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Research Article
Potential Anti-Diabetic Benefits of *Momordica charantia* Extracts on Experimental Diabetes and its Complications

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Abstract
Background and Objective: The *Momordica charantia* L. (Cucurbitaceae) plant is a natural medicine that is commonly used in the treatment of a wide range of ailments, including diabetes. The current study validates two common types of extracts (methanolic and water) of *Momordica charantia* (MC) for antidiabetic effects and compares their efficacy in preventing experimental diabetic complications. **Materials and Methods:** The rats with a fasting serum glucose level above 200 mg dL^{-1} at 96 hrs after streptozotocin (STZ) injection were considered diabetic and included in the study. They were administered with any of the three doses (45, 90 or 180 mg kg^{-1}) of methanolic or aqueous extracts of MC based on their respective experimental groups. After 14 and 28 days, parameters such as body weight, serum glucose, lipid profile, hemoglobin content and antioxidant status were recorded. The observations were statistically examined using a One-way ANOVA and $p<0.05$ indicated significance. **Results:** According to the research findings, rats with STZ-induced diabetes had a significant ($p<0.05$) variance in their body weight (177 ± 5.64), serum glucose levels (216.4 ± 3.96), lipid profiles [cholesterol (107.38 ± 3.70), triglycerides (168.68 ± 14.70)], hemoglobin levels (7.5 ± 0.14) and antioxidant status. These effects were found to become more prominent after 14 days of exposure. By providing either methanolic or water extracts of MC at different doses, the STZ-induced alterations in body weight and hyperglycemia were significantly ($p<0.05$) reversed. Besides, the extracts attenuated the hyperlipidemic, depleted antioxidant stress and improved hemoglobin levels in diabetic rats up to 28 days. Comparing the two extracts, the methanolic extract has shown greater efficacy than the water extract and the effects matches with that of glibenclamide. **Conclusion:** The findings of the current investigation verify the anti-diabetic potential of the MC and show its ability to serve as an alternate therapy for managing hyperglycemia-induced complications. Besides, attenuation in oxidative stress, diminished hemoglobin level, weight loss and hyperlipidemia, both extracts might benefit diabetic conditions by minimizing the complications related to hyperglycemia.

Key words: *Momordica charantia*, oxidative stress, streptozotocin, diabetic complications, antioxidants, natural products

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hyperglycemia induced by anomalies in either insulin action or secretion or both, is a hallmark of the class of medical conditions known as diabetes mellitus. These fundamental problems cause fasting blood and urine glucose levels to rise[1]. Chronic hyperglycemia has been linked to long-term organ damage, dysfunction and failure, particularly in the eyes, kidneys, nerves, heart and blood vessels. Diabetes complications include retinopathy, which causes vision loss; nephropathy, which causes renal failure; peripheral neuropathy, which increases the risk of foot ulcers, amputations and charcot joints and autonomic neuropathy, which causes gastrointestinal, genitourinary and cardiovascular symptoms, as well as sexual dysfunction[2]. Diabetes patients are also at risk for a variety of other diseases, including cardiovascular, renal and organ dysfunction[3].

Globally, the prevalence of diabetes across all age categories was 5.4% in 2020; by 2030, it could increase to 7.2%. The total population of diabetics is expected to increase from 488 million in 2020 to 552 million in 2030[4]. Diabetes has been demonstrated to afflict men more frequently than women and to be more prevalent in urban regions. Between 2000 and 2030, the population of emerging countries living in cities is expected to double. There appears to be a significant global shift in the proportion of persons over the age of 65, which is influencing the prevalence of diabetes[5].

Numerous studies have been conducted to comprehend the pathophysiology of diabetes and its complications; nonetheless, several aspects remain unclear. They have yet to be evaluated for comprehensive diabetes control and associated complications[6]. One of the most widely accepted views is that chronic hyperglycemia and associated impairments in cell signaling function, such as oxidative stress, are linked to complications[7]. As the research developed, experts realized that several plant-based products and their active ingredients can play an important role in the management of diabetes-related complications[8].

Momordica charantia (MC), a member of the Cucurbitaceae family, is grown as a vegetable and medicine in areas of the Amazon, East Africa, Asia, the Caribbean and across South America[9]. Anti-diabetic, antibacterial, anti-tumor, immunomodulation, anti-viral, antioxidant, anti-inflammatory and hepatoprotective properties have been described for MC[10]. Polysaccharides, peptides, terpenoids, saponins, phenolics and steroids are some active ingredients discovered for their pharmacological actions[11]. Favism, diarrhea, vomiting and intestinal cramps were reported as the most common adverse effects[12], but overall, the extracts were determined to be safe for therapeutic usage. The mechanism for antidiabetic effect has been discovered to involve various pathways, including glucose uptake increase, insulin-like activity and insulin resistance alleviation[13].

Diabetes is linked to several complications, including hyperlipidemia, infection and oxidative stress-induced organopathy. Diabetes has also been linked to a decrease in red blood cell count, which can lead to anemia due to low hemoglobin levels[14]. Some established anti-diabetic drugs, such as metformin and glibenclamide, have been shown to have hypoglycemic effects via various pathways[15,16]. Metabolites with diverse pathways were found to be effective because they lower hyperglycemia at different sites[14]. Furthermore, such multifaceted actions have been shown to reduce the rates of insulin resistance, oxidative stress and hyperlipidemia, all of which can contribute to diabetic complications in patients[17].

The literature suggests that MC is one of the most researched plants due to its potential anti-diabetic effects. According to reports, the plant has the potential to be a successful alternative medicine for the treatment of diabetes and its consequences[18]. More research is needed to investigate the effect of various types of MC extracts on streptozotocin-induced type 1 diabetes sequelae, such as hemoglobin content deficiencies. As a result, this study was designed to assess the potential of MC on type 1 diabetes mellitus and associated complications. The study assessed the role of two common types of MC extract (methanolic and water) on diabetes sequelae such as dyslipidemia, hemoglobin depletion, oxidative stress and weight loss in experimental rats.

MATERIALS AND METHODS

Study area: The study was conducted in the research laboratories of Al-Ameen College of Pharmacy, Bangalore, India as per the guidelines of good laboratory practice from May, 2020 to September, 2021.

Plant materials: The methanolic extract (NR-2A, batch No: PT/0605129 A) and successive water extract (NR-2B, batch No: PT/0605129 B) of *Momordica charantia* (fruit) were provided by Natural Remedies Pvt. Ltd., Bangalore, for research purposes. In brief, the extraction process includes maceration water or methanol to prepare, water and methanolic extracts, respectively. Crude botanicals were kept in respective solvents for 48 hrs at 25°C, filtered and subjected to a spray drying technique to preserve the composition of the phytochemicals.

The extracts were dissolved in distilled water with the help of a sonicating at the time of administration to animals and were further diluted as per the dose requirement.

Preparation of drug solutions: Streptozotocin (STZ, Himedia, India, Batch number CMS1758) was dissolved in an ice-cold citrate buffer of pH 4.5 and a single intraperitoneal injection was administered immediately within five min to avoid degradation[19]. After administration, care was taken to avoid any incidences of mortality in experimental rats, as described in the literature[20]. Glibenclamide tablet (Glib, Dionil®, Batch# 1106, Emcure Pharmaceuticals, India) of 5 mg was dissolved in 83.33 mL of distilled water to give a 60 mg mL^{-1} solution. This solution was administered orally at a 0.6 mg kg^{-1} b.wt., dose using a clean and dry oral feeding needle. The NR-2A and NR-2B plant extracts were suspended in distilled water by sonicating for 5 min. With a clean, dry oral feeding needle, these suspensions were given orally at three different dose levels, 45, 90 and 180 mg kg^{-1} b.wt.

Experimental animals: Seventy-two albino Wistar rats (220-250 g) of either sex were obtained from the Central Animal Facility, Natural Remedies Pvt. Ltd., Bangalore and housed three animals per cage with paddy husks as bedding. Animals were housed at a temperature of 25±2°C and a relative humidity of 30-60%. A 12 hrs light/dark cycle was used. The animals had free access to feed and ultraviolet-purified water *ad libitum*. The experiment was conducted per the guidelines of CPCSEA[21] and after approval from the Institutional Animal Ethics Committee of Al-Ameen College of Pharmacy, Bangalore, India.

Experimental grouping: The randomly selected experimental rats were divided into nine groups, comprising 6-8 animals. Group 1 was considered vehicle control, group 2 diabetic and group 3 standard (glibenclamide, 0.6 mg kg^{-1})[22]. Groups 4, 5 and 6 received methanolic (NR-2A) extract at 45, 90 and 180 mg kg^{-1}, respectively, for 28 days. Further, groups 7, 8 and 9 were administered with water (NR-2B) extracts of MC (45, 90 and 180 mg kg^{-1}), respectively, for 28 days[23]. The freshly prepared extract was administered to animals of the respective group orally using a reusable oral feeding needle (20 G).

Induction of type-1 diabetes mellitus: Before administering a single intraperitoneal (i.p.) injection of freshly produced STZ at a concentration of 45 mg kg^{-1}, Wistar rats (220-250 g) were fasted for 14 hrs[19]. The animals were given free access to food and water after receiving STZ. The measurement of fasting serum glucose 96 hrs after STZ injection was used to validate the development of hyperglycemia in the rats. Before blood was extracted from the retro-orbital plexus while the animals were under light ether anesthesia, they fasted once again for 14 hrs. Rats classified as diabetic and participated in the study were those whose fasting serum glucose level at 96 hrs after STZ injection was greater than 200 mg dL^{-1}.

Protocol for collecting biological fluids: Under light ether anesthesia, blood was extracted from the retro-orbital plexus of the rats on days 0, 14 and 28 after the animals fasted for 14 hrs. After clotting for 30 min at room temperature, the blood samples were centrifuged for 10 min at 3000 rpm. The resultant top layer of serum was transferred into dry, clean and labeled micro-centrifuge tubes. The serum samples were kept between 2 and 8°C, examined right away for glucose levels, then kept for 2 weeks at -80°C to estimate lipid profiles[24]. The blood was drawn from the retro-orbital plexus of the rats under light ether anesthesia on days 14 and 28 in EDTA tubes. The blood samples were stored at 2-8°C and analyzed within 1 week for the hemoglobin content. After the end of the 28 day treatment protocol, animals were sacrificed. The vital organs, such as the liver, heart and kidney, were collected and transferred into the homogenization tube containing ice-cold phosphate buffer (pH 7.6). The supernatant was collected after homogenization and stored (2-8°C) and the antioxidant status in different organs, including protein contents, was analyzed within one week after sacrifice.

Parameters analyzed

Body weight: The body weight of each animal was recorded daily and the data on days 0, 14 and 28, i.e., the days corresponding to other parameters, were analyzed and tabulated[25].

Fasting serum glucose estimation: The glucose-GOD/POD kit (Bhat Bio-Tech India (P) Ltd., Bangalore) was used to calculate serum glucose using the GOD/POD (glucose oxidase and peroxidase) method. Catalog no: GLL-1000) on 0, 14 and day 28. The principle of the method includes the enzymatic reaction of glucose with oxygen in the presence of GOD, resulting in the formation of H_2O_2 and gluconic acid. The formed H_2O_2 then reacts with 4 amino antipyrine and phenol in the presence of POD to form chinonimine, a colored complex measured photometrically at 510 nm[26].

Serum lipid profile

Serum cholesterol estimation: Serum cholesterol was estimated by the colorimetric, end-point CHOD-POD (Cholesterol Oxidase and Peroxidase) method using the Cholesterol kit (Bhat Bio-Tech India (P) Ltd., Bangalore). Catalog No: CHL 50) on day 14 and 28. The principle depends on the enzymatic reaction of cholesterol esters in the presence of cholesterol esterase to form cholesterol and fatty acids. The cholesterol further undergoes an enzymatic reaction in the presence of cholesterol oxidase to form cholest-4-ene-one and H_2O_2. The formation of H_2O_2 is determined by the reaction of 4 amino antipyrine and phenol in the presence of peroxidase to form a colored complex called quinoneimine that can be measured colorimetrically[27].

Serum triglyceride estimation: Serum triglyceride was estimated by the enzymatic, endpoint, colorimetric and GPO-POD (glycerol-3-phosphate oxidase-peroxidase) method using the Triglyceride kit (Bhat Bio-Tech India (P) Ltd., Bangalore). Catalog No: TGL 50) on day 14 and day 28. The principle depends on the hydrolysis of triglycerides, which are hydrolyzed to glycerol by lipoprotein lipase. Glycerol released from triglycerides is transformed by glycerol kinase into glycerol-3-phosphate, oxidized to Dihydroxyacetone Phosphate (DHAP) and H_2O_2 in the presence of peroxidase. The quinoneimine indicator is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of triglycerides in the sample and is measured colorimetrically[28].

Hemoglobin estimation: The cyanmethemoglobin method was used to calculate hemoglobin using the Haemoglobin kit (Bhat Bio-Tech India (P) Ltd., Bangalore). Catalog no: HR-1000) on day 14 and day 28. In principle, except sulfhemoglobin, hemoglobin and its derivatives are oxidized to methemoglobin by ferricyanide in the presence of an alkali. Cyanmethemoglobin is then formed from methemoglobin by its reaction with cyanide. The red-colored complex has a peak absorbance of 540 nm, proportional to total hemoglobin concentration[29].

Antioxidant status

Estimation of protein content: The protein content of the tissue was estimated by the Lowry method. The principle depends on the reactivity of peptide nitrogen with copper ions under alkaline conditions. Further, reducing folin-ciocalteu phosphomolybdic phosphotungstic acid to heteropoly molybdenum blue is measured colorimetrically at 680 nm[30].

In brief, the procedure includes the addition of 0.9 L of distilled water with 0.1 mL of 10% tissue homogenate and 5.0 mL of alkaline copper reagent followed by incubation for 10 min at room temperature. To this, 0.5 mL of folin-phenol reagent solution (1:1) was added and the mixture was kept in the dark for 30 min. The absorbance of the solution was measured at 660 nm against the reagent blank. Bovine serum albumin was used as standard (20 mg mL^{-1}) and the protein content was calculated using the formula:

$$\text{Protein content} = \frac{\text{Test}\left(\text{optical density}\right)\times\text{Standard concentration}\;\left(2\text{ mg}/0.1\text{ mL}\right)\times 10}{\text{Standard}\left(\text{optical density}\right)\ \text{mg of protein}}$$

Mg of protein per gram of tissue homogenate

Estimation of Glutathione (GSH): Ellman's reagent (DTNB) was used to estimate glutathione. Glutathione's sulfhydryl groups combine with DTNB to generate a colorful complex that can be colorimetrically detected at 412 nm[31].

The procedure, in brief, was to 0.2 mL of 10% tissue homogenate, add 0.8 mL of trichloroacetic acid solution and centrifuge for 10 min at 3000 rpm. To 0.2 mL of supernatant, add 0.8 mL of phosphate buffer and 2.0 mL of 0.6 mM DTNB (5,5' Dithiobis-(2-Nitro benzoic acid) and mix well. The absorbance of the reaction mixture was read at 412 nm against the reagent blank. Further, reduced glutathione in the concentration of 0.03 µmoles was used as standard and the quantity of reduced glutathione was expressed in terms of µM of GSH per gram of tissue using the formula:

$$\text{Glutathione}\left(\text{reduced}\right) = \frac{\text{Test}\left(\text{optical density}\right)\times\text{Standard concentration}\left(0.03\ \mu\text{ mol}\right)\times 10}{\text{Standard}\left(\text{optical density}\right)\times 0.2\text{ mL of tissue homogenate}}$$

Estimation of lipid peroxides: At pH 3.5, thiobarbituric acid reacts with malondialdehyde (MDA), a secondary lipid peroxidation product, to estimate tissue lipid peroxides. The developed color is a reddish pink, estimated at 532 nm[32].

In brief, the reaction mixture contains 0.1 mL of 10% tissue homogenate+0.2 mL of sodium dodecyl sulfate solution (8.1%)+1.5 mL of acetic acid solution (20%) and 1.5 mL of thiobarbituric acid solution (0.8%) was finally made up to 4 mL with distilled water and heated at 95°C for 60 min. After cooling, 1 mL of distilled water and 5 mL of the mixture

of n-butanol and pyridine (15:1 v/v) were added and the mixture was shaken vigorously. The mixture was centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer was measured at 532 nm against the reagent blank. Tetramethoxypropane was used as standard in the concentration of 3 nM. Lipid peroxides were expressed as nM of MDA per min per mg of protein using the formula:

$$\text{Lipid peroxide} = \frac{\text{Test}\left(\text{optical density}\right)\times\text{Standard concentration}\left(3\ \text{nM}\right)\times 10}{0.2\ \text{mL of tissue homogenate}\times\text{Standard}\left(\text{optical density}\right)\times 60\times\text{Protein}\left(\text{mg}\right)}$$

Statistical analysis: The values are expressed as Mean±SEM. The data were analyzed using one-way ANOVA followed by Dunnett's test using SPSS version 20. Statistical significance was set at $p \leq 0.05$ when groups were compared, such as control vs diabetic and diabetic vs treatment.

RESULTS

Effects on body weight: Figure 1 represented the influence of methanolic and water extracts of MC on body weight. The data of the first-day study (day 0) indicated that the diabetic group had significantly ($p<0.05$) lesser body weight compared to the control. At the two test durations (14 days and 28 days), it was discovered that the diabetic animals' body weight was significantly ($p<0.05$) lower than that of the control animals. Compared to the control group, neither the zero-day nor the 14 day treatments produced a significant difference in body weight. However, after 28 days of treatment, NA-2A (180 mg kg^{-1}) and NR-2B (45 mg kg^{-1}) showed significant ($p<0.05$) improvement in body weight compared to diabetic animals. Other treatments including glibenclamide, irrespective of dosage, did not significantly change the body weight.

Effect on serum glucose: The data recorded to study the influence of methanolic (NR-2A) and water (NR-2B) extracts of MC indicated that on the 0th day (the day the experiment was started), the diabetic animals had significant ($p<0.05$) elevated serum glucose levels compared to the control. Treatment with different doses of methanolic extra and water extract of MC led to a significant ($p<0.05$) decrease in serum glucose levels when compared to the diabetic group. Additionally, after 28 days of treatment, it was discovered that the serum glucose remained significantly ($p<0.05$) lowered with varying

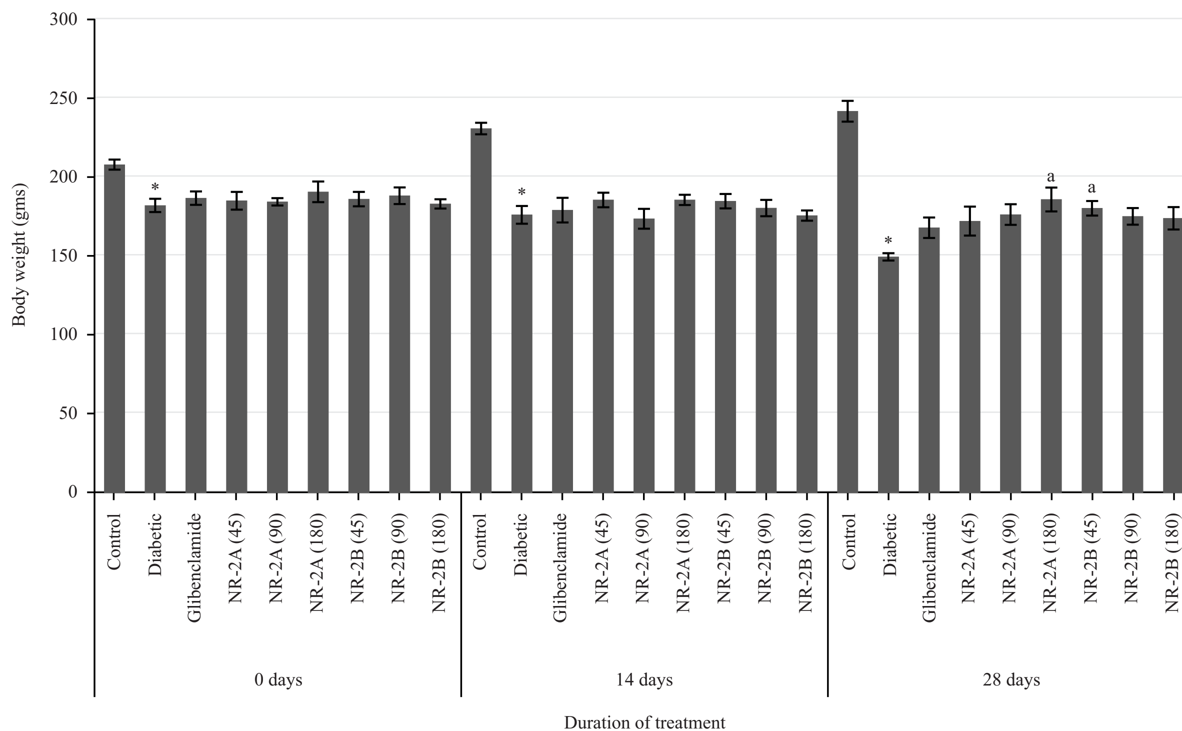

Fig. 1: Effect of methanolic and water extracts of MC on body weight in diabetic rats

NR-2A: Methanolic extract, NR-2B: Water extract, Statistics: One-way ANOVA followed Dunnett's test, Values are represented as Mean±S.E., *$p<0.05$ diabetic vs normal control and [a]$p<0.05$ treatments vs diabetic control

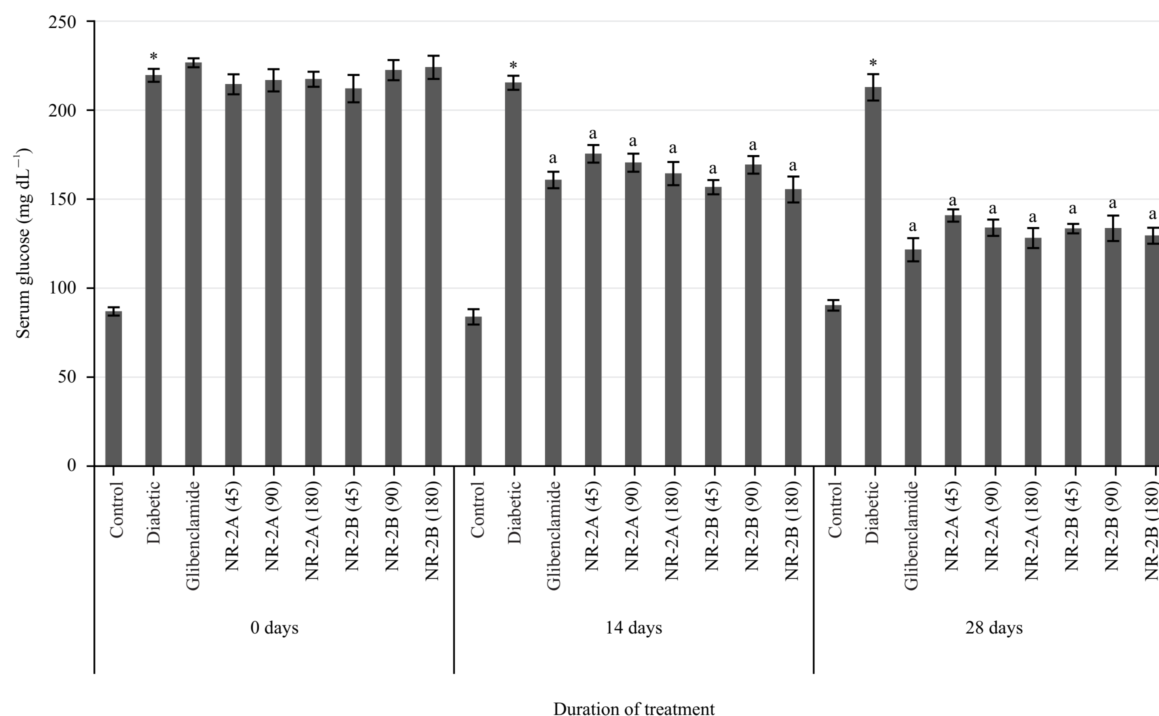

Fig. 2: Effect of methanolic and water extracts of MC on serum glucose in diabetic rats

NR-2A: Methanolic extract, NR-2B: Water extract, Statistics: One-way ANOVA followed Dunnett's test, Values are represented as Mean±S.E., *p<0.05 diabetic vs normal control and [a]p<0.05 treatments vs diabetic control

Table 1: Effect of NR-2A and NR-2B on fasting serum cholesterol and triglyceride levels in rats

	Serum cholesterol (mg dL^{-1})		Serum triglyceride (mg dL^{-1})	
Treatment and dose (mg kg^{-1})	14 days	28 days	14 days	28 days
Control	67.87±6.63	69.27±4.81	66.54±6.74	70.05±4.70
Diabetic (45)	107.38±3.70*	106.47±2.02*	168.68±14.70*	174.00±10.31*
Gliben (0.6)	59.77±4.53[a]	76.33±6.41	87.53±3.21[a]	91.17±3.88[a]
NR-2A (45)	57.47±4.42[a]	67.95±4.28[a]	83.09±13.67[a]	93.51±8.37[a]
NR-2A (90)	52.83±7.99[a]	57.36±7.24[a]	71.87±16.88[a]	89.81±6.70[a]
NR-2A (180)	69.35±3.56[a]	74.87±3.90[a]	81.15±1.69[a]	89.53±4.24[a]
NR-2B (45)	59.93±4.32[a]	68.75±3.46[a]	79.80±7.78[a]	91.02±4.42[a]
NR-2B (90)	53.65±4.61[a]	65.20±6.28[a]	74.23±11.15[a]	87.76±6.16[a]
NR-2B (180)	50.36±5.34[a]	68.67±4.48[a]	70.32±6.52[a]	87.93±7.18[a]

Glib: Glibenclamide, NR-2A: Methanolic extract, NR-2B: Water extract, Statistics: One-way ANOVA followed Dunnett's test, Values are represented as Mean±S.E., *p<0.05 diabetic vs normal control and [a]p<0.05 treatments vs diabetic control

doses of MC's methanolic (NR-2A) and water (NR-2B) extracts. The standard antidiabetic agent (Glib) was also found to reduce the serum glucose level significantly (p<0.05) compared to diabetic animals at different durations of treatments (Fig. 2).

Effect on serum cholesterol and triglycerides: The influence of two extracts (methanolic and water) of MC on the serum cholesterol and triglyceride levels was represented in Table 1. The lipid profile was estimated for two treatment durations, 14 and 28 days. The results showed that after 14 and 28 days, the cholesterol levels of STZ-induced diabetic animals were significantly (p<0.05) higher than those of control animals. After 14 and 28 days, it was discovered that the two extracts, at different tested doses, significantly (p<0.05) reduced elevated cholesterol levels in diabetic animals. The serum triglyceride levels were significantly elevated (p<0.05) in diabetic animals compared to normal animals. At both 14 and 28 days, the elevated serum triglyceride level was significantly (p<0.05) reduced by the methanolic and water extracts of MC in the tested doses. Glib administration to diabetic animals was found to significantly (p<0.05) lower

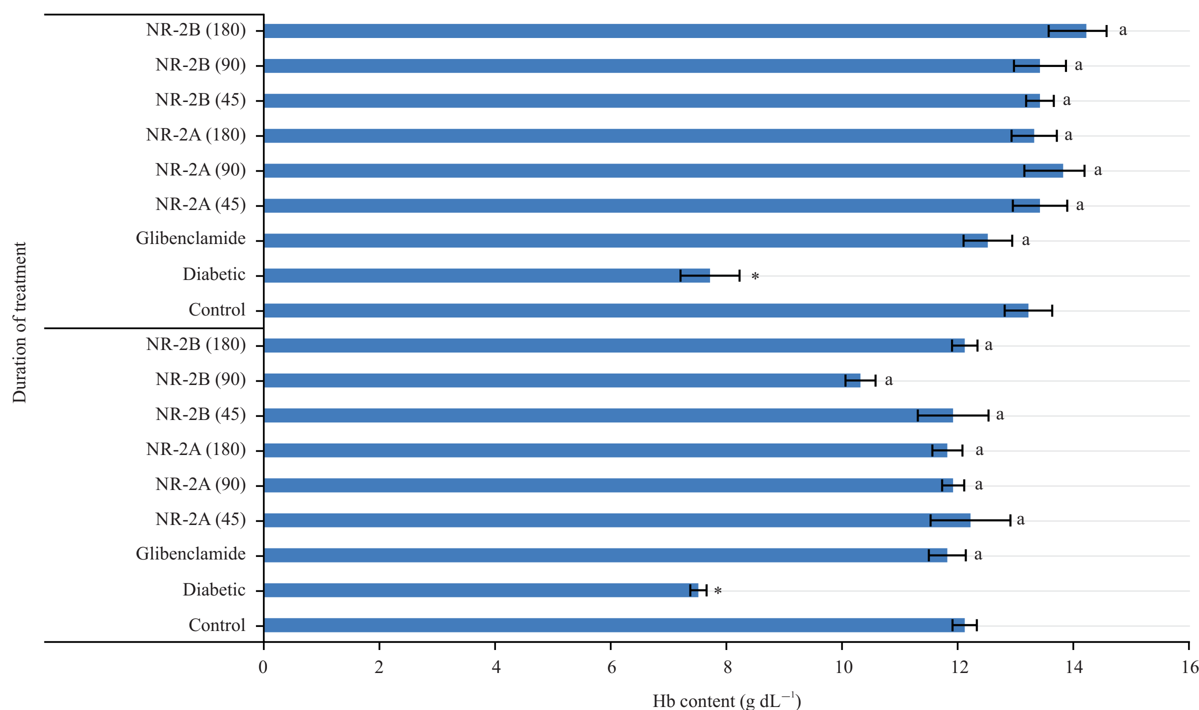

Fig. 3: Effect of methanolic and water extracts of MC on hemoglobin content in diabetic rats

NR-2A: Methanolic extract, NR-2B: Water extract, Statistics: One-way ANOVA followed Dunnett's test, Values are represented as Mean±S.E., *p<0.05 diabetic vs normal control and [a]p<0.05 treatments vs diabetic control

Table 2: Effect of NR-2A and NR-2B on antioxidant status in different organs of rats

Antioxidant status in organs					NR-2A			NR-2B		
		Control	Diabetic	Glib (0.6 mg)	45 mg	90 mg	180 mg	45 mg	90 mg	180 mg
LPO	Liver	0.14±0.015	0.27±0.014*	0.15±0.019	0.12±0.005[a]	0.12±0.007[a]	0.12±0.013[a]	0.12±0.017	0.14±0.023[a]	0.12±0.010[a]
	Kidney	0.05±0.005	0.16±0.010*	0.08±0.009[a]	0.07±0.003[a]	0.08±0.005[a]	0.10±0.008[a]	0.09±0.014[a]	0.07±0.011[a]	0.08±0.005[a]
	Heart	0.19±0.011	1.10±0.043*	0.21±0.024[a]	0.21±0.022[a]	0.20±0.022[a]	0.19±0.019[a]	0.27±0.027[a]	0.27±0.019[a]	0.25±0.026[a]
GSH	Liver	0.97±0.02	0.17±0.04*	0.80±0.06[a]	0.67±0.07[a]	0.73±0.05[a]	0.76±0.05[a]	0.84±0.04[a]	0.87±0.03[a]	0.87±0.09[a]
	Kidney	0.89±0.06	0.22±0.04*	0.61±0.03[a]	0.58±0.04[a]	0.65±0.05[a]	0.69±0.03[a]	0.59±0.07[a]	0.59±0.04[a]	0.62±0.03[a]
	Heart	0.78±0.06	0.32±0.02*	0.58±0.04[a]	0.57±0.05[a]	0.58±0.03[a]	0.65±0.05[a]	0.58±0.05	0.53±0.03[a]	0.55±0.02[a]
Total proteins	Liver	26.17±0.74	13.97±0.74*	23.39±1.52[a]	25.08±0.62[a]	26.05±0.92[a]	25.33±1.26[a]	22.27±1.12[a]	23.32±1.76[a]	25.28±0.99[a]
	Kidney	16.31±0.87	9.39±0.36*	14.02±0.91[a]	15.98±0.65[a]	16.23±0.56[a]	15.76±0.14[a]	15.27±0.47[a]	13.80±0.81[a]	12.56±0.74
	Heart	5.09±0.11	3.37±0.13*	4.55±0.41	4.39±0.12[ab]	4.60±0.18[a]	4.65±0.13[a]	4.56±0.15[a]	4.50±0.18[a]	4.50±0.07[a]

Glib: Glibenclamide, NR-2A: Methanolic extract, NR-2B: Water extract, Statistics: One-way ANOVA followed Dunnett's test, Values are represented as Mean±S.E, *p<0.05 diabetic vs normal control and [ab]p<0.05 treatments vs diabetic control

serum cholesterol and triglyceride levels compared to the STZ group at both treatment durations tested (14 and 28 days).

Effect on hemoglobin contents in diabetic rats: The influence of STZ-mediated diabetes and the role of various drug treatments on the hemoglobin level was indicated in Fig. 3. At days 14 and 28 of the diabetic condition, there was a significant (p<0.05) decrease in hemoglobin content compared to control animals. When given to diabetic animals, the methanolic extract (NR-2A) and water extract (NR-2B) tested at 45, 90 and 180 mg kg^{-1} was found to significantly (p<0.05) increase the hemoglobin content. The enhancement in the hemoglobin content was found to be significant (p<0.05) for both 14 days and 28 days of treatment. Furthermore, the analysis revealed that Glib therapy for 14 and 28 days significantly (p<0.05) increased hemoglobin levels in diabetic animals.

Effect on antioxidant status in different organs: The data representing the antioxidant study was indicated in Table 2. By estimating the levels of LPO, GSH and total proteins in the homogenate of three organs, such as the liver, kidney and

heart, the antioxidant status of the diabetic and various treatments was examined. Compared to the control group, diabetic rats treated with STZ showed significant ($p<0.05$) increases in LPO and decreases in GSH and total protein in three organs. The methanolic extract of MC was found to reverse the oxidative status significantly ($p<0.05$) in diabetic conditions in all tested doses, such as 45, 90 and 180 mg kg^{-1}. On the other hand, it was found that the MC water extract, except 45 mg and 180 mg kg^{-1}, reversed the increased oxidative stress in all tested doses. In diabetic rats, NR-2B at 45 mg did not cause a significant ($p<0.05$) suppression of LPO in the liver or an increase in GSH in the hearts. Furthermore, in the kidney homogenate of diabetic animals, NR-2B at 180 mg kg^{-1} did not result in a significant ($p<0.05$) increase in total proteins. Glib, tested as a standard antidiabetic agent, produced a significant ($p<0.05$) reversal of oxidative changes induced by STZ, except for the LPO level in the liver and total protein in the heart.

DISCUSSION

The present study evaluated the methanolic and water extracts of MC for their anti-diabetic activity, lipid profile and effects on protein content, reduced glutathione, lipid peroxidation and hemoglobin content. The untreated diabetic rats showed progressive and significant ($p<0.05$) body weight loss throughout the study period as against the weight gain of the normal group of rats. Throughout the trial, the diabetic control animals' fasting serum glucose level was observed to be higher than that of the normal control group.

The observations recorded in the present study are from previous research[25,33]. Studies conducted in the past suggested that hyperglycemia and defective insulin functions can negatively influence body weight in both experimental and clinical conditions[33]. These effects are directly dependent on the level of hyperglycemia and the duration of the diabetic condition. Current study observations also indicated that changes in body weight and other complications of hyperglycemia are prominent from 14 days onwards. The serum cholesterol and serum triglyceride of the diabetic group of rats were also found to be significantly ($p<0.05$) increased, which was also in agreement with previous research[34]. The significant ($p<0.05$) reduction in the hemoglobin content and antioxidant status, including diminished protein contents in different organs, showed similarity to that reported in the literature[35]. These observations confirmed that STZ (45 mg kg^{-1}) produces a reproducible and consistent diabetic condition and related complications in rats. The STZ induces diabetic conditions by causing the alkylation of biological macromolecules such as DNA, destroying pancreatic beta cells[36]. Several experimental models have been studied in the literature for the induction of type-1 diabetes mellitus. Current study findings suggested that STZ at 45 mg kg^{-1} by IP route can induce sustained diabetic conditions with related complications in the rats. Further, Gliben (0.6 mg kg^{-1}) tested as a standard antidiabetic agent showed significant ($p<0.05$) protection against all the changes induced by STZ. Studies conducted in the past also indicated that administration of Gliben reduces diabetic complications such as loss of body weight, hyperlipidemia and oxidative stress[15,37,38].

The treatment of NR-2A (the methanolic extract of MC) at different doses showed significant ($p<0.05$) protection from loss of body weight (Fig. 1) and reduced the elevated serum glucose levels (Fig. 2) when compared with the diabetic control group. All doses of the extract have shown a progressive and significant reduction in serum glucose levels and protected rats from losing body weight. Still, the recovery was incomplete, as normal levels could not be reached. Similar observations have been reported in previous studies for the extracts of MC, suggesting the antidiabetic property of the metabolite[10,39,40].

The reduction in the serum glucose levels is probably due to recovery in the degenerated islet cells due to insulin-like molecules and increased glucose utilization by the liver, as reported Cortez-Navarrete *et al.*[13]. Further, NR-2A reduced serum cholesterol and triglyceride levels (Table 1). Previous research suggested a possible mechanism due to an elevation in the HDL cholesterol levels[41] due to the presence of the active components. The NR-2A treatment at all the selected dose levels showed significant ($p<0.05$) recovery from the loss of hemoglobin in diabetic rats (Fig. 3) and could be related to decreased glycosylation of hemoglobin, as reported in the study of Horax *et al.*[42]. Moreover, the low hemoglobin content in diabetes is reported to be associated with a rapid decline in the glomerular filtration rate[43]. The extract of MC appears to benefit this complication and the hyperglycemic condition. The MC also increases the reduced glutathione level as well as protein contents and decreases lipid peroxidation in different organs (Table 2), suggesting a possible beneficial role for NR-2A in managing diabetic complications[44].

The current study indicated that MC methanolic extract has attenuated the STZ-induced type-1 diabetic complications. The action of hyperlipidemia and oxidative stress appears to modulate several diseased states commonly recorded in diabetic conditions[24]. Hyperlipidemia in diabetic conditions can cause hemodynamic changes

leading to myocardial infarction and stroke. These co-morbidities are important contributors to mortality in the population[45]. Besides, improvement in hemoglobin content seems to benefit the patient by augmenting the oxygen-carrying capacity of red blood cells, thereby rejuvenating the functioning of various tissues and organs[46]. Weight loss in diabetic conditions is another concern where studies conducted in the past have recommended the need for nutritional supplementation[47]. The findings of the present study indicated that the methanolic extract of MC has multiple actions in type-1 diabetes mellitus. The extract not only reduced the hyperglycemia but also other related complications that might require additional therapeutic interventions, suggesting that MC could be an effective alternative medicine for diabetes mellitus (both type-1 and type-2). The treatment with NR-2B (a water extract of MC) reversed the STZ-induced changes in body weight, serum glucose, hemoglobin and lipid profile in the same way that the methanolic extract (NR-2A) did. However, water extracts of MC at 45 and 180 mg did not alter the oxidative status in diabetic conditions (Table 2). The observation indicates that although water extract effectively reduces major STZ-induced complications, the extract's role in antioxidant status needs further research.

Although, the study met its objective of verifying the pharmacological effectiveness of several MC extracts in experimental animal models, some limitations need to be addressed in future investigations. First, methanolic extract revealed greater cardioprotective activity compared to water extract; nevertheless, there is no safety evaluation of the extracts, which precludes the therapeutic advantage of one extract over another type of extract/preparation. Second, no active components of each extract were examined, which would have provided insight into the therapeutic impact of specific extract constituents. Finally, the current experiments were conducted on a limited number of animals in a controlled laboratory setting; this needs to be refined before the intended preparation may be used for translational research.

CONCLUSION

The study's findings on the function of *Momordica charantia* (MC) methanolic and water extracts against streptozotocin (STZ)-induced diabetes complications were intriguing. Although, both extracts protected against STZ-induced diabetes complications, the methanolic extract outperformed the water extract. The study confirmed MC's anti-diabetic properties, including type-1 diabetes mellitus and shows it may be a beneficial natural ingredient for controlling hyperglycemia-related problems. Further research on the MC's specific phytochemicals, however, will aid in determining the active elements responsible for these potential pharmacological benefits. The isolation, identification and purification of phytoconstituents contained in MC extracts, as well as safety and efficacy testing, could identify a lead metabolite for treating hyperglycemia complications.

SIGNIFICANCE STATEMENT

The purpose of this study was to investigate the anti-diabetic potential of methanolic and water extracts of *Momordica charantia* (MC) at various doses (45, 90 and 180 mg kg^{-1}) in the presence of streptozotocin-induced diabetic rats. In diabetic rats, both extracts successfully reduced hyperlipidemia, decreased oxidative radicals and increased hemoglobin levels. Administering either extract to rats improved their antioxidant activity in the liver, kidneys and heart in a dose-dependent manner. The results of this study confirm that MC has antidiabetic potential and, following clinical review, could be an excellent complement to the existing arsenal of anti-diabetic drugs.

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