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ANTIDIABETIC AND GLYCOGENESIS 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 antihyperglycemic, oral glucose tolerance test (OGTT) and glycogenesis effects of the different fractions (Petroleum ether, ethyl acetate and chloroform) of methanolic extract of *Momordica charantia.* The different fractions of the extract were administered intraperitoneally as a single dose of 150 mg/kg body weight to alloxan induced as well as glucose induced diabetic rats and found to reduce blood glucose level significantly ($p<0.05$). The different fractions of *Momordica charantia* to the alloxan-induced diabetic rats resulted in the significant elevation of liver glycogen content which was decreased by 50.60% in diabetic control. The plant fractions also improve the glucose tolerance in the glucose induced rats. The effects of plant fractions were compared with standard drug metformin. The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpines, alkaloids and flavonoids etc. were present in the plant which has antidiabetic and glycogenesis properties. Thus, this investigation paves the way for plant based diabetic treatment and indicates that various fractions (Petroleum ether, ethyl acetate and chloroform) of the methanolic extract of *Momordica charantia* have favorable effect in bringing down the severity of diabetes, enhancing glycogenesis activity by increasing the cellular uptake of glucose and also improving glucose tolerance activity.

INTRODUCTION: Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action [1]. It is one of the oldest diseases affecting millions of people all over the world [2]. Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [3]. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals [4]. The number of cases of non-insulin dependent diabetes mellitus (Type-2) has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity, and ageing of populations. In the year 2000, the number of diabetic patients was 151 million and is estimated to rise to 300 million by 2025 [5].

According to the World Health Organization, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of the DM have been reported [6]. Plants are recognized as a wonderful source for medicines. It is estimated that 1200 species of plants are used as folk medicines for diabetes [7]. Most of them lack of scientific evidence for their alleged benefits.

There are various medicinal plants in the world, which are the potential sources of the drugs. The discovery of the widely used hypoglycemic drug, metformin (N, N-dimethylguanylguanidine) came from the traditional approach through the use of *Galega officinialis* [8]. Traditionally various plants are being used to treat diabetic patients. It is believed that, herbal medicine has little side effects as well as it requires no cost in few cases. Thus it can solve the economic problem of the poor. Now a day, the scientists and researchers are looking for natural plant products by research all over the world and a large number of the evidence have shown the immense potential of medicinal plants used traditionally. Therefore, a key strategy in treating patients with type 2 diabetes is maintenance of blood glucose level. Current oral anti-diabetic agents, which include insulin releasers, insulin sensitizers and glucosidase inhibitors, have modest efficacy and limited of modes of action. In addition, current anti-diabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness.[8] Thus, discovery and development of novel drugs for diabetes is still needed.

Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. In Bangladesh about 5 million people are affected with diabetes for various reasons, in recent years the popularity of complementary medicines has increased. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine have been used commonly in India [9].

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 [10, 11]. Research report of BMRC showed that *M. charantia* fruits have significant hypolipidemic and hepatoprotective effects.

In the present study, the antidiabetic and glycogenesis properties of *Momordica charantia* was assessed by evaluating the comparative antihyperglycemic, oral glucose tolerance test (OGTT) and glycogenesis activities in alloxan induced and glucose induced diabetic rats.

MATERIALS AND METHODS:

Plant materials: The fresh fruits of *Momordica charantia* (Local name- Karala) were collected from medicinal plant garden of 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 (Whatmann filter paper no. 1). Then the solvent was allowed to evaporate using rotary evaporator at temperature 40-45 ^{0}C. 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: [12]

Test for saponins*:* 300 mg 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*:* 300 mg extract mixed with 5 ml 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*:* 300 mg 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 turnins 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. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Glycogen test diagnostic kit was o-toluidine reagents. 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 [13].

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 fractionated extracts of *Momordica charantia* were dissolved in 99% DMSO to prepare the solution where each 0.1 ml contained *Momordica charantia* according to the dose of 150 mg/kg body weight [14]. 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 animals: A total number of 50 long-Evans male rats weighing about 150-180 gm, 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^{o}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: 50 long-Evans male rats were randomly assigned into 10 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.)
Group 7	Normal+ Glucose
Group 8	Normal+ Glucose+ Metformin (150mg/kg body wt.)
Group 9	Normal+ Glucose+ Petroleum ether fraction MC (150mg/kg body wt.)
Group 10	Normal+ Glucose+ Ethyl acetate fraction MC (150mg/kg body wt.)

Experimental induction of diabetes: 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 (110 mg/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 animals with blood glucose levels above 11.1 m mol/L was selected for the study. Group 7 animal were used for normal control receives only glucose and groups 8-10 animals were allowed to fast for 12 hrs were rendered diabetic by oral administration of glucose for the test of OGTT.

Antihyperglycemic effect of plant extracts: The groups 2-6 were prepared for resting antihyperglycemic effect after alloxan induction. All the rats were starved at water for 16 hours and then were tested for baseline glucose level. The group 2 was selected for diabetic control group which does not receive extract or metformin. The group 3 stands for metformin control group which was administered metformin intraperitoneally at a dose of 150 mg/kg body weight. The groups 4-6 received the different fractions of extract of the plants. The blood glucose level was then tested by using glucometer (Bioland Glucometer, Germany). In this case, the blood was collected by picking the tail vein in 0, 2, 6, 16 and 24 hours after drug and plant extract administration [15].

Estimation of glycogen content in liver: The liver was collected from hearts after sacrificing the rats of the groups 1-6 which were used for

antihyperglycemic experiment. Then liver glycogen concentrations were analyzed by taking absorbance by UV spectrophotometer using o-toluidine reagent diagnostic kits. This test utilizes the o-toluidine-glucose coupling reaction for the estimation of glycogen after trichloroacetic acid (TCA) extraction, precipitation by alcohol and hydrolysis [16].

Oral glucose tolerance test: Groups 7-10 were selected for OGTT test after starving at water for 16 hours. The base line glucose level was measured by glucometer [17]. Group 7 stands for normal control group received glucose. Group 8 was treated with metformin (150 mg/kg body weight). The extracts of different fractions were then administered intraperitoneally at the dose of 150 mg/kg body weight. 2ml glucose (2 gm/kg body weight) solution was administered orally by intra gastric tube. The blood glucose level was then tested by using glucometer (Bioland Glucometer, Germany). In this case, the blood was collected by picking the tail vein in 0, 30, 60, 90, 150 and 270 minutes.

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 fasting blood glucose (FBG) level, glycogen content in liver and oral glucose tolerance test (OGTT) were investigated in the alloxan-induced diabetic rats using metformin as standard antidiabetic agents.

Effect of different fractions of *M. charantia* on fasting blood glucose (FBG) level in diabetic rats: The mean blood glucose concentration of control and different fractions of *M. charantia* treated animals were estimated on the 2, 6, 16 and 24 hours, respectively as shown in **Fig. 1**. Their baseline glucose concentrations were also measured. Petroleum ether fraction reduced blood glucose level to 61%, 27%, 15% and 13% in 2, 6, 16 and 24 hours, respectively. Maximum reduction of blood glucose level by 87% was observed for petroleum ether fraction in 24 hours of the experiment. Ethyl acetate fraction reduced blood glucose level to 75%, 48%, 30% and 25% in 2, 6, 16 and 24 hours, respectively. Maximum reduction of blood glucose level by 75% was observed for ethyl acetate fraction in 24 hours of the experiment. $CHCl_3$ fraction reduced blood glucose level to 80%, 50%, 32% and 32% in 2, 6, 16 and 24 hours, respectively. Maximum reduction of blood glucose level by 68% was observed for petroleum ether fraction in 24 hours of the experiment.

Incase of metformin, (Fig. 1) it reduced blood glucose level to 66%, 38%, 20% and 25% in 2, 6, 16 and 24 hours, respectively. Here, metformin caused maximum reduction of blood glucose level by 80% in 16 hours. The results were compared with diabetic control group.

Effect of different fractions of *M. charantia* on the level of glycogen in diabetic rats: In this study, it was found that the level of glycogen in liver was reduced to 49% in diabetic rats as compared to the normal control group. In the treatment of diabetic rats with metformin standard, petroleum ether and ethyl acetate fractions of *M. charantia,* the level of glycogen content was improved to 86%, 80% and 83%, respectively as shown in the **Fig. 2**. In this case chloroform fraction *of M. charantia* had more significant activity in glycogen synthesis. The results were compared with diabetic control group.

Effect of different fractions of *M. charantia* on fasting blood glucose (FBG) level in the glucose-induced hyperglycemic rats: In case of glucose-induced hyperglycemia, metformin reduced blood glucose level to 62%, 58% and 53% in 30, 60 and 90 minutes, respectively. So, metformin caused maximum reduction of blood glucose level of 53% in 90 minutes. Petroleum ether fraction reduced blood glucose level to 96%, 78% and 68% in 30, 60 and 90 minutes, respectively. Ethyl acetate fraction reduced blood glucose level to 72%, 69% and 70% in 30, 60 and 90 minutes, respectively as shown in the **Fig. 3**. Here the maximum reduction of 31% was observed by Petroleum ether fraction in 60 minutes. The results were compared with normal control group which received glucose only.

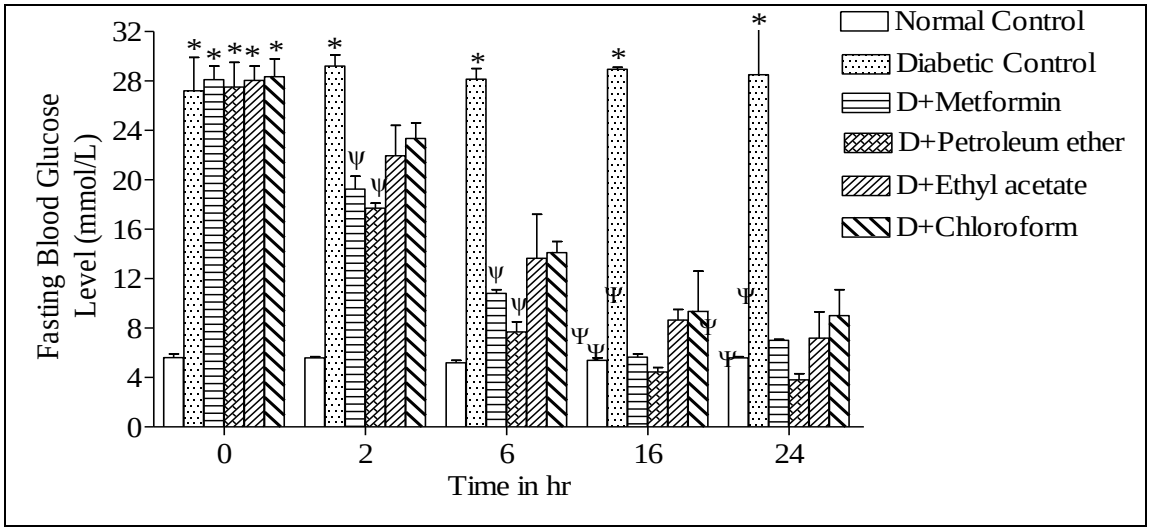

FIG. 1. EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* ON THE FBG LEVEL ON DIABETIC RATS COMPARED TO NORMAL RATS.

*** INDICATES SIGNIFICANT CHANGE IN BLOOD GLUCOSE LEVEL COMPARED WITH NORMAL CONTROL GROUP ($p<0.05$).**

Ψ INDICATES SIGNIFICANT CHANGES IN FBG LEVEL IN DIABETIC RATS AFTER TREATMENT ($p<0.05$).

THE RESULTS ARE EXPRESSED AS MEANS ± SEM.

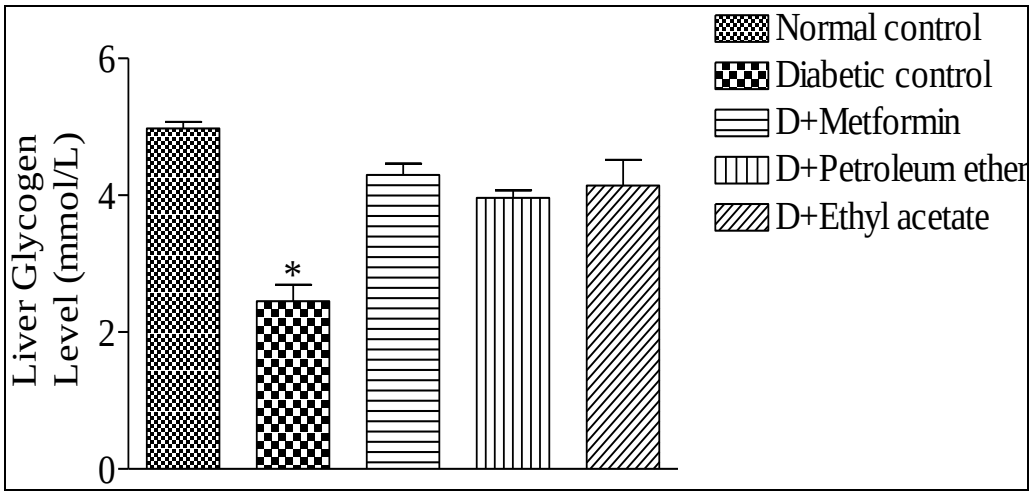

FIG. 2: EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* ON THE GLYCOGEN LEVEL IN LIVER ON DIABETIC RATS COMPARED TO NORMAL RATS.

*** INDICATES SIGNIFICANT DIFFERENCE ($p<0.05$) FROM WITHOUT ALLOXAN TREATED TIME CONTROL.**

THE RESULTS WERE EXPRESSED AS MEANS ± SEM.

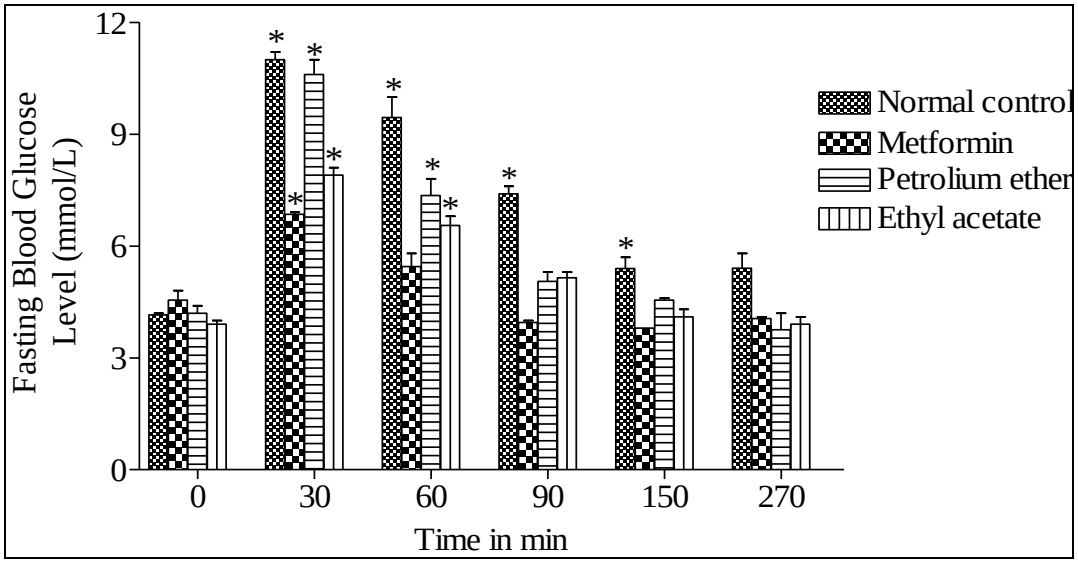

FIG. 3: EFFECT OF DIFFERENT FRACTIONS OF *M. CHARANTIA* ON THE GLUCOSE-INDUCED HYPERGLYCEMIA IN NORMAL RATS. * INDICATES SIGNIFICANT CHANGES IN FBG LEVEL COMPARED TO NORMAL RATS AFTER TREATMENT (P<0.05). THE RESULTS ARE EXPRESSED AS MEANS ± SEM.

Phytochemical Screening: The phytochemical screening tests indicated the different constituents such as saponins, tanins, triterpines, alkaloids and flavonoids were present in the plant *M. charantia* which have the antidiabetic and glycogenesis properties. The results are summarized in Table 1.

TABLE 1: THE PHYTOCHEMICAL CONSTITUENTS OF THE EXPERIMENTAL PLANT FRACTIONS OBTAINED BY PHYTOCHEMICAL SCREENING TESTS

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

(+) = Present; (-) = Absent

DISCUSSION: Diabetes is a major threat to global public health that is rapidly getting worse and biggest impact in on adult of working age in developing countries. The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great interest. The antihyperglycemic activity of *Momordica charantia* was evaluated with scientific approaches. Management of diabetes without any side effects is still a challenge for the medical system. This leads to an increasing search for improved antidiabetic drugs. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny. The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years [18].

In the light of the literature on *Momordica charantia* we made an attempt to study of the effect of different fractions of methanolic extract of *M. charantia* in diabetic rats. In this study the different fractions of plant extract reduced blood glucose level significantly in the hyperglycemic rats. The significant antihyperglycemic activity of petroleum ether, ethyl acetate and chloroform fraction of *M. charantia* as shown in Fig. 1 may be due to the presence of hypoglycemic saponins, tanins, triterpines, alkaloids and flavonoids etc. (Table 1). Further, *M. charantia* has been reported

to have P-insulin (or v-insulin) which is a large polypeptide that is structurally and pharmacologically comparable to insulin [19]. Besides, having a role in the renewal of beta cells in diabetic rats or alternately may permit the recovery of partially destroyed β-cells [20]. It could be conceived that the plant extracts may also contain some biomolecules that may sensitize the insulin receptor to insulin or stimulates the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level.

In diabetes, the glycogen content of the skeletal muscles and liver, markedly depleted [21] and the reduced level of hepatic glycogen are due to inadequate insulin secretion, which results in the inactivation of glycogen synthatase system [3]. In the present study decreased levels of glycogen and glycogen synthase were observed in diabetic control rats. It may be due to insufficient secretion of insulin in the diabetic state as stated earlier. It was reported that the treatment with *M. charantia* the accumulation of glycogen and its content rised to of normal level (Fig. 2).

This may be due to the presence of hypoglycemic saponins, tannins, triterpenes, alkaloids and flavonoids etc. and activation of glycogen synthatase system. Intraperitoneal administration of petroleum ether and ethyl acetate fractions of *M. charantia* recovered this problem and helped in cellular uptake of glucose and subsequently synthesis the liver glycogen again by the modulatory effects of constituents of the fractions through induction of insulin secretion. Decreased in the activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animals resulting in depletion of liver and muscle glycogen content [21]. Treatment with plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx.

Oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's main source of energy [17]. Juice of unripe fruits of *M. Charantia* has been reported to produce fall in fasting blood glucose (FBG) level and improve glucose tolerance in rats [22, 23, 24, 25]. In our study, it was observed that various fractions have also hypoglycemic effect in glucose induced hyperglycemic rats. The fractions of plant extract enhanced glucose utilization. So the blood glucose level was significantly reduced in the glucose loaded rats (Fig. 3). This may be due to the presence of hypoglycemic saponins, tannins, triterpenes, alkaloids and flavonoids etc. (Table 1).

CONCLUSION: Thus, in the light of our pharmacological studies it was observed that the administration of plant fractions of *M. charantia* extract demonstrated antihyperglycemic activity by producing significant restoration of blood glucose level as well as illustrated some beneficial effects such as increase liver glycogen synthesis activity by cellular uptake of glucose in alloxan-induced diabetic rats and also improvement of oral glucose tolerance in glucose induced diabetic rats. Further comprehensive pharmacological investigations are needed to elucidate the exact chemical compounds responsible for antihyperglycemic activity, liver glycogen synthesis activity as well as improvement of oral glucose tolerance and also their exact mechanism of actions.

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