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HYPOLIPIDEMIC AND HEPATOPROTECTIVE EFFECTS OF DIFFERENT FRACTIONS OF METHANOLIC EXTRACT OF MOMORDICA CHARANTIA (LINN.) IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The study was undertaken to investigate the hypolipidemic and hepatoprotective effects of the different fractions (Petroleum ether, ethyl acetate and chloroform) of methanolic extract of *Momordica charantia.* The different fractions of extract were administered intraperitoneally as a single dose of 150 mg/kg body weight to alloxan induced diabetic rats and found to reduce blood lipid level (Total cholesterol and Triglycerides) significantly (p<0.05). The plant fractions also exhibited correction of altered biochemical parameters viz., SGOT and SGPT levels in diabetic rats. The effect of plant fractions were compared with standard drug metformin. The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpenes, alkaloids and flavonoids etc. were present in the plant which has hypolipidemic and hepatoprotective properties. Thus, this study indicates that various fractions (Petroleum ether, ethyl acetate and chloroform) of the methanolic extract of *Momordica charantia* have favorable effect in hypolipidemic and hepatoprotective activities.

INTRODUCTION: Hypercholesterolemia and hyper-triglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia. The frequency of hyperlipidemia in diabetes is indeed very high depending on the type of diabetes and its degree of control. Diabetes mellitus is one of the oldest diseases affecting millions of people all over the world [1]. Diabetes is a metabolic disorder featured by hyperglycemia as well as hyperlipidemia. The alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and insulin action [2].

Although numerous oral hypoglycemic and hypolipidemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [3]. Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic, hypolipidemic and hepatoprotective potential in the experimental animals [4].

Hyperlipidemia is the current medical as well social problem, specially associated with diabetes mellitus leading to increasing morbidity and mortality. The major risk factors of hyperlipidemia are associated with atherosclerosis which predisposes ischemic heart disease and cerebrovascular disease. The study of the effect of *M. charantia* on lipid profile in hyper cholesterolemic diabetic patients and showed that *M. charantia* significantly reduces the lipid level [5].

There was a significant rise in SGOT and SGPT levels in diabetic rats, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores [6]. Medicinal plants and herbs are of great importance to the health of the individuals and communities. A scientific investigation of traditional herbal remedies for diabetes may provide valuable lead for the development of alternative drug and therapeutic strategies.

M. charantia (Family- Cucurbitaceae) is a creeping or climbing annual weak herb. It is extensively cultivated in Bangladesh, India, China and other parts of Southeast Asia. Medicinal properties of this fruits were studied for antihyperglycemic, hypolipidemic and hepatoprotective effects to animals and also to the human subjects[7, 8]. Research report of BMRC showed that *M. charantia* fruits have significant hypolipidemic and hepatoprotective effects.

In the present study, the hypolipidemic and hepatoprotective properties of the fruits of *Momordica charantia* was assessed by evaluating the comparative hypolipidemic {Total cholesterol (TC), Triglyceride (TG)} and hepatoprotective { Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT)} activities in normal and alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant materials: The fresh fruits of *Momordica charantia* (Local name- Karala) were collected from medicinal plant garden, Rajshahi University, Bangladesh. The plant parts were dried completely under the mild sun and crushed with an electric grinder in to coarse powder and used for cold extraction. The authenticity of the *Momordica charantia* was identified by Mr. AHM Mahbubur Rahman, Department of Botany, University of Rajshahi, Bangladesh.

Preparation and fractionation of crude extracts: The crude extract was obtained through cold extraction process. The coarse powder was submerged in methanol and allowed to stand for 10 days with occasional shaking and stirring. When the solvent became concentrated, the alcohol content was filtered through cotton and then through filter paper (Whatman filter paper no. 1). Then the solvent was allowed to evaporate using rotary evaporator at temperature 40-45^oC. Thus, the highly concentrated crude extract was obtained. That was then fractionated using petroleum ether, ethyl acetate and $CHCl_3$. The fractions were then evaporated by rotary evaporator. The dried fractions of extract were then preserved in the freeze for the experimental uses.

Phytochemical screening methods: The following Phytochemical screening methods were used for the tests [9]:

Test for saponins*:* 300mg of extract in 5 ml water was boiled for two minutes. Mixture was cooled, mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

Test for tannins*:* To an aliquot of the extract added sodium chloride to make to 2% strength. This was filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

Test for triterpenes*:* 300mg extract mixed with 5ml chloroform and warmed for 30 minutes. To the chloroform solution small volume of concentrated sulfuric acid was added and mixed properly. The appearance of red color indicated the presence of triterpenes.

Test for alkaloids*:* 300mg extract was digested with 2 molar HCl. The acidic filtrate was mixed with amyl alcohol at room temperature and the alcoholic layer was examined. Pink color indicated the presence of alkaloids.

Test for flavonoids*:* The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnings and potassium hydroxide solution. Red color indicated the presence of flavonoids.

Drugs and chemicals used: The standard drug metformin was the generous gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. Total cholesterol (TC) and triglyceride (TG) wet reagent diagnostic kits were the products of Cresent diagnostic kits. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. SGPT and SGOT wet reagent diagnostic kits were purchased from AMP Medizintechnik GmbH; Austria. Dimethyl sulfoxide (DMSO) was purchased from Loba Chemie, Bombay, India and used to dissolve metformin and the different fractions of extract of *M. charantia* since these substances are insoluble in water and other available inert solvents [10].

Preparation of dosage of active drug and plant extract:

Metformin*:* Metformin was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form using sterilized water in such a concentration that, each 0.1 ml of solution contained metformin according to the dose of 150 mg/kg body weight since metformin is effective in such dose.

Momordica charantia: The dried fractions of extract of *M. charantia* were dissolved in 99% DMSO to prepare the solution where each 0.1 ml contained *M. charantia* according to the dose of 150 mg/kg body weight[11]. 0.1 ml of each solution was administered intraperitoneally to every 100 gm body weight of the rats during treatment to achieve required dose of fractions of plant extract.

Selection of animal: A total number of 30 long-Evans male rats weighing about 150-180gm, age 2 months were purchased from animal house of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Prior to commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well ventilated animal house at room temperature of 25°C and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water. All the rats were kept in cages with wide square mesh at bottom to avoid coprophagy and maintained with natural 12 hour light and dark cycle.

Grouping of experimental rats: 30 long-Evans male rats were randomly assigned into 6 groups, 5 rats in each group.

Group 1	Normal Control
Group 2	Diabetic Control
Group 3	Diabetic+ Metformin (150mg/kg body wt.)
Group 4	Diabetic+ Petroleum ether fraction MC (150mg/kg body wt.)
Group 5	Diabetic+ Ethyl acetate fraction MC (150mg/kg body wt.)
Group 6	Diabetic+ Chloroform fraction MC (150mg/kg body wt.)

Experimental induction of diabetes and collection of serum: Group 1 animals were used for normal control receives only vehicle (DMSO). Groups 2-6 animals were allowed to fast for 12 hrs and were induced diabetic by injection intraperitoneally a freshly prepared solution of alloxan (110mg/kg body wt.) in normal saline after base line glucose estimation was done. The alloxan treated animals were allowed to feed over night to overcome drug induced hypoglycemia. After 48 hours blood glucose content was measured by using Bioland G-423 test meter (Bioland, Germany) using blood sample from the tail vein of the rats. When the condition of diabetes was established (blood glucose levels above 11.1 mmol/L) the drug (metformin) and fractions of plant extract were administered to the respective groups of animals selected for the study. After completing the 24 hours blood glucose level testing the rats were sacrificed and 7 ml of blood was collected directly from heart of each rat by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was obtained. The serum was then used for the experimental analysis.

Test of hypolipidemic effects of the plant extracts (TC and TG test): The blood serum collected from hearts after sacrificing the rats was used for testing the serum total cholesterol (TC) and triglyceride (TG) levels. The concentrations were analyzed by taking absorbance by UV spectrophotometer (Shimidzu UV-1200, Tokyo, Japan) using commercial wet reagent diagnostic kits (Boehringer Mannheim GmbH, Germany) according to manufacturer's protocol.

Hepatoprotective activity test (SGOT and SGPT test): In this case, the blood serum was used for testing of the SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) levels. The concentrations were analyzed by taking absorbance by UV spectrophotometer (Shimidzu UV-1200, Tokyo, Japan) using commercial wet reagent diagnostic kits (AMP Medizintechnik GmbH; Austria) according to manufacturer's protocol.

Statistical analysis: The results were expressed as mean±SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ($p<0.05$).

RESULTS: The effect of the different fractions of methanolic extract of *Momordica charantia on* the serum total cholesterol (TC), serum triglyceride (TG), SGOT and SGPT levels were investigated in the alloxan-induced diabetic rats using metformin as a standard agent.

Effect of different fractions of *Momordica charantia* on TC and TG levels in diabetic rats: The mean serum total cholesterol and triglyceride levels of control and treated animals after 24 hours are shown in **Fig. 1 and 2**, respectively. Hypolipidemia was observed in animals treated with the different fractions. In case of the effects of metformin and different fractions of *M. charantia* on total cholesterol level in diabetic rats the metformin, petroleum ether, ethyl acetate and $CHCl_3$ fractions of *M. charantia* reduced total cholesterol level to 47%, 18%, 16% and 21%, respectively. However, the effects of metformin and different fractions of *M. charantia* on serum triglyceride level in diabetic rats the metformin, petroleum ether, ethyl acetate and $CHCl_3$ fractions of *M. charantia* reduced serum triglyceride level to 39%, 23%, 27% and 31%, respectively. The results were compared with diabetic control group.

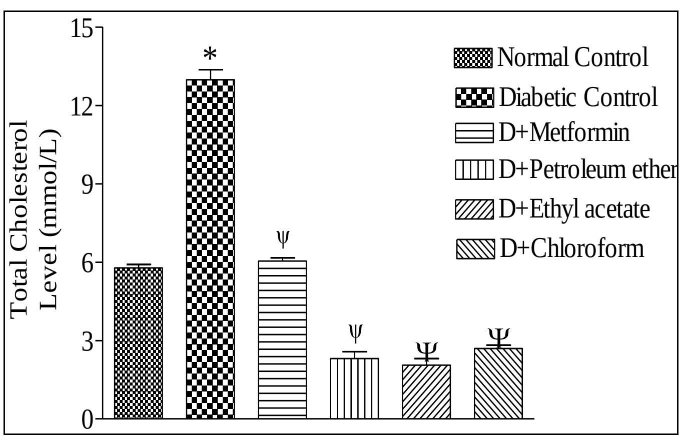

FIG. 1: EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* ON THE TOTAL CHOLESTEROL LEVEL IN DIABETIC RATS

* indicates significant change compared with normal control group (p<0.05).

ψ indicates significant changes in diabetic rats after treatment (p< 0.05). The results are expressed as means ± SEM.

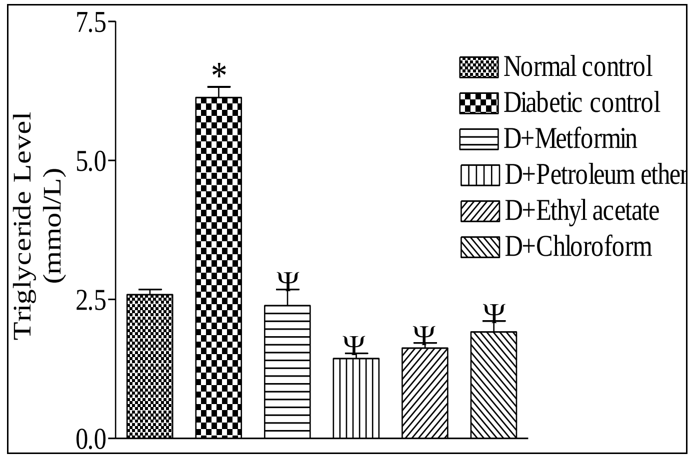

FIG. 2: EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* ON THE TRIGLYCERIDE LEVEL IN DIABETIC RATS

* indicates significant change compared with normal control group (p<0.05). The results are expressed as means ± SEM.

Effect of different fractions of *Momordica charantia* on liver enzymes (SGOT and SGPT) in diabetic rats: In diabetic rats SGOT and SGPT levels were raised in comparison to normal rats. Following intraperitoneally administration of different plant fractions SGOT and SGPT levels were significantly reduced compared with diabetic control group as shown in the Table 1.

TABLE 1: EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* EXTRACTS ON SGPT AND SGOT LEVEL

Group	SGOT (Unit/ml)	SGPT (Unit/ml)
Normal Control	18.0	21.5
Diabetic Control	46.1*	26.8
D + Metformin	23.2 Ψ	15.7 Ψ
D + Petroleum ether	20.5 Ψ	21.0 Ψ
D + Ethyl acetate	22.0 Ψ	17.3 Ψ

* indicates significant difference (*p*<0.05) from normal control group. Ψ indicates significant difference (*p*<0.05) from the diabetic control. Data are expressed as means ± SEM

Phytochemical Screening: The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpenes, alkaloids and flavonoids were present in the plant

M. charantia which have the hypolipidemic and hepatoprotective properties. The results are summarized in **Table 2**.

TABLE 2: THE PHYTOCHEMICAL CONSTITUENTS OF THE EXPERIMENTAL PLANT FRACTIONS OBTAINED BY PHYTOCHEMICAL SCREENING TESTS

Partitionates	Saponin	Tannins	Triterpenes	Alkaloids	Flavonoids
Chloroform	+	+	+	-	+
Ethyl acetate	+	-	+	+	+
Petroleum ether	+	+	+	+	+

(+) = Present; (-) = Absent

DISCUSSION: Hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases. The epidemiologic data shows that the prevalence of dyslipidaemia in Chinese adults aged 18 and above is 18.6%, which is to say the number of dyslipidemic patients has reached 160 million [12]. It is also reported that almost 12 million people die of cardiovascular diseases and cerebral apoplexy each year all over the world. Therefore, it is very important to pay attention to early stage prevention and control of hyperlipidemia in a comprehensive way. However, the risk of hyperlipidemia would be reduced by consumption of flavonoids and their glycosides, supported by abundant studies [13, 14, 15, 16].

In the light of the literature on *Momordica charantia* we made an attempt to study the effect of different fractions of methanolic extract of *M. charantia* in diabetic rats. In diabetic rats there was a significant increase in lipids (total cholesterol and triglycerides) [17]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [18]. Intraperitoneal administration of different fractions of methanolic extract of *M. charantia* resulted in a significant reduction of serum lipid levels in rats viz. total cholesterol and triglyceride levels (Fig. 1 and 2). Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant activity [19]. *M. charantia* plant fractions demonstrated the presence of flavonoids and other different constituents such as saponins, tannins, triterpenes and alkaloids (Table 2). With respect to the lipid lowering capacity of these plant fractions, it could be suggested that the constituents of these plant fractions may acted as inhibitors for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in *de novo* cholesterol biosynthesis as has been suggested for some plants earlier [20, 21].

The increase in the activities of SGOT and SGPT is found in diabetic rats[22]. The higher levels of SGOT and SGPT may give rise to a high concentration of glucose. In other words, the gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids. Following intraperitoneal administration of different plant fractions significantly reduced the SGOT and SGPT levels (Table 1). The hepatoprotective activity of *Momordica charantia* may be attributed due to the presence of flavonoids, ascorbic acid [23] and other components such as saponins, tannins, triterpenes and alkaloids etc. (Table 2).

CONCLUSION: In conclusion, the administration of plant fractions produced significant reduction of blood lipid level as well as SGOT and SGPT level. It causes rapid induction of hypolipidemia as well as hepatoprotective effect in diabetic rats. Thus, in the light of our pharmacological studies the study of various fractions of *M. charantia* extract might offer a natural key in hypolipidemic and hepatoprotective activity. Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the exact mechanism of actions.

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