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# Biochemical Study of Aqueous Extract of *Momordica charantia* in Alloxan Induced Albino Mice (*Mus musculus*)

Prafull Kumar Tandan[1], Anupam Bharti[2] & Navodita Priyadharshini[3]
[1,2]*Research scholar, University Department of Zoology,T.M.B.U*
[3]*Assistant Professor, University Department of Zoology, T.M.B.U*

***Abstract***
***Background:*** *Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which leads to severe complications affecting multiple organs. Alloxan is a diabetogenic compound that induces diabetes by selectively destroying pancreatic β-cells. Momordica charantia (bitter melon) is widely known for its antidiabetic properties, but its role in restoring serum protein levels in diabetic conditions remains unclear.*
***Aim:*** *This study aims to investigate the biochemical changes in serum protein expression upon alloxan-induced diabetes and the potential restorative effects of Momordica charantia in albino mice (Mus musculus).*
***Method:*** *Albino mice were divided into four groups having 10 mice each: Control (N), Control + M. charantia (NM), Alloxan-induced diabetic (DM), and Alloxan + M. charantia (AM). Blood glucose levels were monitored at 0, 7, 14, and 21 days to evaluate the hypoglycemic effect of M. charantia. Serum proteins were analyzed using SDS-PAGE to identify proteins lost in the diabetic group (DM) and their possible restoration in the treatment group (AM) (Poovitha & Parani, 2020).*
***Results:*** *Blood glucose levels significantly increased in the NA group compared to controls, confirming successful diabetes induction. The AM group showed a marked reduction in glucose levels over 21 days, indicating the hypoglycemic effect of M. charantia. SDS-PAGE analysis revealed that specific proteins (~66 kDa, albumin) disappeared in DM but reappeared in AM, suggesting the therapeutic potential of M. charantia in restoring serum protein levels.*
***Conclusion:*** *This study demonstrates that Momordica charantia effectively reduces blood glucose levels and restores specific serum proteins lost due to alloxan-induced diabetes. These findings suggest that M. charantia has both hypoglycemic and protective effects on serum protein expression, making it a promising candidate for diabetes management.*
***Keywords:*** *Diabetes mellitus, Momordica charantia, Alloxan, SDS-PAGE, Serum protein, Albino mice, Albumin, Blood glucose*

## I. Introduction

Diabetes mellitus is a complex metabolic disorder primarily resulting from dysregulation of glucose homeostasis due to impaired insulin signaling. At the molecular level, diabetes is characterized by chronic hyperglycemia, oxidative stress, and inflammation, leading to complications affecting multiple organs. Type 1 diabetes (T1D) is an autoimmune disorder where pancreatic β-cells are destroyed by T-cell-mediated immune responses, specifically involving CD4+ and CD8+ T cells, cytokines like IFN-γ and TNF-α, and the activation of apoptotic pathways such as the mitochondrial and endoplasmic reticulum stress pathways (Cnop et al., 2005). In contrast, Type 2 diabetes (T2D) is primarily driven by insulin resistance, where insulin receptor substrate (IRS) signaling is impaired, leading to reduced glucose uptake and increased hepatic gluconeogenesis (Saltiel & Kahn, 2001).

At the cellular level, β-cell dysfunction in T2D is associated with oxidative stress-induced mitochondrial damage, endoplasmic reticulum stress, and β-cell apoptosis via activation of caspases (Poitout & Robertson, 2008). Additionally, insulin resistance is linked to chronic low-grade inflammation mediated by pro-inflammatory cytokines such as IL-6, TNF-α, and resistin, which interfere with insulin receptor phosphorylation and downstream signaling through the PI3K-Akt pathway (Hotamisligil, 2006). The metabolic imbalance in diabetes also leads to increased advanced glycation end-products (AGEs), which further contribute to vascular and cellular damage through receptor-mediated activation of inflammatory cascades (Singh et al., 2014).

Traditional medicinal plants have been extensively studied for their potential role in diabetes management. Many plant extracts contain bioactive compounds such as flavonoids, alkaloids, tannins, and saponins that exhibit hypoglycemic, antioxidant, and anti-inflammatory properties (Patel et al., 2012). *Momordica charantia* (bitter melon) is one of the most widely researched medicinal plants for diabetes. Studies suggest that its bioactive compounds, including charantin, polypeptide-p, and vicine, contribute to its glucose-lowering effects by enhancing insulin secretion, promoting glucose uptake, and modulating key metabolic enzymes (Joseph & Jini,

2013). In alloxan- and streptozotocin-induced diabetic models, bitter melon extract has demonstrated a significant reduction in blood glucose levels, confirming its antidiabetic potential (Sathishsekar & Subramanian, 2005).

In addition to *Momordica charantia*, several other medicinal plants have shown promising effects in diabetes management. *Trigonella foenum-graecum* (fenugreek) contains saponins and alkaloids that enhance insulin sensitivity and reduce postprandial hyperglycemia (Puri et al., 2002). *Gymnema sylvestre*, known as the "sugar destroyer," has been reported to regenerate pancreatic β-cells and improve glucose homeostasis (Tiwari et al., 2014). *Withania somnifera* (ashwagandha) has also been shown to lower fasting blood glucose levels and reduce oxidative stress in diabetic models (Mishra et al., 2016). These findings highlight the potential of plant-based therapies in diabetes management.

Serum proteins play a crucial role in maintaining physiological homeostasis, and alterations in their levels can serve as biomarkers for disease progression and treatment efficacy. Diabetes has been shown to cause significant changes in serum protein expression, particularly affecting albumin, transferrin, and other glycoproteins involved in metabolic regulation (Gupta et al., 2011). Reduced albumin levels in diabetic conditions are often associated with increased glycation, leading to impaired protein function and systemic inflammation (Sundaram et al., 2013). Transferrin, a key iron-transporting protein, is also affected in diabetes, with alterations in its glycosylation pattern linked to oxidative stress and diabetic complications (Ahmed et al., 2020).

Previous studies using SDS-PAGE analysis have demonstrated that alloxan-induced diabetes leads to the loss of specific serum proteins, which can be restored upon treatment with medicinal plant extracts. For instance, *M. charantia* has been shown to enhance protein synthesis and protect against protein degradation in diabetic models (Grover & Yadav, 2004). Similarly, fenugreek and *G. sylvestre* extracts have been reported to restore normal serum protein levels by modulating hepatic and renal functions (Patel et al., 2012). These studies suggest that medicinal plants may exert their therapeutic effects not only by reducing blood glucose levels but also by maintaining serum protein integrity.

## II. Materials and Methods

**Experimental Animals**

Healthy male albino mice, aged four weeks and weighing between 25–30 g, were obtained from the Animal House of the University Department of Zoology, T.M. Bhagalpur University, Bhagalpur. The mice were maintained under standard laboratory conditions at room temperature, with access to a standard rodent diet and water provided ad libitum.

**Preparation of Plant Sample**

Fresh *Momordica charantia* (bitter melon) was sourced from the local market in Bhagalpur, Bihar. The seeds were removed, thoroughly washed with distilled water, and sun-dried until completely dehydrated. The dried seeds were then ground into a coarse powder using a mechanical grinder. To prepare the aqueous extract, the powder was dissolved in one liter of distilled water and filtered using filter paper. The resulting extract was stored in a refrigerator for subsequent use. For oral administration, the dosage was standardized to 100 mg/kg body weight (Kumar, 2013).

**Induction of Diabetes Using Alloxan**

The experimental mice were fasted for 18 hours before diabetes induction. Alloxan monohydrate was used to induce diabetes via intraperitoneal injection following a previously established protocol (Wilson et al., 1972). The total alloxan dose (450 mg/kg body weight) was administered in three separate injections of 150 mg/kg body weight at 48-hour intervals. Mice with fasting blood glucose levels equal to or exceeding 200 mg/dL were confirmed as diabetic and subsequently included in the experimental study (Lensen, 2008).

**Experimental group**

The experimental animals were divided into four groups. The control group (N) consisted of healthy, non-diabetic mice receiving a standard diet and water ad libitum without any treatment. The control + *Momordica charantia* group (NM) included non-diabetic mice administered an aqueous extract of *M. charantia* (100 mg/kg body weight) orally for the study duration. The alloxan-induced diabetic group (DM) comprised mice rendered diabetic through intraperitoneal administration of alloxan monohydrate (450 mg/kg body weight in three doses) without any further treatment. Lastly, the alloxan + *M. charantia* group (AM) consisted of diabetic mice receiving *M. charantia* extract (100 mg/kg body weight) orally to assess its potential restorative effects.

**Estimation of serum glucose**

It is estimated by the Dr. Morepen Glucometer kit ( Kumar, 2020). It will be also estimated by the method of o-toluidine using a modified reagent.

**Estimation of plasma protein**

Blood serum protein will be detected by gel Electrophoresis technique. SDS-PAGE will be used to analyze the integrity of the proteins. (Poovitha & Parani, 2020).

**Statistical Analysis**

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 16.0. One-way analysis of variance (ANOVA) was conducted to compare differences between groups. A *p*-value of less than 0.05 was considered statistically significant. Results were presented graphically using SPSS 16.0.

## III. Result

**Authentication of Plant Materials**

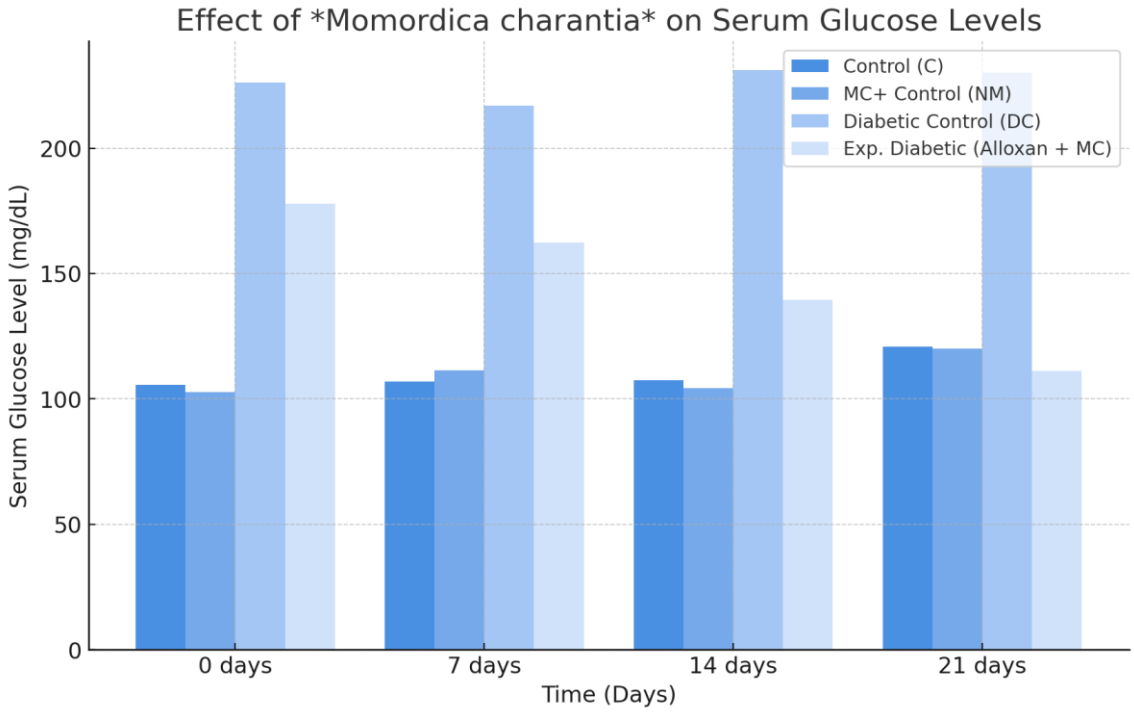
The plant material, *Momordica charantia* (bitter melon), was procured from the local market in Bhagalpur, Bihar. The collected plant was taxonomically identified and authenticated by Professor H.K. Chourasia from the Department of Botany, T.M. Bhagalpur University, Bhagalpur.

**Effect of Momordica charantia seed extract on serum glucose**

| Days | Control (C) | NM (M.C + Control) | DC (Diabetic Control) | AM (Alloxan + M.C) |
|---|---|---|---|---|
| 0 days | 105.67 ± 7.25 | 102.83 ± 6.43 | 226.17 ± 14.19 | 177.83 ± 6.911 |
| 7 days | 107 ± 12.61 | 111.33 ± 6.43 | 217 ± 14.19 | 162.33 ± 6.6 |
| 14 days | 107.50 ± 12.61 | 104.33 ± 6.67 | 231.10 ± 9.61 | 139.50 ± 7.39 |
| 21 days | 120.83 ± 7.35 | 120.01 ± 7.19** | 230.17 ± 10.41 | 111 ± 12.91** |

(**Significant at p < 0.05)

**Table 1**




**Graph 1: Effect of Momordica charantia on serum Glucose levels**

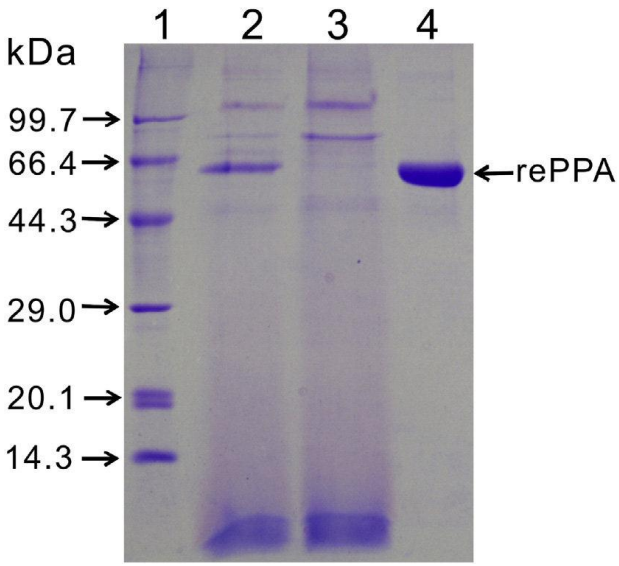
As per the Table 1, the diabetic control (DC) group exhibited the highest glucose levels (226.17 ± 14.19 mg/dL), whereas the normal control (C) and M. charantia-treated control (NM) groups had significantly lower levels (105.67 ± 7.25 mg/dL and 102.83 ± 6.43 mg/dL, respectively). The alloxan-induced diabetic group treated with *M. charantia* (AM) initially showed elevated glucose levels (177.83 ± 6.911 mg/dL), indicating successful diabetes induction.

By day 7, the glucose levels in the AM group declined to 162.33 ± 6.6 mg/dL, whereas the DC group remained high at 217 ± 14.19 mg/dL. The NM group showed a slight increase (111.33 ± 6.43 mg/dL), but it remained within the normal range. At 14 days, the glucose levels of the AM group continued to decrease significantly to 139.50 ± 7.39 mg/dL, demonstrating the hypoglycemic potential of *M. charantia*. The DC group, however, maintained hyperglycemia (231.10 ± 9.61 mg/dL). By the end of the experiment (21 days), the AM group exhibited a substantial reduction in glucose levels (111 ± 12.91 mg/dL), nearing normal values and showing a statistically significant difference ($p < 0.05$) compared to the DC group (230.17 ± 10.41 mg/dL). Meanwhile,

the NM group (120.01 ± 7.19 mg/dL) remained comparable to the control group (120.83 ± 7.35 mg/dL), suggesting that *M. charantia* had no hypoglycemic effect in non-diabetic mice.

**Effect of Momordica charantia seed extract on Serum protein**

The SDS-PAGE analysis of serum protein expression revealed notable differences across the experimental groups. The control group exhibited a normal serum protein profile with distinct bands, including albumin (~66 kDa). A similar protein profile was observed in the *Momordica charantia* control group, indicating that *M. charantia* alone did not significantly alter serum protein expression. However, in the diabetic control group, a specific protein band (~66 kDa) disappeared, and the overall intensity of other protein bands was reduced, suggesting protein degradation and alterations in serum protein composition due to alloxan-induced diabetes. Interestingly, in the experimental diabetic group treated with *M. charantia*, the missing protein band (~66 kDa) reappeared, and band intensities were restored to near-normal levels. This suggests a protective or restorative effect of *M. charantia* on serum protein integrity, potentially through mechanisms that enhance protein synthesis or prevent protein degradation. These findings highlight the potential of *M. charantia* in mitigating diabetes-induced changes in serum protein expression.




## IV. Discussion

**Effect of *Momordica charantia* on Blood Glucose Levels**

The present study demonstrates the hypoglycemic potential of *Momordica charantia* (MC) seed extract in alloxan-induced diabetic mice. The significant reduction in blood glucose levels in the experimental diabetic group (AM) over 21 days suggests that MC has antihyperglycemic properties. This aligns with previous research that attributes MC's glucose-lowering effect to its bioactive compounds, including charantin, polypeptide-p, and vicine, which enhance insulin secretion, improve glucose uptake, and inhibit key gluconeogenic enzymes (Joseph & Jini, 2013). Studies have also shown that MC modulates AMP-activated protein kinase (AMPK) signaling, a key regulator of glucose metabolism, leading to improved insulin sensitivity and glycemic control (Cheng et al., 2020).

**Impact of *Momordica charantia* on Serum Protein Expression**

The SDS-PAGE analysis revealed a notable alteration in serum protein expression in diabetic mice. The absence of a ~66 kDa protein band in the diabetic control (DM) group suggests that diabetes induces protein degradation or suppression of protein synthesis. Previous studies indicate that chronic hyperglycemia leads to excessive glycation of serum proteins, increasing their susceptibility to degradation and oxidative damage (Ahmed et al., 2020). Notably, the reappearance of this protein band in the AM group suggests that MC has a restorative effect on protein synthesis or prevents degradation. Similar findings have been reported in studies investigating medicinal plants such as *Trigonella foenum-graecum* and *Gymnema sylvestre*, which modulate hepatic protein synthesis and maintain serum protein homeostasis in diabetic conditions (Patel et al., 2012).

**Molecular Mechanisms of *Momordica charantia* in Diabetes Management**

The observed effects of MC on glucose regulation and protein preservation can be attributed to its multiple molecular mechanisms. Studies indicate that MC influences the insulin receptor substrate (IRS) signaling

pathway, enhancing insulin sensitivity and glucose transport via upregulation of GLUT4 translocation (Kumar et al., 2015). Additionally, MC exhibits antioxidant properties that counteract oxidative stress-induced damage to pancreatic β-cells and other metabolic tissues (Sathishsekar & Subramanian, 2005). The protective effect on serum protein expression may be mediated through its ability to reduce advanced glycation end-products (AGEs) formation, a key factor contributing to protein dysfunction in diabetes (Singh et al., 2014).

**Comparison with Other Antidiabetic Therapies**

MC has shown comparable efficacy to conventional antidiabetic drugs in several preclinical and clinical studies. Research suggests that MC extract can exert similar glucose-lowering effects as metformin by modulating key metabolic pathways (Haque et al., 2019). Moreover, unlike synthetic drugs, MC provides additional benefits, including hepatoprotective and nephroprotective effects, making it a promising candidate for diabetes management with minimal side effects (Tiwari et al., 2014).

**Clinical Relevance and Future Perspectives**

The findings of this study contribute to the growing body of evidence supporting the use of medicinal plants in diabetes management. However, further studies involving human clinical trials are necessary to validate the therapeutic potential of MC. Future research should focus on elucidating the precise molecular targets of MC's bioactive compounds and optimizing its dosage for maximum efficacy.

## V. Conclusion

The present study demonstrates the hypoglycemic and protein-restorative effects of *Momordica charantia* seed extract in alloxan-induced diabetic mice. The significant reduction in serum glucose levels and the reappearance of a ~66 kDa protein band in treated diabetic mice suggest that *M. charantia* not only improves glycemic control but also protects against diabetes-induced protein alterations. These findings highlight its potential as a natural antidiabetic agent. However, further clinical studies are required to confirm its efficacy and elucidate the precise molecular mechanisms involved.

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