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Scientific Research

Comparative Study between the Effect of *Momordica charantia* (Wild Type) Fruits and *Coccinia cordifolia*'s Leaf on Hypoglycemic and Hypolipidemic Activities of Alloxan Induced Type 2 Diabetic Long-Evans Rats

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Abstract

Aims: The study was aimed to compare the hypoglycemic and hypolipidemic effect of *Momordica charantia* (wild type) fruits and *Coccinia cordifolia*'s leaf in alloxan induced type 2 diabetic Long-Evans rats. Methods: All rats were divided into groups A to H (n = 48, 06 rats per group). They were made diabetic by intraperitoneally injecting alloxan monohydrate 150-mg/Kg-body weight. Groups A and B were provided with normal diet and glibenclamide plus normal diet respectively. C to H groups were provided with different percentages (70%, 50% and 30% of regular diet) of samples. Different biochemical parameters (blood glucose level, serum total cholesterol, triglyceride (TG), LDL, HDL, serum insulin, hepatic glycogen) were observed for 21 consecutive days (at eight days interval). Results: Oral administration of both of the *Momordica charantia* (wild type) fruits and *Coccinia cordifolia*'s leaf significantly ($p < 0.05$) improved hypoglycemic status by decreasing fasting blood glucose level. Hypolipidemic status also observed, and found improved significantly ($p < 0.05$) by decreasing the level of serum total cholesterol, triglyceride (TG), serum insulin level and LDL in type 2 diabetic rats. HDL levels were increased slightly and hepatic glycogen

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level was more or less unchanged in both groups at 22nd day. Conclusion: Though both samples show hypoglycemic and hypolipidemic activities, 50% wild type fruits of *Momordica charantia* were more significant ($p < 0.05$) to ameliorate the diabetic state in type 2 diabetic Long-Evans rats.

Keywords

Diabetics, Alloxan Monohydrate, Hypoglycemia, Hypolipidemia

1. Introduction

Diabetes is a major degenerative disease in the world today [1], affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [2]. Diabetes mellitus is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others [3]. It is the most common endocrine disorder and by 2025 three hundred million will subsequently have the disease [4] and the largest number of diabetic people will be in India, China and United States [5] [6]. Type 2 diabetes has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes mellitus (NIDDM). A recent epidemiological study in Bangladesh reported the prevalence of type 2 diabetes exceeding 11% and the prevalence of impaired fasting glucose, which precedes the onset of diabetes, exceeding 6% in Dhaka city [7]. In Bangladesh Institute of Research and Rehabilitation in Diabetes Endocrine and Metabolic Disorders (BIRDEM), a total of about 20,000 new cases of diabetes are diagnosed every year. As for biological test system, most of the cases—rats, mice, rabbits or guinea pigs etc. are used in the laboratory. In case of animal model for diabetes, alloxan, streptozotocin (STZ) etc. are injected intraperitoneally (IP) or intravenously (IV) to make the animal as a diabetic subject. More than 400 species have been reported to display hypoglycemic effects, but only a few of them have been investigated [8]-[11]. More than 800 plant species are showing hypoglycaemic activity [12]. Among them *Momordica charantia* (wild type) and *Coccinia cordifolia*'s leaf are commonly found in Bangladesh and show hypoglycemic and hypolipidemic activities more potently than *Momordica charantia* (hybrid type) and *Coccinia cordifolia*'s roots [13] [14]. There has been increasing demand for the use of plant products with antidiabetic activity due to their low cost, easy availability and lesser side effects. The aim of this comparative study is to compare the chronic effectiveness of wild type of *Momordica charantia* (so called tropical vine) and leaf of *Coccinia cordifolia* (so called aggressive vine) on glycemic, lipidemic, insulinemic status in type 2 diabetic model rats.

2. Research Design and Methods

2.1. Study Site and Desing

The wild type *M. charantia* fruits and *Coccinia cordifolia*'s leaves were collected from Raer bazaar, Dhaka, Bangladesh. These were identified by Bangladesh National Herbarium, Dhaka. The study was conducted in the Animal Research Section, Institute of Food Science & Technology (IFST) at Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. From the sample collection to comparative study the experiment was as follow (**Figure 1**).

2.2. Preparation of Experimental Sample

The leaves of *Coccinia cordifolia* and wild type of *M. charantia* fruits were washed and cut into small pieces and then dried using oven at 37˚C temperature. The dried samples were grinded to make powder after that screened to get fine powder.

2.3. Preparation Model Animals, Dose and Route of Administration

Total 56 fasted healthy female rats (Long-Evans) of local strain (body weight 140 gm to 190 gm) were made diabetic by injecting alloxan monohydrate intraperitoneally (150-mg/Kg-body weight) which destroys the ß cells of pancreas and produces diabetes mellitus. Three days after injection 51 rats were found survived. Then their

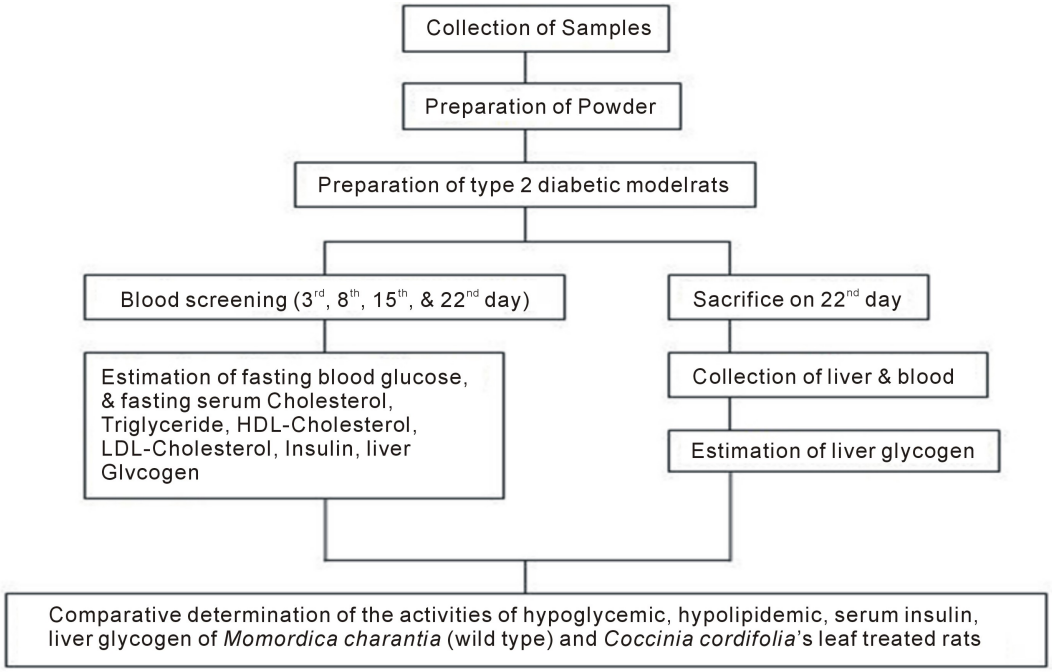


Figure 1. Flow chart of study.

fasting blood glucose level was measured and selects 48 as diabetic (blood glucose levels = 6 mmol/L) to convey the study. To observe the hypoglycemic activity the prepared samples (powder of *Coccinia cordifolia* leaves and wild type of *M. charantia* fruits) were administered orally at a dose of daily 70 g/kg (70%), 50 gm/kg (50%) and 30 gm/kg (30%) body weight for 21 days.

2.4. Grouping of Rats

The experimental rats were kept under observation for a week and maintained at aconstant room temperature of 25˚C ± 5˚C with humidity of 40% to 70% with natural 12 h day-night cycle. Total 48 rats were divided into 08 (eight) groups (six rats per group) as follow:

1) Group-A: fed normal diet.
2) Group-B: fed with glibenclamide plus normal diet.
3) Group-C: fed powder of *M. charantia* fruits 30% plus 70% normal rat diet.
4) Group-D: fed powder of *M. charantia* fruits 50% plus 50% normal rat diet.
5) Group-E: fed powder of *M. charantia* fruits 70% plus 30% normal rat diet.
6) Group-F: fed powder of *C. cordifolia* leaf 30% plus 70% normal rat diet.
7) Group-G: fed powder of *C. cordifolia* leaf 50% plus 50% normal rat diet.
8) Group-H: fed powder of *C. cordifolia* leaf 70% plus 30% normal rat diet.

2.5. Measurement of Biochemical Parameters

Biochemical parameters were measured by collecting fasting blood samples by amputation of the tail tip (about 0.2 ml blood collected) under diethyl ether anesthesia. Just before cutting the tail was immersed into warm water (400C) for approximately 22 seconds for vasodilatation. The level of blood glucose was determined 3rd (initial), 8th, 15th and 22nd day sample. At the final day (on 22nd) day blood sample was collected by sacrificing the rats. Blood glucose level of rat was measured by glucometer (OneTouch Ultra).Serum total cholesterol by enzymatic-colorimetric (Cholesterol Oxidase/Peroxidase, CHOD-PAP) method (Randox Laboratories Ltd., UK) using autoanalyzer (AutoLab). Serum HDL-cholesterol by enzymatic colorimetric (Cholesterol CHOD-PAP) method (Randox Laboratories Ltd., UK) using micro-plate reader (Bio-Tek, USA). Serum triglyceride (TG) by enzymatic colorimetric (GPO-PAP) method (Randox Laboratories Ltd., UK) using auto analyzer (Auto Lab). Serum LDL cholesterol was calculated by manually. The calculated formula was:

$$\text{LDL-C}=\text{TC}-\left(\frac{\text{TG}}{5}+\text{HDL}\right)$$

Serum insulin by Rat Insulin enzyme linked immunosorbent assay (ELISA) method (Crystal Chem Inc., USA). Liver glycogen levels were estimated by Anthrone-sulphuric acid method.

2.6. Statistical Analysis

Experimental data were analyzed using the Statistical Package for Social Science(SPSS) software for windows version 14 (SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean (±SD) and to compare the differences between different variables. Error bars were calculated by using standard deviations. Independent Student's t-test and ANOVA (analysis of variance) followed by Bonferroni post hoc test were performed for Statistical analysis of the results. A p value of less than 0.05 was considered significant.

3. Results

3.1. Effect of *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder on Body Weight of Model Rats

Body weight of each rat was taken at seven days interval. It is evident from the **Table 1** that at 8th day body weights were decreased from group-B to group-H but in group-A there was a consistent tendency to increase in body weight. At the end of study period we found that 50% of *M. charantia* fruits (wild type) powder have showed more considerable rise (5.66% rise in case of *M. charantia* and 2.76% in case of *C. cordifolia*) in body weight than 50% of *C. cordifolia*'s leaf powder.

3.2. Effect of *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder on Blood Glucose level (BGL) of Model Rats

The chronic effects of *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder on fasting glucose levels of type 2 diabetic model rats has been presented in **Table 2**. At the initial day fasting blood glucose levels were comparatively higher indicating the presence of functioning ß cells. But after 21 days (on 22nd day) of chronic feeding, *Coccinia cordifolia* powder (leaf) had significant effect ($p < 0.05$) on lowering of fasting glucose levels of type 2 diabetic rats. The standard drug glibenclamide, which served as positive control, also showed significant ($p < 0.05$) hypoglycemic effect. A gradual decrease in blood glucose level (at all study day) was observed from initial to 22nd day. The decreased percentage was higher in 50% of both leaf & fruit but *M. charantia* fruits (wild type) showed more decrease (58.39%) in blood glucose level than *C. cordifolia*'s leaf (47.19%).

3.3. Comparison of Total Cholesterol (CH) & Triglyceride (TG) Levels of Model Rats after *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder Administration

Total Cholesterol (TC) and TG level were decreased from initial to 22nd day among all the groups. The data-from **Figure 2(a)** shows that after 21 days (on 22nd day), the decreasing tendency of TC levels were by 5.25%, 12.33% & 5.87% respectively for 70%, 50% & 30% of *M. charantia* fruits (wild type) and7.38%, 11.24% & 9.33% respectively for 70%, 50% & 30% of *C. cordifolia*'s leaf and 15.10% decreased was found in glibenclamide treated groups. After 21 days (on 22nd day), decreasing tendency of TG levels (from **Figure 2(b)**) were 6.91%, 25.39% & 9.59% and 8.00%, 23.80%, 9.52% in respect of 70%, 50% & 30% of *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf respectively and 18.05% decreased was found in glibenclamide treated groups. So 50% powder of *M. charantia* fruits (wild type) was found more potent than *C. cordifolia*'s leaf to decrease TC and TG level of diabetic rats.

3.4. Comparison of the Effect of *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder on Lipidemic Status (LDL and HDL) of Model Rats

The effect of *M. charantia* (wild type) and *C. cordifolia*'s leaf on atherogenic lipids (LDL-Cholesterol) is depicted in **Figure 3(a)**. It seems that, there were significant changes in case of LDL-cholesterol level among all the test groups after 21 days of chronic experiment. The standard drug control group had pronounced 35.03%

Table 1. Chronic effect of *M. charantia* (Wild) fruits and *C. cordifolia*'s leaf powder on body weight (BW) of alloxan induced type 2 diabetic rats.

Group	BW_Initial day (gm)	BW_8th day(gm)	BW_15th day(gm)	BW_22nd Day (gm)
Group-A (Normal diet) (n = 06)	160.4 ± 3.35 (100%)	166.7 ± 2.86 (103.92%)	171.8 ± 4.58 (107.10%)	177.9 ± 5.48 (110.91%)
Group-B (Glibenclamide) (n = 06)	169 ± 2.53 (100%)	165.0 ± 2.23 (97.63%)	166.6 ± 2.18 (98.57%)	169.1 ± 2.71 (100.05%)
Group-C (30% *M. charantia* fruits) (n = 06)	154.2 ± 3.32 (100%)	152.5 ± 4.48 (98.70%)	154.9 ± 4.66 (100.45%)	160.1 ± 7.1 (103.82%)
Group-D (50% *M. charantia* fruits) (n = 06)	157 ± 3.80 (100%)	156.6 ± 2.66 (99.74%)	160.1 ± 3.17 (101.97%)	165.9 ± 3.22 (105.66%)
Group-E (70% *M. charantia* fruits) (n = 06)	156 ± 4.10 (100%)	153.2 ± 5.21 (98.20%)	154.1 ± 4.48 (98.78%)	160 ± 3.73 (102.56%)
Group-F (30% *C. cordifolia*'s leaf) (n = 06)	159 ± 2.4 (100%)	155.0 ± 3.25 (97.48%)	160.1 ± 1.80 (100.69%)	162.3 ± 3.69 (102.07%)
Group-G (50% *C. cordifolia*'s leaf) (n = 06)	152 ± 2.56 (100%)	147.1 ± 2.27 (96.77%)	151 ± 3.10 (99.34%)	156.2 ± 2.49 (102.76%)
Group-H (70% *C. cordifolia*'s leaf) (n = 06)	165 ± 4.03 (100%)	158.1 ± 5.49 (95.81%)	166.6 ± 3.73 (100.96%)	169.3 ± 5.00 (102.60%)

Table 2. Chronic effect of *M. charantia* (Wild) fruits and *C. cordifolia*'s leaf powderon fasting blood glucose Conc. (Glu.) Of alloxan induced type 2 diabetic model rats.

Group	Glu_Initial Day (mMol/l)	Glu_8th day (mMol/l)	Glu_15th day (mMol/l)	Glu_22nd day (mMol/l)
Group-A (Normal diet) (n = 06)	5.20 ± 0.35 (100%)	5.5 ± 0.38 (105.77%)	5.30 ± 0.31 (101.92%)	5.22 ± 0.30 (100.39%)
Group-B (Glibenclamide) (n = 06)	15.00 ± 1.3 (100%)	11.0 ± 0.5 (73.33%)	9.0 ± 0.8 (60.0%)	5.76 ± 0.5 (38.4%)
Group-C (30% *M. charantia* fruits) (n = 06)	15.06 ± 0.52 (100%)	12.78 ± 0.97 (84.86%)	10.92 ± 1.27 (72.51%)	7.63 ± 1.15 (50.66%)
Group-D (50% *M. charantia* fruits) (n = 06)	14.9 ± 0.45 (100%)	12.5 ± 0.42 (83.89%)	8.34 ± 0.18 (55.97%)	6.20 ± 0.33 (41.61%)
Group-E (70% *M. charantia* fruits) (n = 06)	15.68 ± 1.58 (100%)	13.0 ± 1.88 (82.90%)	9.0 ± 0.90 (57.39%)	6.68 ± 0.51 (42.60%)
Group-F (30%*C.cordifolia*'s leaf) (n = 06)	15.32 ± 1.04 (100%)	13.30 ± 1.33 (86.81%)	11.72 ± 0.97 (76.50%)	11.2 ± 0.79 (73.10%)
Group-G (50% *C. cordifolia*'s leaf) (n = 06)	17.42 ± 0.54 (100%)	13.45 ± 1.01 (77.21%)	11.78 ± 0.93 (67.62%)	9.20 ± 0.65 (52.81%)
Group-H (70% *C. cordifolia*'s leaf) (n = 06)	13.98 ± 1.70 (100%)	12.62 ± 1.78 (90.27%)	10.6 ± 1.14 (75.82%)	8.20 ± 0.19 (58.65%)

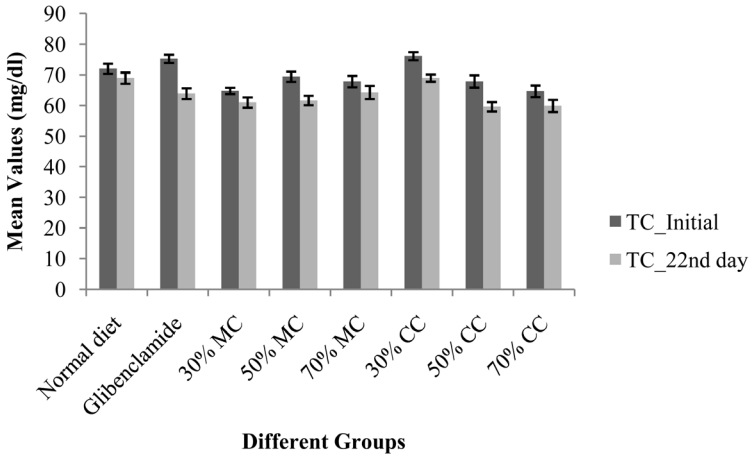
LDL-cholesterol decreasing effect from the initial value. 50% powder of *M. charantia* fruits (wild type) fruits and *C. cordifolia*'s leaf showed more efficient LDL-cholesterol decreasing effect than other presentence and groups of the study.

The effect of *M. charantia* (wild type) fruits and *C. cordifolia*'s leaf on atherogenic lipids (HDL-cholesterol) is depicted in **Figure 3(b)**. The standard drug control group had HDL-cholesterol increasing effect. In the cases of 50% powder of *M. charantia* (wild type) and *C. cordifolia*'s leaf treated group little effect to increase value from the initial value was found.

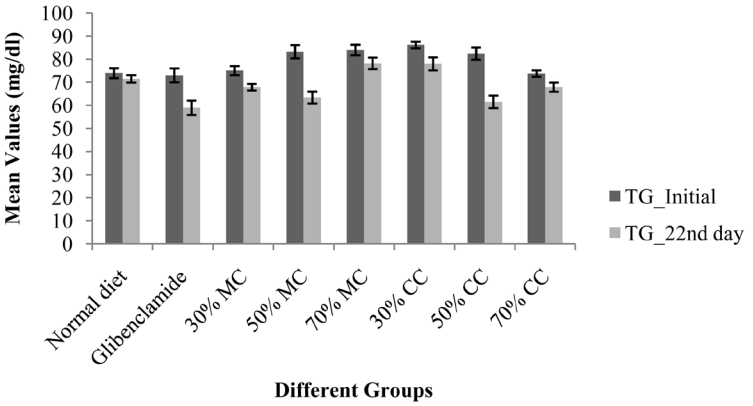
Though the effect was not much significant but 50% powder of *M. charantia* (wild type) was more efficient to decrease LDL level (34.63%) and increase HDL level (5.71%) than *C. cordifolia*'s leaf.

3.5. Comparison of Serum Insulin Levels of Model Rats after Providing *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder

The chronic effect of *M. charantia* (wild type) and *C. cordifolia*'s leaf powder on insulinemic status of type 2



(a)



(b)

Figure 2. (a) Comparison of TC level of different groups (Group A to group H) provided with *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder. Here, MC: *M. charantia* fruits (wild type) and CC: *C. cordifolia*'s leaf. (b): Comparison of TG level of different groups (group A to group H) after providing *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder; Here, MC: *M. charantia* fruits (wild type) and CC: *C. cordifolia*'s leaf.

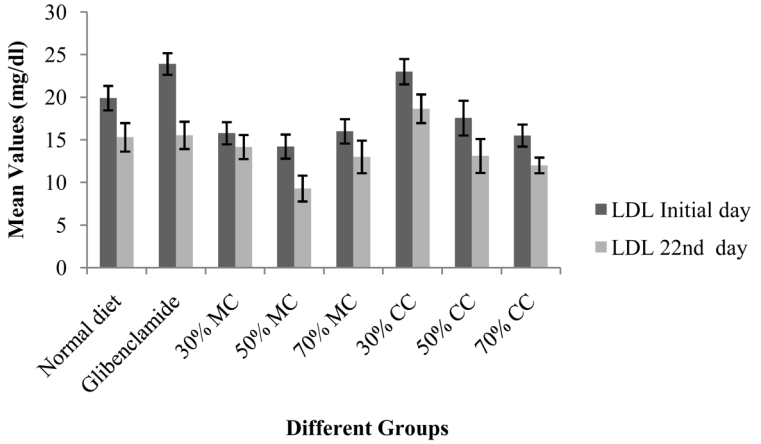
diabetic model rats is shown in **Figure 4**. Serum insulin level increases significantly ($p < 0.05$) with providing wild type of *M. charantia* fruits (40.71%) than leaf of *C. cordifolia*'s (17.34%). So compared with *M. charantia* fruits and *C. cordifolia*'s leaf, wild type of *M. charantia* fruits is more effective than *C. cordifolia*'s leaf to effect on serum insulin level.

3.6. Hepatic Glycogen Content after Providing *M. charantia* Fruits (Wild Type) and *C. cordifolia*'s Leaf Powder to the Type 2 Diabetic Model Rats

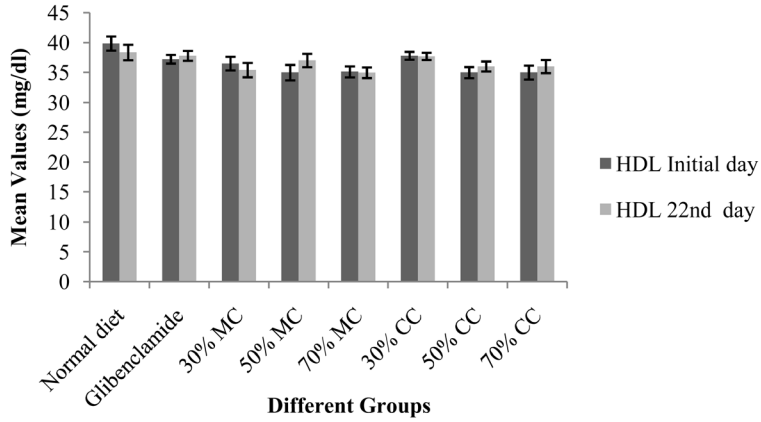
Table 3 shows the content of liver glycogen in normal control and treated diabetic rats (Glibenclamide treated, *M. charantia* (wild) and *C. cordifolia*'s leaf treated). In comparison with the normal control and treated groups it was observed that the glycogen content of rat liver was not significantly ($p < 0.05$) changed.

4. Discussion

The study was carried out to compare the Hypoglycemic and Hypolipidemic effect of *M. charantia* (wild type) fruit and *C. cordifolia*'s leaf in type 2 diabetic model rats. Chronic experiment with 30%, 50% and 70% of sample was done and 50% of *M. charantia* (wild type) fruit showed more prominent results to improve glycemic,



(a)



(b)

Figure 3. (a) Chronic effect of *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder on LDL level; Here, MC: *M. charantia* fruits (wild type) and CC: *C. cordifolia*'s leaf. (b) Chronic effect of *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder on HDL level; Here, MC: *M. charantia* fruits (wild type) and CC: *C. cordifolia*'s leaf.

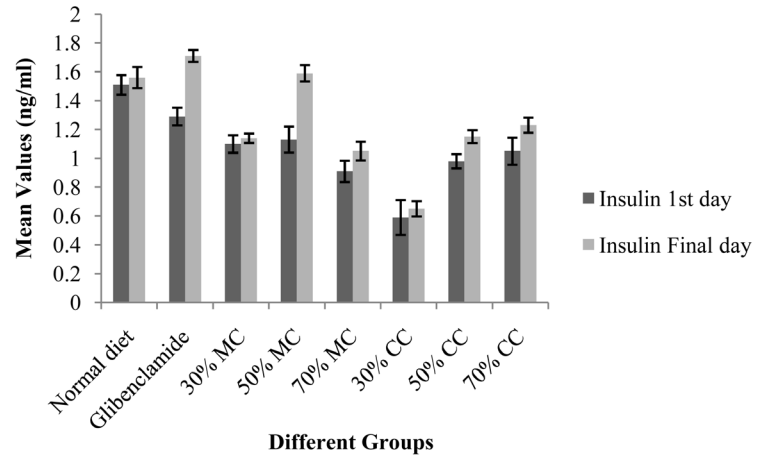


Figure 4. Chronic effect of *M. charantia* fruits (wild type) and *C. cordifolia*'s leaf powder on serum insulin level; Here, MC: *M. charantia*fruits (wild type) and CC: *C. cordifolia*'s leaf.

Table 3. Chronic effect *M. charantia* fruits (wild type) and*C. cordifolia*'s leaf powderon hepatic glycogen content of type 2 diabetic model rats.

Groups	Glycogen_22nd (mg/gm of tissue)
Group-A (Normal diet) (n = 06)	36.88 ± 2.29
Group-B (Glibenclamide) (n = 06)	32.45 ± 2.50
Group-C (30% *M. charantia* fruits) (n = 06)	31.58 ± 1.58
Group-D (50% *M. charantia* fruits) (n = 06)	31.08 ± 2.21
Group-E (70% *M. charantia* fruits) (n = 06)	32.63 ± 1.74
Group-F (30% *C. cordifolia*'s leaf) (n = 06)	32.12 ± 0.8
Group-G (50% *C. cordifolia*'s leaf) (n = 06)	31.43 ± 0.94
Group-H (70% *C. cordifolia*'s leaf) (n = 06)	33.94 ± 1.2

lipidemic status in type 2 diabetic model rats among other study groups. From **Table 1** it is evident that body weight of the study groups is restored after providing the *M. charantia* (wild type) fruit and *C. cordifolia*'s leaf. High blood glucose level is destructive effect of type 2 diabetics which is more significantly ($p < 0.05$) down regulated (from **Table 2**) in *M. charantia* (wild type) fruit groups than the *C. cordifolia*'s leaf groups. The possible mechanism by which powder brings about its hypoglycemic activity may be the potentiating of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing ß-cells or by its release from the bound insulin [15]. TC and TG level was also down regulated (in **Figure 2(a)** & **Figure 2(b)**) by both *M. charantia* (wild type) fruit and *C. cordifolia*'s leaf but 50% of *M. charantia* (wild type) fruit was more significant ($p < 0.05$) than 50% of *C. cordifolia* leaf. In the case of LDL it was found (from **Figure 3(a)**) that 50% wild type of *M. charantia* shows more decrease (34.63%) than *C. cordifolia*'s (25.29%) leaf. In case of HDL level (from **Figure 3(b)**) *M. charantia* (wild type) fruits showed more increase (5.71%) than the *C. cordifolia*'s (2.88%) (but the increase was not so significant). Increased in serum insulin level was observed from **Figure 4**. In comparison with *M. charantia* fruits and *C. cordifolia*'s leaf, wild type of *M. charantia* fruits was more effective than *C. cordifolia*'s leaf to increase serum insulin level. The increase in insulin, which results inactivation of glycogen synthetase system, may be due to improvement of glycogenesis. Hepatic glycogen content was not significantly changed with none of *M. charantia* (wild type) and *C. cordifolia*'s leaf as shown in **Table 3**.

5. Conclusion

From the study we can conclude that powder of *M. charantia* (wild type) fruits and *C. cordifolia*'s leaf exhibited promising hypoglycemic and hypolipidemic activities in alloxan induced Type 2 diabetic Long-Evans rats. Hence both *M. charantia* (wild type) fruits and *C. cordifolia*'s leaf can be helpful in the management of diabetes mellitus and other associated complications. In this study it is found that powder of *M. charantia* (wild type) fruits has more hypoglycemic and hypolipidemic activity than the powder of *C. cordifolia*'s leaf. Further investigations with large scale of animals may be more helpful to explicate the mechanism of action and active principles to project this plant as a therapeutic target in diabetes research.

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