Impact of Bitter Gourd (*Momordica charantia*) Hypoglycemic Formulation on Biochemical Parameters in Alloxan-Induced Diabetic Albino Rats

Abstract

Diabetes mellitus is a prevalent endocrine disorder characterized by chronic hyperglycemia. This study evaluates the hypoglycemic effects of a methanolic extract of *Momordica charantia* (bitter gourd) on blood glucose levels in alloxan-induced diabetic albino rats. The objective was to assess the efficacy of this extract compared to Glizid-M, a standard antidiabetic drug, in reducing hyperglycemia. Rats with alloxan-induced diabetes were administered *Momordica charantia* methanolic extract at three dosages: 150 mg/kg, 300 mg/kg, and 600 mg/kg. The highest dosage (600 mg/kg) demonstrated a statistically significant reduction in blood glucose levels compared to the lower doses (300 mg/kg and 150 mg/kg). These findings suggest a dose-dependent efficacy of *Momordica charantia* in lowering blood glucose levels in diabetic rats. This research contributes to the scientific validation of *Momordica charantia* as a potential therapeutic agent for diabetes management.

Keywords: Diabetes Mellitus, Alloxan-Induced Diabetes, *Momordica charantia*, Hypoglycemic Activity, Blood Glucose Regulation

Introduction

Diabetes Mellitus (DM) represents a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The two primary forms of diabetes are Type 1 Diabetes Mellitus (T1DM), formerly known as insulin-dependent diabetes mellitus (IDDM), and Type 2 Diabetes Mellitus (T2DM), formerly referred to as non-insulin dependent diabetes mellitus (NIDDM). Both types of diabetes have profound implications for health, leading to significant metabolic abnormalities such as dysregulated lipogenesis and protein glycosylation. The long-term complications associated with diabetes include cardiovascular disease, peripheral vascular disease, retinopathy, neuropathy, and nephropathy, all of which contribute to increased morbidity, mortality, and premature mortality, particularly among young adults. Additionally, diabetes can exacerbate reproductive issues, affecting both maternal and neonatal health (American Diabetes Association, 2021).

In India, the prevalence of diabetes is projected to increase dramatically, with estimates suggesting a rise of 195% by 2025 (King et al., 1998). This escalating epidemic impacts both urban and rural populations, imposing significant social, psychological, and financial burdens (Mohan et al., 2006). Effective management of diabetes has traditionally focused on pharmacological interventions aimed at controlling blood glucose levels and mitigating associated complications (Tiwary and Madhusudana, 2002). Recent advances have refined therapeutic regimens, but the quest for novel and complementary treatment options continues.

Herbal remedies have gained traction as potential adjuncts to conventional diabetes therapies, due to their accessibility, cost-effectiveness, and lower side-effect profiles. Among these, *Momordica charantia* (bitter gourd), a member of the Cucurbitaceae family, has been extensively studied for its anti-diabetic properties. In India and several other Asian countries, bitter gourd is not only a staple vegetable but also a traditional remedy

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for diabetes (Garau et al., 2003; Chandrashekar et al., 1989). Clinical trials suggest that its aqueous extract may offer superior efficacy compared to dried powder or dietary consumption (Karumanayake et al., 1990; Ahmed et al., 2004).

Momordica charantia influences glucose metabolism through multiple mechanisms. It has been shown to lower blood glucose levels by inhibiting disaccharidase enzymes, which prevents the hydrolysis of disaccharides into glucose (Oishi et al., 2007; Kumar Shetty et al., 2005). Additionally, it affects glucose transport channels, thereby reducing glucose absorption into the bloodstream (Singh et al., 2004). These effects are beneficial for both T1DM and T2DM patients, aiding in the prevention of postprandial hyperglycemia.

In T1DM, characterized by autoimmune destruction of pancreatic β-cells leading to insufficient insulin production, *Momordica charantia* has been reported to enhance insulin secretion (Yibchok-Anun et al., 2006; Fernandes et al., 2007). Evidence suggests the presence of an insulin-like molecule within bitter gourd (Khanna et al., 1981). While bitter gourd can supplement insulin therapy, it does not replace the need for insulin administration entirely.

For T2DM, often associated with obesity and characterized by insulin resistance in the liver, skeletal muscle, and adipose tissue, *Momordica charantia* has demonstrated potential in reversing insulin resistance (Nerurkar et al., 2008; Klomann et al., 2010). Its consumption may also mitigate secondary complications such as hypertension, dyslipidemia, and oxidative stress, which are common in T2DM patients (Satishekhar and Subramanian, 2005; Klomann et al., 2010). Furthermore, bitter gourd's impact on weight management and its potential anticancer properties add to its therapeutic appeal (Nerurkar et al., 2008).

Thus, the growing body of evidence underscores the significance of *Momordica charantia* as a promising candidate for diabetes management, warranting further exploration to substantiate its efficacy and integrate it into broader therapeutic strategies.

1. Materials and Methods

2.1 Plant Materials

The whole fruit of *Momordica charantia* Linn. (bitter gourd) utilized in this study was procured from a local vegetable market in Darbhanga, Bihar, India. Identification and authentication of the plant material were conducted by the Department of Botany, L. N. Mithila University, Darbhanga, Bihar, India.

2.2 Methanolic Extraction of Bitter Gourd Slices

The bitter gourd was thoroughly cleaned and sliced. The slices were air-dried at ambient temperature before being pulverized into a coarse powder. One kilogram of this coarse powder was subjected to methanolic extraction using a Soxhlet apparatus. The methanol extract was then concentrated under reduced pressure and subsequently dried in a vacuum desiccator to obtain the final extract.

2.3 Experimental Animals

Albino rats (weighing 100-150 g) of both sexes were procured from a local animal supplier in Darbhanga, Bihar, India. Prior to the commencement of experiments, the rats were acclimatized for seven days under controlled hygienic conditions and were provided with a standard rodent pellet diet (Gold Mohar, Lipton India Ltd.).

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2.4 Oral Glucose Tolerance Test (OGTT)

After a 30-day treatment with the methanolic extract of bitter gourd, an oral glucose tolerance test (OGTT) was performed. The rats were fasted for 14-16 hours before administering an oral glucose load (2 g/kg) as described by Du Vigneaud and Karr (1925) and Al Awadi et al. (1985). Blood glucose levels were measured at 30, 60, 90, and 120 minutes post-glucose administration using the tail puncture method. Blood glucose concentrations were quantified using a glucometer (Dr. Morepen Gluco One, Delhi).

2.5 Acute Oral Toxicity of Bitter Gourd

The acute oral toxicity of the bitter gourd extract was evaluated by administering doses ranging from 2500 to 3000 mg/kg body weight to different groups of rats (8 rats per group). Mortality was monitored over a 24-hour period. Acute oral toxicity was assessed following the methodology described by Litchfield and Wilcoxon (1949).

2.6 Experimental Design

A total of six groups of albino rats, each consisting of eight rats, were subjected to the following treatment regimens:

• **Group A**: Normal Control
• **Group B**: Alloxan-treated control (150 mg/kg, i.p. body weight)
• **Group C**: Alloxan (150 mg/kg, i.p. body weight) + Bitter gourd extract (150 mg/kg body weight, oral treatment)
• **Group D**: Alloxan (150 mg/kg, i.p. body weight) + Bitter gourd extract (300 mg/kg body weight, oral treatment)
• **Group E**: Alloxan (150 mg/kg, i.p. body weight) + Bitter gourd extract (600 mg/kg body weight, oral treatment)
• **Group F**: Alloxan (150 mg/kg, i.p. body weight) + Glizid-M (standard drug, 5 mg/kg body weight, oral treatment)

Bitter gourd extract and the standard drug Glizid-M were administered via intubation. Group A served as the normal control, while Groups B through F consisted of diabetic rats. Groups C to F, having previously received alloxan, were treated with fixed doses of bitter gourd extract (150, 300, and 600 mg/kg body weight) and the standard drug Glizid-M for 30 days.

2.7 Induction of Diabetes in Experimental Animals

Diabetes was induced in rats through a single intraperitoneal injection of alloxan-monohydrate (150 mg/kg body weight) as per the protocol outlined by R.V. Aruna et al. (1999). Alloxan was weighed individually for each animal, dissolved in 0.5 ml of saline immediately before administration. After 72 hours, rats exhibiting elevated blood glucose levels (>290 mg/dl) were selected. Treatment commenced immediately, excluding the normal

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control and diabetic control groups. Throughout the study, all experimental animals were provided with standard hygienic water and pellet diet. Alloxan is a toxic glucose analogue that selectively destroys pancreatic β-cells, resulting in insulin-dependent diabetes mellitus, similar to Type 1 diabetes in humans (Lenzen, 2008).

2.8 Differences Between Healthy and Alloxan-Diabetic Rats

Healthy Rats

- The pancreas contains functional islets of Langerhans that regulate blood glucose levels.
- Following the ingestion of a pellet diet, glucose subunits are cleaved and absorbed into the bloodstream.
- β-Cells of the pancreas release insulin, facilitating glucose uptake and its storage as glycogen in the liver and other tissues.
- When blood glucose levels drop, glucagon is secreted from the α-cells of the pancreas to mobilize stored glucose from the liver.
- This interplay between insulin and glucagon helps maintain blood glucose levels within a narrow range despite dietary intake and physical activity.

Alloxan-Diabetic Rats

- The pancreas is compromised with damaged islets of Langerhans, impairing its ability to regulate blood glucose levels effectively.
- Glucose subunits from the pellet diet are not properly cleaved or absorbed.
- The lack of insulin secretion disrupts glucose processing, leading to glucose-starved cells that rely on lipid breakdown for energy.
- Lipid catabolism produces ketone bodies, resulting in acidified blood and potentially causing diabetic ketoacidosis, coma, or death.

2.9 Collection of Blood Samples

Rats were briefly warmed before being anesthetized with chloroform and placed in a container for a short duration. A 1 cm section of the tail was quickly removed using a surgical razor. Blood was collected into EDTA vials for hematological tests and into clean, non-EDTA bottles to allow clotting. Serum was separated from the clot by centrifugation and transferred to clean bottles for biochemical analysis. A cauterizing agent (styptic pencil, Silver nitrate) was used to stop the bleeding. When multiple samples were required in a short period, the original wound could be reopened by removing the clot. Additional blood samples could be obtained by removing an extra 3-4 mm of tail (Janet Hoff, 2000).

2.10 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using the Chi-square test. P-values < 0.01 and 0.05 were considered statistically significant.

3. Results and Discussion

Diabetes Mellitus represents a group of metabolic syndromes characterized by increased systemic inflammation and oxidative stress, leading to severe complications such as blindness, diabetic foot disease, kidney failure, myocardial infarction, and encephalopathy. Recent research has demonstrated that upregulated inflammation is

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a primary contributor to metabolic syndrome diabetes. This condition is associated with various factors including insulin resistance, elevated blood lipid levels, ectopic fat deposition, reduced adiponectin levels, increased lipoprotein oxidation, oxidative stress, abnormal mitochondrial function, decreased cellular ATP, altered HDL function, and elevated homocysteine levels (Akhtar et al., 1981; Shibib et al., 1993).

Alloxan, a toxic glucose analogue, induces inflammation and generates highly reactive free radicals as by-products of mitochondrial oxidative-reduction reactions (MORR). In healthy cells, these free radicals are neutralized by protective enzymes such as superoxide dismutase (SOD) and catalase. However, in severe diabetic conditions, the activity of these protective enzymes is diminished, leading to damage of macromolecules such as DNA, RNA, and proteins, ultimately causing pancreatic beta-cell dysfunction and death (Lenzen, 2008).

The acute oral toxicity study of *Momordica charantia* methanolic extract revealed no mortality at doses up to 2500 mg/kg. However, mortality was observed at a dose of 3000 mg/kg in alloxan-induced diabetic rats. Significant increases in fasting blood glucose levels were observed in the diabetic control group compared to the normal control. The methanolic extract of *Momordica charantia* demonstrated hypoglycemic activity on the 10th, 20th, and 30th days of treatment. Notably, the hypoglycemic effect of *Momordica charantia* methanolic extract was found to be more effective than that of Glizid-M (Panacea Biotec) (Table 1, Figure 1).

The extract also significantly reduced serum urea, serum creatinine, and serum cholesterol levels in a dose-dependent manner over 30 days of treatment. Additionally, serum protein levels increased significantly in the extract-treated diabetic rats compared to those treated with Glizid-M (Table 2, Figure 2). The oral glucose tolerance test (OGTT) results showed improved glucose tolerance in diabetic rats treated with *Momordica charantia* methanolic extract, with high doses showing significant hypoglycemic effects after 120 minutes post-glucose load (Table 3, Figure 3).

These findings support the therapeutic potential of *Momordica charantia* in managing blood glucose levels and improving biochemical profiles in diabetic conditions. Akhtar et al. (1981) found that a dose of 150 mg/kg body weight was effective in alloxan-diabetic rabbits, while lower doses had no effect. Shibib et al. (1993) reported that the hypoglycemic effects of bitter gourd are attributed to the suppression of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase, alongside accelerated glucose metabolism through glucose-6-phosphate dehydrogenase.

4. Conclusions

The methanolic extract of *Momordica charantia* demonstrated greater effectiveness than Glizid-M in reducing circulating blood glucose levels and improving the biochemical profile of diabetic rats. Further studies are needed to explore additional therapeutic potentials of *Momordica charantia*.

Table-1- Effect of *Momordica charantia* methanolic extract and Glizid –M (Standard drug) on blood glucose level in alloxan induced diabetic albino rats.

Group		Blood glucose levels (mg/dl)		
	0 Day	10^{th} Day	20^{th} day	30^{th} day
Group –A Normal control	90.18±1.07	92.22±6.15	88.62±0.12	87.24±0.66
Group –B Diabetic control	298.10±1.18	310.12±2.12	309.22±3.16	299.14±0.86
Group-C Alloxan +Methanolic Extract (150mg/kg)	295.18±1.77	280.12±2.16	205.22±2.18	190.62±1.8

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Group –D Alloxan+Methanolic Extract (300mg/kg)	285.12±1.66	218.02±1.22	195.11±0.17	170.62±0.02
Group –E Alloxan +Methanolic Extract (600mg/kg)	289.16±0.67	210.02±2.67	140.40±3.16	95.85±7.80
Group –F Alloxan+Glizid-M (Standard drug)	285.12±6.45	209.45±3.18	135.18±6.12	110.10±1.18

Values are mean ± SEM, n=8,P<0.05 Vs Diabetic control.

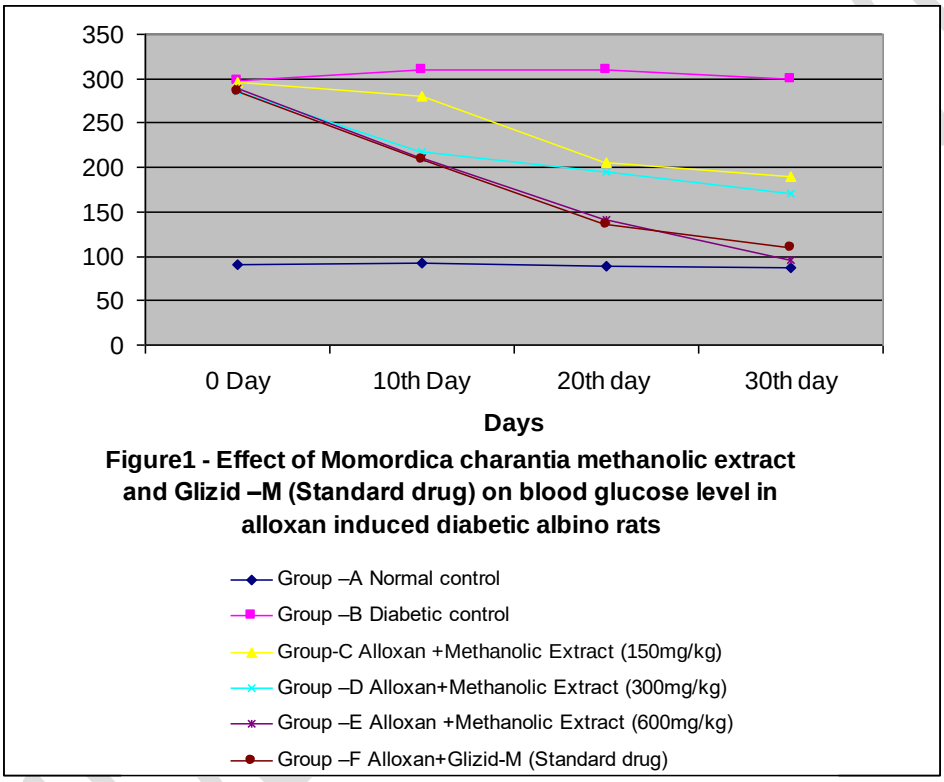

Figure1 - Effect of Momordica charantia methanolic extract and Glizid –M (Standard drug) on blood glucose level in alloxan induced diabetic albino rats

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Table-2 Effect of ***Momordica charantia*** methanolic extract on biochemical profile in alloxan induced diabetic rats.

Group Treatment	Serum-Urea (mg/dl)	Serum creatinine (mg/dl)	Serum cholesterol (mg/dl)	Serum protein(mg/dl)
Group A - Normal control	34.01 ± 1.17	0.46 ± 0.09	100.45 ± 1.07	7.18 ± 0.18
Group B - Diabetic control	70.18 ± 2.10	1.58 ± 0.09	205.91 ± 2.46	4.90 ± 0.87
Group C - Alloxan+Methanolic Extract(150mg/kg)	60.20±1.20	1.30±0.7	180.80±2.17	4.71±0.87
Group D - Alloxan+Methanolic extract (300mg/kg)	50.18±1.70**	1.10±0.02**	110.70±4.18**	6.18±0.17**
Group E –Alloxan + Methonolic Extract (600 mg / kg)	30.00±1.80**	0.55±2.7**	105.40±2.07**	8.90±2.17**
Group F - Alloxan+Glizid-M (standard drug)	35.30±1.75***	0.60±2.07***	107.80±2.09***	7.01±2.07***

Values are mean ± SEM, n=8**P<0.001 and ***P<0.05 Vs Diabetic control

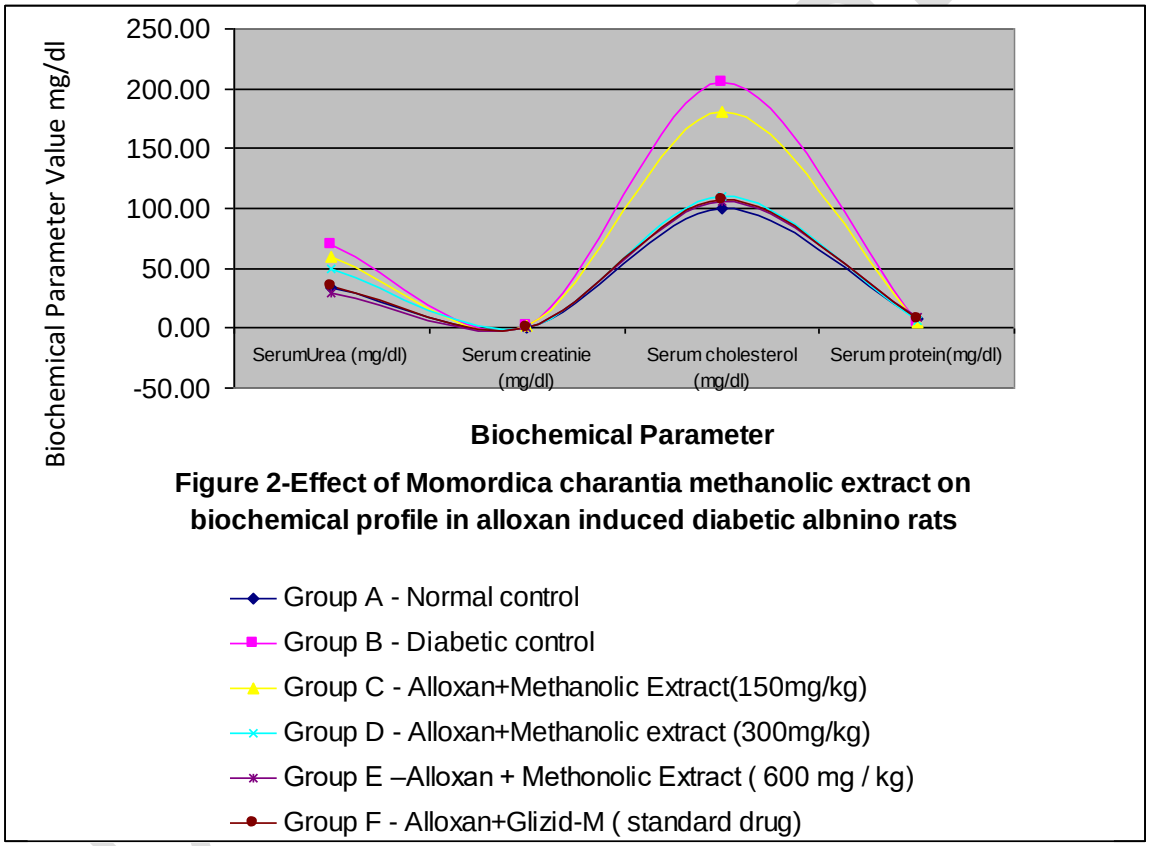

Figure 2-Effect of Momordica charantia methanolic extract on biochemical profile in alloxan induced diabetic albnino rats

Table 3 – Effect of *Momordica charantia* methanolic extract on oral glucose tolerance test in alloxan induced diabetic albino rats.

	Blood glucose level (mg/dl)				
Group	0 min	30 min	60 min	90 min	120 min
Group A normal control	90.60±2.60	190.18±4.01	145.09±2.07`	110.20±3.10	95.02±2.09

Group B diabetic control	295.81±7.45	338.8±1.60	370.09±1.2	342.09±9.12	320.20±7.18
Group C alloxan+Methanolic extract (150mg/kg)	120.18±7.42	240.78±7.16	260.60±6.12	205.70±2.60	170.18±4.62
Group C alloxan +Methanolic extract (300mg/kg)	169.12±6.92	220.16±6.12	200.02±5.12	180.18±3.17	160.20±3.28
Group D alloxan+methanolic extract (600mg/kg)	95.02±6.75	210.18±3.14	140.17±3.48	130.18±6.18	95.10±3.28
Group F alloxan +glizid-M (standard drug)	110.02±7.86	240.26±4.12	210.18±1.18	160.18±3.12	110.19±4.29

Values are mean ± SEM, n=8, P<0.05 Vs diabetic control

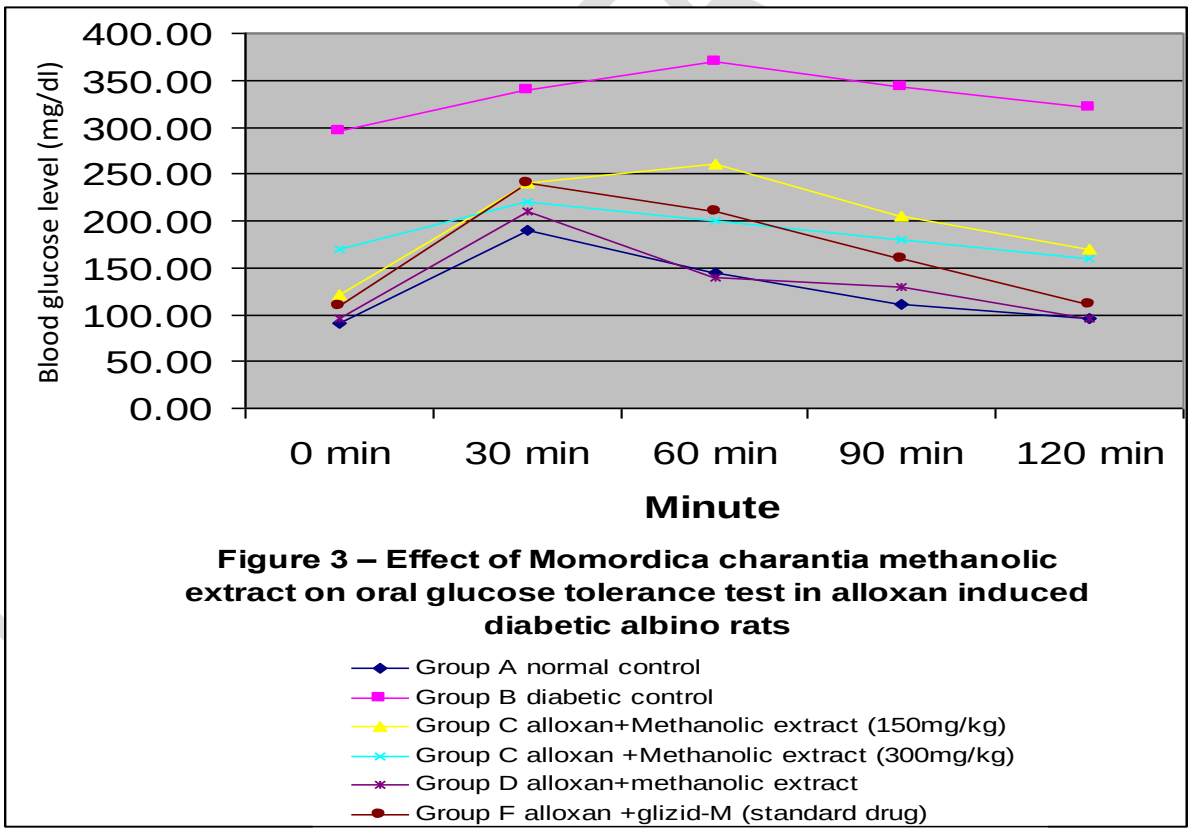

Figure 3 – Effect of Momordica charantia methanolic extract on oral glucose tolerance test in alloxan induced diabetic albino rats

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