



EVALUATION OF ANTI-DIABETIC ACTIVITY OF MALUS DOMESTICA IN COMPARISON WITH GLIBENCLAMIDE

Rizwan Ali¹, Syeda Afroz^{2*}, Shahnaz Usman³, Saima Mahmood Malih⁴, Sumaira Khadim⁵, Iqra Rana⁶, Aisha Ehtasham⁷, Hafiza Amna Batool⁸, Shameela Abbasi⁹, Wardha Jawaid.¹⁰

^{1,2,6,7,8,9,10}Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi.

³Faculty of Pharmacy . Saleem Habib University. Karachi .

⁴Dow college of Pharmacy.Faculty of Pharmaceutical Sciences. DUHS. Karachi

⁵Iqra University. Karachi.

***Corresponding author:** Dr.Syeda Afroz.

*Department of Pharmacology. Faculty of Pharmacy and Pharmaceutical Sciences. University of Karachi. safroz@uok.edu.pk

ABSTRACT

The increasing frequency of diabetes mellitus has generated an urgent demand for new and efficient treatment approaches, especially those originating from natural sources. *Malus domestica* (apple) has attracted interest for its possible antidiabetic effects because of its rich presence of bioactive compounds, such as polyphenols, flavonoids, and dietary fibers, which are recognized for their roles in regulating glucose metabolism and providing antioxidant defense. This research aimed to explore the hypoglycemic properties of *Malus domestica* extract using an alloxan-induced diabetic rat model, which is commonly employed to simulate type 1 diabetes by specifically destroying pancreatic beta cells. The extract was obtained through the Soxhlet extraction method, which guarantees an optimal concentration of bioactive components. The study was carried out over a period of 15 days, during which the rats were categorized into four groups (n=4 for each group): a control group that received no interventions, a diabetic control group, a standard treatment group that was given Glibenclamide (5 mg/kg), and treatment groups that received 200 mg/kg and 400 mg/kg of *Malus domestica* extract. Fasting blood glucose levels were recorded at baseline and at various points during the study to evaluate the hypoglycemic effects of the treatments. The results showed that *Malus domestica* extract dramatically reduced fasting blood glucose levels in diabetic rats; the 400 mg/kg dose had the most impact, almost matching that of the common oral hypoglycemic medication Glibenclamide. The inhibition of enzymes that break down carbohydrates, which lowers the absorption of glucose, increased insulin sensitivity, and antioxidant activity, which shields beta cells in the pancreas from damage caused by oxidative stress, are some possible reasons for this effect. These encouraging outcomes indicate that *Malus domestica* extract could act as a natural treatment option for managing diabetes. Nonetheless, additional clinical studies are needed to establish its long-term effectiveness, ideal dosage, and safety profile in humans, setting the stage for its possible inclusion in complementary diabetes treatment approaches.

Keywords: *Malus domestica*, diabetes mellitus, alloxan-induced diabetes, hypoglycemic effect,

1. INTRODUCTION

Diabetes mellitus (DM) is a long-term metabolic condition marked by sustained high blood sugar levels due to problems with insulin secretion, insulin action, or both. This disease is linked to serious complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy, making it a major global health issue. Traditional drug treatments, including sulfonylureas (e.g., glibenclamide), biguanides, and insulin therapy, continue to serve as the foundation of diabetes management. Nevertheless, these therapies often come with side effects like hypoglycemia, weight gain, and gastrointestinal discomfort, which highlight the need for safer and more effective alternatives. Medicinal plants have attracted significant focus in diabetes management because of their bioactive compounds, which show potential hypoglycemic, antioxidant, and anti-inflammatory effects. Among these, *Malus domestica* Borkh. (apple), part of the Rosaceae family, is acknowledged as a nutrient-rich fruit containing a variety of phytochemicals advantageous for human health. Widely grown in temperate areas, apples are eaten fresh and are also transformed into numerous products like juices, jams, ciders, wines, dried apples, and teas (Patocka et al., 2020). All parts of the fruit, aside from the seeds, are consumable and have historically been linked to improving immunity, increasing stress resilience, and fostering general health. Flavonoids (such as quercetin-3-glucoside, quercetin-3-rhamnoside, and quercetin-3-galactoside), phenolic acids (such as chlorogenic acid, gallic acid, and coumaric acid), and procyanidins are among the many antioxidants found in apples. These bioactive substances reduce inflammation, alter vital metabolic pathways, and aid in the prevention and cellular repair of oxidative damage (Akaniro & Odibo). *Malus domestica* has been shown to have preventive effects against neurodegeneration, Alzheimer's disease, cardiovascular disease, and type 2 diabetes in recent studies (Geană et al., 2021). Phloridzin, a dihydrochalcone with well-established antidiabetic effects, is found in high concentrations in apple leaves in particular. This suggests that plant-derived raw materials high in phloridzin may be useful in preventing diabetes (Liaudanskas et al., 2014).

Numerous studies have indicated the potential of apples to combat diabetes through various mechanisms such as enhancing insulin sensitivity, inhibiting enzymes that digest carbohydrates, modifying glucose uptake, and reducing oxidative stress. The therapeutic benefits of apples are further amplified by the presence of polysaccharides. Polysaccharides, which are composed of more than 10 monosaccharide units connected by glycosidic bonds, have been the focus of extensive research as significant bioactive elements in natural products.

These compounds have demonstrated promising effects in managing glucose metabolism and lowering glycation levels in individuals with diabetes (Li et al., 2017). In spite of these encouraging results, there is scant research assessing the direct hypoglycemic properties of *Malus domestica* extract in models of experimental diabetes. This research seeks to examine the effectiveness of *Malus domestica* extract in a rat model of diabetes induced by alloxan. Alloxan is a recognized diabetogenic substance that specifically eliminates pancreatic β -cells, resulting in insulin deficiency and high blood sugar levels. The apple extract was created with the Soxhlet device and given at doses of 200 mg/kg and 400 mg/kg to diabetic rats for a duration of 15 days. The standard antidiabetic medication glibenclamide (5 mg/kg) was utilized as a reference to assess the effectiveness of the extract in reducing fasting blood glucose levels. This study seeks to evaluate the glycemic reaction to *Malus domestica*, aiming to shed light on its possible role as a natural treatment for diabetes and assist in the creation of functional foods or nutraceuticals for metabolic conditions.

2. MATERIAL AND METHODS

Fresh apples (*Malus domestica* Borkh.) were obtained from a local market and authenticated by a botanist to ensure the validity of the plant material. The *Malus domestica* were thoroughly washed, peeled, and the edible portion was extracted for further processing.

Preparation of Extract

A mechanical grinder was used to grind the fresh *Malus domestica* flesh into a fine powder after it had been allowed to air dry at room temperature for a few days. Ethanol was used as the solvent for the Soxhlet extraction of the powdered material. The extraction process continued until the solvent in the siphon tube became clear. The extract was then concentrated under reduced pressure using a rotary evaporator to obtain the crude ethanolic extract, which was stored in an airtight container at 4°C until further use.

Animals

Thirty healthy albino rats, weighing between 180 and 220 grams, were kept in standard laboratory conditions that included a 12-hour light/dark cycle, a temperature of $22 \pm 2^\circ\text{C}$, and a relative humidity of $55 \pm 10\%$. The rats were randomly assigned to six groups, with five rats in each group. All experimental protocols were carried out in accordance with institutional ethical guidelines for animal research.

Acute Toxicity Studies

Acute toxicity studies were performed in accordance with OECD guidelines to evaluate the safety of *Malus domestica* extract in Wistar rats. The extract was given orally in doses of 200 mg/kg, 400 mg/kg, 1000 mg/kg, and 2000 mg/kg, whereas a control group was given distilled water. The animals were monitored for immediate toxic effects over a 24-hour period and followed for 14 days to assess any delayed toxicity. No deaths or notable toxic reactions were seen at 200 mg/kg and 400 mg/kg, which were utilized in the antidiabetic research. At 1000 mg/kg, there were mild behavioral abnormalities such as decreased movement and drowsiness, whereas at 2000 mg/kg, there were indicators of distress including lethargy and decreased food intake, but no fatalities. Although greater dosages may result in slight behavioral alterations, the data indicate that *Malus domestica* extract is safe at therapeutic concentrations with no significant toxicity seen.

Induction of Diabetes in Rats

Diabetes was induced in rats that had been fasted overnight by administering a single intraperitoneal injection of freshly prepared Alloxan monohydrate (150 mg/kg body weight) diluted in normal saline. To counteract the initial hypoglycemia induced by Alloxan, the rats received a 5% glucose solution following the injection. Blood glucose measurements were taken using a glucometer after a duration of 72 hours. Rats with blood glucose levels exceeding 250 mg/dL were identified as diabetic and included in the study. Blood sugar levels were reassessed after three days to confirm the onset of diabetes.

Experimental Design

The diabetic rats were randomly divided into six groups ($n = 5$ per group):

- Group I (Normal Control): Received no treatment.
- Group II (Diabetic Control): Received no treatment.
- Group III (Standard Drug): Received Glibenclamide (5 mg/kg body weight).
- Group IV: Received *Malus domestica* ethanolic extract (200 mg/kg body weight).
- Group V: Received *Malus domestica* ethanolic extract (400 mg/kg body weight).

Blood sugar levels were checked every week during the study period. Blood samples were obtained from the rat's tail vein with a thin needle after gently warming the tail to enhance blood circulation. The tail was cleaned with 70% ethanol prior to creating a small puncture. Blood was promptly placed on a glucometer test strip, and glucose levels were assessed with a commercially available glucometer designed for small animals. Rats were deprived of food overnight prior to blood collection to guarantee precise fasting glucose measurements. Blood glucose measurements were taken on days 0 (baseline), and 15 to assess the effect of *Malus domestica* extract on glycemic regulation.

Statistical Analysis:

To evaluate the overall variations in blood glucose levels between groups, data were analyzed using One-Way ANOVA. The normal control group served as the reference for pairwise comparisons between the diabetes control and treatment groups using Dunnett's test. Statistical significance was defined as a p -value < 0.05 , and the results were presented as mean $\pm$ SEM using SPSS.

3. RESULT

The impact of ethanolic extract from *Malus domestica* on fasting blood glucose levels in diabetic rats induced by alloxan was assessed over a 15-day observation period, with evaluations conducted on days 0, 10, and 15. At the initial assessment (day 0), all diabetic groups displayed similarly high blood glucose levels, confirming that diabetes had been successfully induced. By day 10, a notable decrease in blood glucose levels was recorded in the groups treated with *Malus domestica* extract when compared to the diabetic control group. This effect was found to be dose-dependent, with the 400 mg/kg group demonstrating a more significant reduction than the 200 mg/kg group. By day 15, the 400 mg/kg group's blood glucose levels had significantly dropped ($p < 0.05$), reaching those of the Glibenclamide-treated group (5 mg/kg). Although the effect was less noticeable than with the higher dose, the 200 mg/kg group also demonstrated a substantial decrease in glucose levels when compared to the diabetic control group. The evolution of diabetes in untreated rats was confirmed by the diabetic control group, which maintained consistently high glucose levels throughout the trial. These results indicate that *Malus domestica* extract has a marked hypoglycemic effect in alloxan-induced diabetic rats, with the 400 mg/kg dosage showing the strongest glucose-reducing activity, similar to Glibenclamide.

4. DISCUSSION

The current research examined the blood sugar-lowering effects of ethanolic extract from *Malus domestica* (apple) in diabetic rats induced by alloxan. The results revealed a notable decrease in fasting blood glucose levels, especially at a dosage of 400 mg/kg, which had an effect comparable to that of the standard antidiabetic medication, glibenclamide (5 mg/kg). These results underscore the potential of *Malus domestica* as a natural treatment option for managing diabetes. Alloxan is a commonly recognized compound that induces diabetes by specifically damaging pancreatic β -cells, resulting in decreased insulin production and high blood sugar levels. In our investigation, the diabetic control group consistently displayed elevated blood glucose levels, validating the efficacy of alloxan in triggering diabetes. In contrast, rats that received the *Malus domestica* extract exhibited a reduction in blood glucose levels that was dependent on the dosage, suggesting that apple extract may assist in glucose regulation. *Malus domestica*'s high dietary fiber, flavonoid, and polyphenol content may be the cause of its hypoglycemic action. Quercetin, phlorizin, and catechin are examples of polyphenols that have been shown to improve glycemic management by increasing insulin sensitivity, inhibiting enzymes that break down carbohydrates, and lowering oxidative stress. Due to their antioxidant qualities, flavonoids may improve the secretion and function of insulin by reducing oxidative damage to pancreatic β -cells. Dietary fiber also lessens postprandial glucose rises by delaying the absorption of glucose. The findings are consistent with earlier research showing the antidiabetic effects of apples and their bioactive components. Studies indicate that eating apples is linked to a reduced risk of type 2 diabetes, mainly because of their potential to regulate glucose metabolism and enhance insulin response. The occurrence of phloridzin in apple skins has been directly associated with lowered intestinal glucose uptake and heightened glucose elimination, aiding in glycemic regulation. Notably, the 400 mg/kg dosage of *Malus domestica* extract achieved the most pronounced decrease in glucose levels, almost equaling the effect of glibenclamide. This implies that a greater concentration of apple bioactives might offer enhanced therapeutic advantages. Nevertheless, the 200 mg/kg dosage also showed a meaningful reduction in blood glucose levels, suggesting a positive impact even at a lower concentration. Even though the study offers encouraging information about *Malus domestica*'s potential as an antidiabetic, more investigation is required to pinpoint its exact modes of action. Its impact on oxidative stress indicators, glucose absorption routes, and insulin secretion should all be investigated in future research. To confirm these results in human populations and establish the ideal

dosage for therapeutic usage, clinical trials are also required. However this study indicates that in rats with diabetes induced by alloxan, *Malus domestica* extract has notable hypoglycemic effects. The results provide credence to apples' possible use as a functional food in the treatment of diabetes. Apple extract may be used in conjunction with traditional antidiabetic treatments as a safer and more convenient option for glycemic control because of its natural source and low risk of adverse effects.

5. CONCLUSION

The results of this research indicate that the ethanolic extract of *Malus domestica* (apple) has considerable hypoglycemic effects in diabetic rats induced by alloxan. The decline in fasting blood glucose levels, especially at the 400 mg/kg dosage, indicates that apple extract might act as a potential natural treatment for managing diabetes. The noted effects can be linked to the existence of bioactive substances like polyphenols and flavonoids, recognized for improving insulin sensitivity, blocking carbohydrate-digesting enzymes, and lowering oxidative stress. While the 200 mg/kg dose of *Malus domestica* also shown a substantial decrease in blood glucose levels, the 400 mg/kg dose had the strongest glucose-lowering impact, matching that of the common medication glibenclamide (5 mg/kg). These results demonstrate *Malus domestica*'s potential as a functional food for the treatment of diabetes and support its involvement in glycemic regulation. Additional studies are necessary to clarify the specific ways apple bioactive influence glucose metabolism and insulin activity. Moreover, clinical trials are required to confirm these results in human groups and establish the best dosages for therapeutic use. Due to its natural source, safety, and availability, *Malus domestica* extract could potentially act as a supportive method alongside standard diabetes therapies, presenting a hopeful option for enhancing glycemic regulation.

Blood Glucose Levels (mg/dl)				
Groups	Treatment	0 th Day	10 th Day	15 th Day
Group -I	Saline	100.0 ± 0.74	99.0 ± 0.63	99.0 ± 0.74
Group -II	Alloxan(150mg/kg)	149.8 ± 0.92	149.4 ± 1.12	148.8 ± 0.80
Group -III	Glibenclamide(5mg/kg)	147.8 ± 2.50	144.2 ± 3.44	146.6 ± 2.19
Group- IV	EEAC (200mg/kg)	128.0 ± 0.89	127.6 ± 1.35	124.4 ± 1.00
Group- V	EEAC (400mg/kg)	124.4 ± 4.66	122.8 ± 5.12	118.6 ± 5.28

Values are expressed as mean ± SEM (n=5) one way ANOVA followed by Dunnett's test, P<0.05

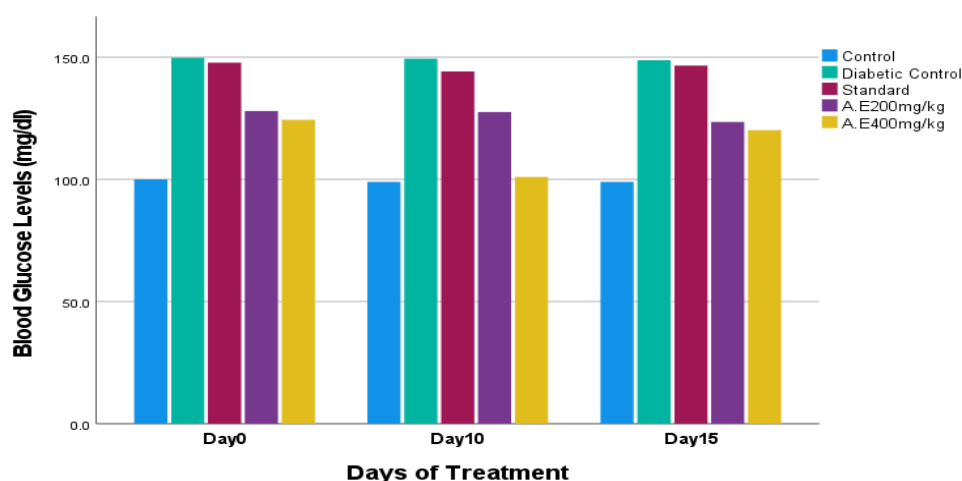


Figure 1: Effect of *Malus domestica* pulp extract on blood glucose levels in Alloxan induced diabetic rats.

References

- Geană, E. I., Sandu, A. I., Stanciu, S., & Ionete, R. E. (2021). Characterization of apple (*Malus domestica*) polyphenols and their antioxidant activity. *Molecules*, 26(10), 2951. <https://doi.org/10.3390/molecules26102951>

2. Liaudanskas, M., Viškelis, J., Raudonis, R., Kviklys, D., Uselis, N., & Janulis, V. (2014). Phenolic composition and antioxidant activity of *Malus domestica* leaves. *The Scientific World Journal*, 2014, 306217. <https://doi.org/10.1155/2014/306217>
3. Li, X., He, T., Wang, X., Shen, M., Wu, T., & Gong, P. (2017). Polysaccharides: A potential application of natural products in metabolic diseases. *Frontiers in Pharmacology*, 8, 374. <https://doi.org/10.3389/fphar.2017.00374>
4. Patocka, J., Wu, W., Nepovimova, E., Kuca, K., & Oleksak, P. (2020). Apples and their health benefits: A review. *Nutrients*, 12(3), 858. <https://doi.org/10.3390/nu12030858>
5. Akaniro, O. U., & Odibo, F. J. (2020). Antioxidant and nutritional properties of *Malus domestica*: A review. *Journal of Medicinal Plants Research*, 14(6), 320-328. <https://doi.org/10.5897/JMPR2020.6955>
6. Ahrén, B. (2007). Dipeptidyl peptidase-4 inhibitors: Clinical data and clinical implications. *Diabetes Care*, 30(6), 1344–1350. <https://doi.org/10.2337/dc07-0227>
7. Akaniro, I. R., & Odibo, A. O. (n.d.). Analysis of the phytochemical composition of three selected fruits: *Musa acuminata*, *Malus domestica*, and *Citrus paradisi*.
8. Alam, F., Islam, M. A., Kamal, M. A., & Gan, S. H. (2018). Updates on managing type 2 diabetes mellitus with natural products: Towards antidiabetic drug development. *Current Medicinal Chemistry*, 25(39), 5395–5431. <https://doi.org/10.2174/0929867325666170914160037>
9. Asgary, S., Rahimi, P., Mahzouni, P., & Madani, H. (2012). Antidiabetic effect of hydroalcoholic extract of *Carthamus tinctorius* L. in alloxan-induced diabetic rats. *Journal of Research in Medical Sciences*, 17(4), 386.
10. Asif, M. (2011). The role of fruits, vegetables, and spices in diabetes. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 1(1), 27.
11. American Diabetes Association. (2004). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 27(Suppl 1), S5–S10. <https://doi.org/10.2337/diacare.27.2007.S5>
12. Bahrami, G., Izadi, B., Miraghaee, S. S., Mohammadi, B., Hatami, R., Sajadimajd, S., & Batooie, N. (2021). Antidiabetic potential of the isolated fractions from the plants of *Rosaceae* family in streptozotocin-induced diabetic rats. *Research in Pharmaceutical Sciences*, 16(5), 505. <https://doi.org/10.4103/1735-5362.324855>
13. Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., & Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. *Pharmaceuticals*, 14(8), 806. <https://doi.org/10.3390/ph14080806>
14. Brands, A. M., Kessels, R. P., de Haan, E. H., Kappelle, L. J., & Biessels, G. J. (2004). Cerebral dysfunction in type 1 diabetes: Effects of insulin, vascular risk factors, and blood glucose levels. *European Journal of Pharmacology*, 490(1–3), 159–168. <https://doi.org/10.1016/j.ejphar.2004.02.049>
15. Buchanan, T. A., & Xiang, A. H. (2005). Gestational diabetes mellitus. *The Journal of Clinical Investigation*, 115(3), 485–491. <https://doi.org/10.1172/JCI24531>