glucose reading. The body weights was increased by 4.4% in normal control group, in diabetic control group decreased by 1.35%, in T1 group decreases by 19 % and in T2 group by decrease 37%. Serum insulin level was also improved in both treatment groups but comparatively in T2 group, its improvement was more. The study demonstrated that ethanolic extract of Momordica charantia has potential antidiabetic property in Type2 diabetes mellitus, thus justifying the traditional usage of plant as food medicine.

Key words: Ethnomedicine; Diabetes mellitus; Momordica charantia; Ethanolic extract; Serum insulin; Bhimber; Azad Kashmir; Hyperglycemia

Introduction

Diabetes mellitus is a group of metabolic diseases; it leads to hyperglycemia due to defects in insulin secretion, insulin action, or both (Anon., 2012). Hyperglycemia complications may generate neuropathy, foot ulcer, retinopathy, nephropathy, hypertension, hyperlipidemia, heart diseases, cataract formation, and hardening of skin (Patel et al., 2011; Luo et al., 2004). A report depicted that around the globe people are affected by diabetes @ 2.8% of total world population and it is predicted that this number will increase to more than 5.5% by the year 2025 (Patel et al., 2012; Gerstain et al., 2007). Its prevalence may rise up to 7.7% by year 2030 (Shaw et al., 2010). For diabetic treatment there tree methods viz: treatment by oral administration of hypoglycemic allopathic drugs, islets transplantation or stem cell therapy and herbal treatment. Herbal or natural medicines are also known as “alternative medicines.” Herbs and herbal medicines are considered to be safe as compared to the synthetic products which are becoming uncommon due to their high price and side effects. The use of herbal medicines is rising in modern world due to their cost effectiveness, easy availability and having no side effects (Singh et al., 2009). From time immemorial human being use plants for food and medicine (Hussain et al., 2009). Many plants used as herbal medications against diabetic disorders in different areas of world. Various herbs have been explored for their antidiabetic potential, for e.g. Cyompsis tetragonoloba, Cichorium intybus, Ficus carica, Picralima nitida, Phyllanthus amarus, Abutilon indicum, Clausena anisata, Alpinia galangal, Abelmoschus moschatus, Acacia arabica, Achyranthes aspera, Allium cepa, Ajugaivga, Allium sativum, Aloe barbadensis, Brassica nigra, Mangifera indica, Momordica charantia (Shinwari, 2010; Patel et al., 2012; Bhandari and Sharma, 1999; Perez et al., 2000; Agva et al., 2001; Akhtar et al., 2002). Momordica charantia commonly known as bitter gourd (family Cucurbitaceae) has medium size plant/vein present in warmer habitat/regions of world and they have high economic importance (Cousens, 2008). Its two varieties are commonly grown, one is Momordica muricata whose fruit is round and small and other is Momordica charantia having long and fusiform fruit (Chakravarty, 1990). Momordica charantia has been acknowledged as a medicinally important plant globally and in our homeland too (Mushtaq et al., 2009). Plants have been in life of man for cure of different diseases since time of emergence on land (Querishi et al., 2009). M. charantia has been documented as antidiabetic, anticancer, antiviral, antitumor, antileukemia, antibacterial, antimutagenic, antioxidant, antiulcer, anti-inflammation, anticholestrolemic, immunostimulant, hypotensive, antifertility, anti-HIV and insectisidal (Zahra et al., 2012; Sofowora, 2006; Taylor,2005). This plant is also known as “Plant Insulin” herb because it is peptide-p which cures hyperglycemia disorders. To our best of knowledge, there are no available reports on the anti-hyperglycemic effect of the fruits of indigenous plant from Azad Kashmir. Hence, the present study was carried out to determine the effect of ethanolic fruit extract of Momordica charantia L. on blood glucose level in control, normal alloxan-induced diabetic rabbits.
Materials and Methods

Instrumentation and Chemicals: Different instruments were used for analyses which include Soxhlet apparatus (Bomex), Hot plate (Velp Scientific), Rotary Evaporator (Heibolth), Glucometer (ApexBio). Alloxan (Applichem), Lignocane gel (Howards), hydrochloric acid (Merck Co), ethanol glycogen, mercuric chloride (Merck Co), potassium Co), glucose standard, Lignocane gel (Howards), Glucometer (ApexBio). Alloxan (Applichem (Bomex), Hot plate (Velp Scientific), Rotary Evaporator were used for analyses which include Soxhlet apparatus.

Plant material: Momordica charantia L. fruits were collected from different villages of district Bhimber, Azad Kashmir, Pakistan from March 2011 to March 2012. The plant samples were identified by Dr M Ishtiaq (Taxonomist, Deptt of Botany). The collected samples were pressed, poisoned, and accessed on herbarium sheets. Thevoucher specimens were placed in herbarium of department of Botany for future reference and study.

Preparation of extract: Fresh unripe fruit of Momordica charantia were washed carefully in order to remove all impurities. Whole fruit was cut into thin slices and dried in shadow. In order to avoid the fruit from fungal attack 70% ethanol was sprayed in the room in which fruit was placed. When they became completely dried, they were ground and their extract was prepared by soxhlet apparatus as described by Akueshi et al., 2002. Briefly, 10 grams powder of fruit was placed in the upper tube of soxhlet apparatus and 100 mL ethanol was placed in flask of apparatus. Extract was prepared for 12 hours and after that, it was placed on the hot plate on 100°C. Then it is filtered and transferred to the evaporator where the extract was finally prepared and it is saved at 4°C.

Phytochemical screening: Phytochemical analysis of ethanolic extract of Momordica charantia was conducted to check the chemicals present in it and for this qualitative analysis was performed. Phytochemical analysis of tannins, saponins, flavonoids, anthraquinones and alkaloids were evaluated according to the methods of Trease & Evans (2002), Sofowora (2006) and (Hasan & Khatoon, 2012) were used.

Experimental animals: Female domestic rabbits (Oryctolagus cuniculus) weighing approximately 1-2 Kg were used in this study and they were brought in the lab one week prior to the start of research in order to minimize the stress effect. During whole study they were fed with green vegetables, grains and Cynodon dactylon. The study was conducted in Laboratory of Department of Botany (Bhimber). The experiments were performed after approval of the protocol by the Departmental Animal Ethics Committee (DAEC), supervised by University Ethical Committee (UAEC) and animal care was taken as per the national guidelines for animal care.

Induction of diabetics in rabbits

Optimization of dose: Initially different doses of alloxan were given to make the rabbits diabetic. For this purpose, animals were divided into four groups. First group (T1-group) was given a dose of 60mg/kg alloxan, second (T2-group) a dose of 75 mg/kg, third (T3-group) a dose of 90mg/kg and fourth (T4-group) a dose of 105mg/kg. None of the rabbit from T1-group became diabetic. Only one rabbit from the T2-group treated became diabetic. 70% rabbits died belonging to T3-group, which is treated with105mg/kg dose of alloxan. All rabbits of T4-group remained alive and this dose was also proved effective (Alam et al., 2005).

Alloxan induction: Lignocane and xylene were applied on ear of rabbit which made their veins more prominent and alloxan monohydrate was injected intravenously to the rabbits after 12 hour fasting and the procedure followed for it was as given by Akhtar, 1982. Required dose (55mg/kg) of Alloxan was dissolved in 0.9 % NaCl saline solution immediately before use, and it was injected in marginal ear through 3cc syringe. Fasted blood glucose levels were assessed 48 hours after alloxan injection as well as glycosuria to confirm the diabetic states. The rabbits were kept for 15 days to stabilize the diabetic condition (Jyoti et al., 2002). Only rats with a fasting blood glucose level of at least 200 mg/dl and positive urine glucose were used in the experiment.

Criteria for diabetic model: For the present study, only one criterion was set and when diabetes level of rabbits exceeds 300mg/dl they were considered as diabetic. At least three readings were taken before starting of treatment; if these reading exceed the required criteria then treatment was started.

Treatment of diabetic animals: Experimental animals (Rabbits) were divided into four groups each comprising of five rabbits named viz: T1-group normal control; T2-group diabetic control; T3-group with dose of 1g/day; T4-group with dose of 3g/day. In the experiment testing for hypoglycaemic effect of the plant in normal and diabetic rats after single oral administration was determined. The control and diabetic groups (rabbits) received 1.5% dimethyl sulphoxide in distilled water. Two sampling groups each of normal and diabetic rats were given 1g and 3g/kg of the plant extracts, respectively. Their positive control groups were administrated with 5 mg/kg glibenclamide (Daonil®), a standard oral hypoglycaemic agent for comparative analysis. Blood glucose levels were determined at time zero and subsequently at time 0.5, 1, 2, 3, 5, and 8 h for day 1, day 7 and day 15. On 7th day and 15th day, their blood samples were collected and analyzed for blood and urine glucose levels. Body weight, food, and water intakes were also monitored.

Functional analysis during treatment

Blood sampling and measurement of blood and urine glucose: For glucose level, analysis blood samples (10 μl) were obtained from ear vein of rabbits, and glucose level was determined by glucometer (Sigma Co). The urine glucose level was assessed using glucose indicator sticks (Bio-rad) before and after treatment.
Serum insulin test: On 7th and 15th day during the diabetic treatment of rabbits by ethanolic extract of *Momordica charantia*, approximately three cc. blood samples was collected from their thigh vein. Serum was separated by centrifuge machine and sent for their serum insulin test from Armed Forces Institute of Pathology (AFIP) Rawalpindi.

Statistical analysis

In statistical analysis all values are expressed as mean ± S.E.M. and all results of experiment were evaluated by using two-way ANOVA by using software “Graph Pad prism 6.” if probability factor p<0.05 or less were considered significant.

Results

The efficacy of extracts of *Momordica charantia* as antihyperglycemic herbal therapy on experimental animals (rabbits) was monitored and analyzed on 7th and 15th day of experiment. The results of 7 day dose administration depicted that glucose level in T3-group was 157±12.6 (with 54.8% reduction) and in T4-group was 131.6±9.6 (with 61.35% reduction). The mimic effect of herbal extract on urine glucose level was also seen positive with reduction of 67.3% in T3-group and 80.4% in T4-group, respectively (Table 1), and these results are also demonstrated and explained in Fig. 1.

The final and second reading was measured after 15th days treat of ethanolic extract on rabbit models. The results explored showed that blood glucose in T3-group was 150±1.5 (with 55.8% reduction) and in T4-group was 90.0±6.5 (with 71.9% reduction), respectively. A considerable reduction in urine glucose was also found in 72.7% reduction in T3-group and 83.3% reduction in T4-group (Table 2). Serum insulin level of normal control and treatment groups were monitored at 7th and 15th day of experiment to evaluate the effect of alcoholic extract of *Momordica charantia* (Figs 2 & 3). There was no remarkable difference observed in food, water intake and movements yet results showed that mammoth changes in weight of various was noticed. The final reading for weight was conducted on day 15 that demonstrated that rabbits treated with 1g (T3-group) gained mass of 1.26±0.029 and rabbits treated with 3g (T4-group) raised weight of 1.6±0.058, and these were significantly lower than (T2-group) named as diabetic group with (0.00±0.005) as shown in Fig. 4.
Discussion

Diabetes mellitus is a complex syndrome involving malfunctioning of pancreas along with disturbed carbohydrates and fat metabolism. It is a global burden including developed as well as developing countries. Its victims are increasing day by day. Literature showed that it is considered as a killer disease and it affects people of any age group (Patel et al., 2006). Diabetic patient is on leap risk of many other diseases like blindness, coronary heart diseases, renal failure, gangrene, and many other diseases (Wolff, 1993).

Dietary fats are also important because the fatty acid present in it affects the glucose metabolism by changing the function of cell membrane and insulin signaling. Insulin sensitivity increases by replacing saturated fats with unsaturated fats (Sunil et al., 2011).

Since ancient time people were interested in exploring new medicines, using plant extracts (Gilani et al., 2010; Qasim et al., 2010). They found that large number of plants contain such compound, which are effective against different diseases (Verpoorte, 1998). Modern medicines are also based on plant extracts and many prescriptions contain one or more ingredient from natural flora (Thorfeldt, 2005). Literature predicted that nearly 400 plants are available in nature which have hypoglycemic as well as hypolipidemic properties. These plants are effective to restore the ability of the function of pancreas. Most plants contain alkaloid, flavonoids, terpenoids, carotenoids etc. (Malviya et al., 2010; Siddiqui et al., 2014).

Hypoglycemic effect of Momordica charantia is very obvious as demonstrated in our results. The fresh fruit’s ethanolic extract has remarkable positive impact on hyperglycemic conditions. The results of our study proved that it has very vital potential for cure of diabetes in rabbits and these findings corroborate with past research of Alion and these findings corroborate with past research of Alion (Wolff, 1993). Many studies showed that extract of Momordica charantia have many bioactive compounds that have hypoglycemic activity (Yibchok-Anun et al., 2006) and our research also demonstrated presence of various flavonoids, saponins, glyco-alkaloid, alkaloids, terpenoids, tannins and other secondary compounds. The efficacy of these compounds as hypoglycemic agent, painkiller, bacterial infection, and diuretic has been proved by previous researchers (Raman & Lau, 1996; Okwu & Josiah, 2006; Couzens, 2008; Aiyelaagbe & Osamudiamen, 2009), hence our findings are recommendatory of those past research work on this medicinal herb/vine (Agawa et al., 2001).

Our study depicted that an analysis conducted at day 7 when dose of 1g/kg was administered, a decrease of 54.8% in T 3-group and 61.35% in T 4-group was present and similarly Lal & Chaudhary (1968) also noticed more than 10% decrease in the blood glucose level after continuous administration of M. charantia extract for fifteen days. In 2 nd test conducted on 15 th with dose of induction of 3g/kg, it was explored that 55.8% decrease in T 1-group and 71.9% decrease in T 4-group and these results are also coincided with work of Biyani et al. (2003) also reported that 48% decrease in the blood glucose level by oral administration of extract.

The experimental findings proved that body weight is also affected by M. charantia extract dose. In T 1-group which was considered as normal control 4.4% increase in the weight was noticed. While in diabetic control group (T 2-group) 1.35% decrease in the weight was observed which is due to disturbed blood glucose level. A decrease of 1.19% body weight was observed in the group treated with 1gm/kg extract and 37% decrease in weight was noticed in the group treated with 3gm/kg extract (Fig. 4). This property of plant is also proved with results of Chen et al., 2003, who claimed that all compounds present in the M. charantia normalize the blood glucose level by reducing the obesity. Hence, people can normalize their blood glucose level by reducing their weights (Bouche et al., 2004). Many scientists reported that all compounds present in the M. charantia have hypoglycemic as well as hypolipidemic property and it reduces the cholesterol level of serum and liver (Akueshi et al., 2002; Chen et al., 2003).

Blood serum insulin was tested on 7 th and 15 th day of experiment. There was not observed any prominent change in the serum insulin level of normal (T 1-group) but in diabetic control (T 2-group) a rise up to 0.00 or 0.01 was seen and it persisted at 15 th day trial. In T 3-group which was treated with 1gm/kg extract depicted 13% improvement in blood serum insulin between 7 th and 15 th day, and in T 4-group with a treatment of 3gm/kg extract showed 17% improvement in serum Insulin level on day 7 th and 15 th day of experiment (Fig. 3). Many studies showed that blood glucose level is associated with Beta cells and insulin level also depends upon these cells. In alloxan-induced diabetic rabbits, this level decreased due to destruction of Beta cells. With the use of compound recipe of plants improvement of blood glucose and serum insulin level was observed by the slowly improvement of Beta cells (Wadood et al., 2007). However, further comprehensive study is recommended for knowing of mechanism how the extract of plant effect on anatomy and endomorphology of pancreas or islets of langerhan. For this extraction of herb in different solvents and their phytochemical analysis up to single bioactive constituent will be leaping step to discover new drug for this global plethora of diabetics which is killing thousands of people daily.

Table 1. Effect of Momordica charantia ethanolic extract on blood and urine glucose levels after 7 days oral administration to alloxan – induced diabetic rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Dose (g/kg)</th>
<th>Blood glucose (mg/dL)</th>
<th>Urine glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>T 1-group</td>
<td>5</td>
<td>-</td>
<td>95.0 ± 0.8</td>
<td>94.3 ± 0.9</td>
</tr>
<tr>
<td>T 2-group</td>
<td>5</td>
<td>-</td>
<td>352 ± 1.5</td>
<td>358.0 ± 1.2</td>
</tr>
<tr>
<td>T 3-group</td>
<td>5</td>
<td>1.0</td>
<td>348 ± 10.5*</td>
<td>157.0 ± 12.6*</td>
</tr>
<tr>
<td>T 4-group</td>
<td>5</td>
<td>3.0</td>
<td>340.5 ± 13.5*</td>
<td>131.6 ± 9.6*</td>
</tr>
</tbody>
</table>

T 1-group=Normal; T 2-group=Diabetic normal; T 3-group=T1 (with 1 g/kg dose); T 4-group=T2 (with 3g/kg dose); N=Number of rabbits used = 5 per dose; values expressed as mean ± SEM; * P<0.05; % reduction of blood and urine glucose was with respect to initial values.
Table 2. Effect of Momordica charantia ethanolic extract on blood and urine glucose levels after 15 days oral administration to alloxan – induced diabetic rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Dose (g/kg)</th>
<th>Blood glucose (mg/dL) Before treatment</th>
<th>Blood glucose (mg/dL) After treatment</th>
<th>% Reduction</th>
<th>Urine glucose (mg/dL) Before treatment</th>
<th>Urine glucose (mg/dL) After treatment</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-group</td>
<td>5</td>
<td>-</td>
<td>80.0 ± 0.2</td>
<td>75.3 ± 0.5</td>
<td>-0.05%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T2-group</td>
<td>5</td>
<td>-</td>
<td>343 ± 0.9</td>
<td>348.0 ± 1.6</td>
<td>+0.01%</td>
<td>4.8 ± 0.2</td>
<td>5.0 ± 0.4</td>
<td>+1.6%</td>
</tr>
<tr>
<td>T3-group</td>
<td>5</td>
<td>1</td>
<td>340 ± 1.0*</td>
<td>150 ± 1.5*</td>
<td>-55.8%</td>
<td>4.4 ± 0.6*</td>
<td>1.2 ± 0.5*</td>
<td>-72.7%</td>
</tr>
<tr>
<td>T4-group</td>
<td>5</td>
<td>3</td>
<td>320.5 ± 8.7*</td>
<td>90.0 ± 6.5*</td>
<td>-71.9%</td>
<td>3.6 ± 0.8*</td>
<td>0.6 ± 0.5*</td>
<td>-83.3%</td>
</tr>
</tbody>
</table>

*T1-group=Normal; T2-group=Diabetic normal; T3-group=T1 (with 1 g/kg dose); T4-group=T2 (with 3g/kg dose); N=Number of rabbits used = 5 per dose; values expressed as mean ± SEM; * P<0.05; % reduction of blood and urine glucose was with respect to initial values.

Conclusion

It was concluded that rural area people still rely on folklore herbal recipes and food medicines to cure diabetics. Pharmacological exploration demonstrated that Momordica charantia is very effective herbal therapy against diabetes. Its phytochemical analysis ethanolic extract demonstrated the presence of various compounds like alkaloids, saponins, sterols, steroid, terpenoids, flavonoids, tannins, phlobatannins and cardiac glycosides which are effective remedy of diabetes. Its fruit extract reduces the blood glucose level and body weight; and it improves serum insulin level without any side effects in contrast to allopathic medicines on general body systems. Its perseverance use can repair the beta cells of pancreas and which leads towards a healthy life. So, it is recommended that patients of pre-diabetes and diabetes may use this herbal therapy to remedy health disorders instead of using allopathic medicines and insulin injections, which are costly and bunch of side effects. There is hitherto space to evaluate the fruit using more solvents and also investigate other cultivars/populations from different villages of the area and country to broaden the horizon of ethnopharmacological research on this plant.

References


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