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Original Research Article

Evaluation Of *Rauvolfia Tetraphylla* Leaf Extract For Antidiabetic Activity In Alloxan Induced Albino Rats

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ABSTRACT:

Diabetes mellitus (DM), one of the most devastating illnesses, has a major influence on one's health and quality of life. Phytotherapy has a good prospect in the treatment of diabetes since it is less toxic and has fewer adverse effects than synthetic medications. In Asian, Oriental, and Latin American nations, *Rauvolfia tetraphylla L.* (RT) is extensively used in traditional ethnomedicine. In the current study, in vivo anti-diabetic activity of RT leaf extract was evaluated following standard procedures. Intraperitoneal injection of 90 mg/kgbwt of alloxan monohydrate was used to induce hyperglycemia. The experiment was conducted with six groups of male Wister rats (120.20±15.25 g) and administered 50, 100 and 150 mg/kgbwt of the RT extract, standard drug-glibenclamide (6 mg/kgbwt) treated group, normal sterile saline treated group and one non-diabetic untreated group, respectively. After 21 days, rats receiving 150 mg/kgbwt of the fraction had blood glucose levels of 109.002.33 mg/dL as compared to 78.911.90 mg/dL for the group receiving glibenclamide. Triglycerides, total cholesterol and LDL cholesterol levels were decreased while HDL cholesterol levels were raised as a result of the RT extract treatment. There was a significant increase ($p<0.05$) at 150 mg/kgbwt in the levels of catalase (34.36±0.30 U/mL), superoxide dismutase (70.66±0.56 U/mL) and reduced glutathione (100.64±3.25 µg/mL) activities compared with 24.86±0.76 U/mL, 33.32±0.73 U/mL and 63.82±2.10 µg/mL respectively for the control groups. The administration of diabetic rats with plant extracts over a 21 days period returned blood glucose levels close to normal control group (89±0.88 mg/dL). For all the parameters evaluated, the results were observed significant and in close comparison with the standard antidiabetic drug, glibenclamide. Along with all other anti-diabetic parameters, the pathological morphology of the kidney, liver, and pancreas is normalized. This indicates that the extracts of RT have diminishing effect on severity of diabetes.

Keywords: *Rauvolfia tetraphylla*; Leaf Extract; Antidiabetic Activity; Albino Rats

1. INTRODUCTION

Many of the plants that are employed in native folk medicine around the world have proven effective sources of medicinal substances. More than half of the medications that are commercially accessible come from plants or act as imitations of plants. Natural medicines have been employed as diabetic and cancer prevention measures. Research on medicinal plants and the derivatives of those plants has demonstrated their significance as a source of potent medications. More than half of the medications used for treatment are made naturally from the medicinal plants that they are derived

from. Kishore *et al*.,(2021 &2022) investigated on a plant *Cyphostemma* and proven the anti-cancer and anti-bacterial activity. Mishra shanti bhushan *et al*., 2009 reported that *Allium cepa Linn, Brassica juncea, Embellica officinalis Gaertn, Momordica charantia* and *Murraya koenigii* concluded that have anti-diabetic activity. Diabetes mellitus (DM), a multisystem illness characterized by lack of insulin secretion (Type-I) or insensitivity (Type-II) [1]. It is frequently accompanied with comorbidities like as hyperlipidemia, diabetic nephropathy and cardiovascular illnesses according to alarming figures from the World Health Organization, posing a health hazard to the worldwide population [2, 3]. Biguanides, glibenclamide, and Dipeptidyl peptidase-4 (DPP-4) inhibitors, which are the first-line glucose-lowering medications on the market, have excellent therapeutic results for DM but come at a high cost or with adverse effects [4-6]. Diabetes can be treated with insulin and a variety of synthetic oral hypoglycemic drugs, but insulin cannot be taken orally and synthetic drugs can have harmful side effects. Given the long-term need for therapy, finding effective, nontoxic, and affordable antidiabetic agents is critical. As a result, complementary and functional foods, as well as alternative pharmaceuticals, have emerged as novel therapeutic options for the management of diabetes. The quest for safe and effective medications has been a major focus of current research. The search for safe and functional anti-diabetic agents from natural materials is gaining traction. Many studies are now being conducted to investigate plant natural products containing particular phytocomponents with anti-diabetic potential as an alternative treatment [2]. *Rauvolfia tetraphylla L*. is an important medicinal plant belonging to the family Apocynaceae. It is a small evergreen, heavily branched woody shrub that is indigenous to the Northern and Southern America, West Indies, as well as many other nations, including India, Bangladesh, Pakistan, Bhutan, Indonesia, China and Myanmar. It is present in the plains of several Indian states, including Telangana, Andhra Pradesh, Karnataka, Orissa, Tamil Nadu, West Bengal, Kerala, Jammu and Kashmir, Bihar, Madhya Pradesh and Assam [7, 8]. R. tetraphylla is well known for its significant medicinal value, including those for fever, hypertension, cholera, eye disease, intestinal diseases, diarrhoea, and dysentery [9, 10]. The root extract is utilized as a treatment for high blood pressure, diabetes, stomach ache, and mental disorders in several regions of India. [11, 12]. Recent studies have revealed the insecticidal, antibacterial, anti-inflammatory and cytotoxic properties of R. tetraphylla [13, 14]. Furthermore, it is a good source of bioactive compounds and phytochemical components. Leaves have been found to contain alkaloids such 10-methoxytetrahydroalstonine, isoreserpiline, an isomeric combination of 11- and 10-demethoxyreserpiline, α-yohimbine, reserpiline, curan-17-oic acid, and 18, 19-Secoyohimban [15]. According to a comparison study, the root of *R. tetraphylla* has a higher content of reserpine than other parts [16]. There have been reports of indole alkaloids from the plant's seed coat, including ajmalcine, yohimbine, demethyl serpentine, and mitoridine [17]. *R. tetraphylla* has been found to contain a variety of phytochemicals, including tannins, steroids, alkaloids, flavonoids, phenols, sugars, reducing sugars, saponins, and more [18]. Research should be done to find solutions to the problem of diabetes, which has emerged as a severe global health issue that is raising concern among various nations. Research into herbal medicines as a diabetes alternative therapy should be prioritised in order to minimize reliance on synthetic pharmaceuticals, given the abundance of natural plant compounds having anti-diabetic potential. In the current investigation, rats that had been given alloxan to induce diabetes were used to examine the antidiabetic effects of RT extract. The effectiveness of the plant treatments was compared to that of metformin, a widely used synthetic anti-diabetic medication.

2. MATERIALS AND METHODS

Materials and chemicals

Alloxan, Metformin, and d-glucose were purchased from Sigma Chemical Co. All other chemicals used were of analytical grade.

Plant material preparation

Fresh leaves of *Rauvolfia tetraphylla L*. were collected from Nallamala forest, Mahaboobnagar, Telangana, India. The plants were identified and authenticated by Retd Prof. Rama Krishna, Department of Botany, Osmania University, Hyderabad and voucher specimens were deposited as herbarium. The plant leaves were washed, rinsed with distilled water, air dried under shade at room temperature (25±2.0°C), cut into pieces, pulverized and stored at a dry place for further use.

Extraction of plant phytochemicals

Extraction was carried out by methanol solvent using soxhlet apparatus as described by Anuj SK, *et., al*. [19] and Igbinosa.O *et, al.* [20] and Kishore *et al.,(*2021) with minor modifications. Phytochemicals from ground sample of *R. tetraphylla* were extracted using methanol (100g/1000mL) for 10 h up to 22 cycles at 40 ^{0} C until in the extractor siphon became colorless. The solvent was then removed from the extract using a rotary evaporator operating at 45°C. The extract was freeze-dried and preserved for future research at 4°C.

Acclimatization of animals

From the National Institute of Nutrition, 36 male Albino rats weighing 116 2.84 g were acquired (Hyderabad, India). All animals were housed in hygienic, PVC-coated stainless steel cages with a 12-hour light/dark cycle in an air-conditioned animal house with typical climatic parameters like a constant temperature of 20°C to 25°C. Before and during the trial, animals had an usual pellet meal and had unrestricted access to water. Prior to receiving experimental treatments, animals were fasted for 12 hours while still having appropriate access to water. The trials were all conducted during the day. The animals were given a 7-day acclimatization period before the experiment. The Department of Zoology at Osmania University in Hyderabad's Ethical Committee on the Use of Animals for Research gave its approval to the current work. Rats were handled in compliance with accepted laboratory animal care guidelines.

Induction of diabetes in rats

All animals, excluding those in the control group, were given a 12-hour fast before receiving an intraperitoneal injection of 90 mg/kg body weight of freshly prepared, ice-cold alloxan monohydrate made with sterile water (2%). When the diabetic condition had stabilised after a week, fasting blood glucose levels (FBG) were assessed. Diabetes was defined as having a fasting blood glucose level above 11.1 mmol/L, and animals with this condition were used in the research.

Experimental design

For a total of 21 days, the experiment was run. To acclimate to the surroundings, the rats were maintained for 7 days. Throughout the course of treatment, a daily oral dose of the leaf extract was given at the same time. For 21 days, a daily oral dose of the plant extract, dissolved in sterile saline, was given. Six groups, each with six rats, were formed at random from male Albino rats weighing between 120.20±15.25 g:

Group-1: Normal saline treated rats
Group-2: Normal saline treated alloxan induced diabetic rats (Diabetic Control-DC)
Group-3: Alloxan induced diabetic rats treated with glibenclamide synthetic drug, (5 mg/kgbwt)
Group-4: Alloxan induced Diabetic rats administered 50 mg/kgbwt of the RT leaf extract
Group-5: Alloxan induced Diabetic rats administered 100 mg/kgbwt of the RT leaf extract
Group-6: Alloxan induced Diabetic rats administered 150 mg/kgbwt of the RT leaf extract

At the start and end of the trial, the experimental animals' body weights were measured. The animals' tails were punctured to collect blood, and the blood glucose level was measured every five days for the duration of the trial.

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Collection and preparation of blood and tissues

Rats were starved for the night on day 21 and then killed by cervical decapitation around 24 hours following the final dose. Rats from each group had their intra-cardiac punctures performed, and blood was drawn into sterile, plain tubes with EDTA. The serum was extracted from the blood using a clean Pasteur pipette and stored frozen until it was utilised for biochemical tests. For liver enzyme assays and histopathology, the liver, kidney, and pancreas from each rat in each group were removed, cleaned in normal saline, and stored in 20% sucrose solution [21].

Biochemical assays

Using a commercial Agappe biochemical kit and following the procedure described by Nauck *et al.*, the lipid profile of the serum samples was evaluated [22].

Measurement of *In-vivo* antioxidant parameters

After being sacrificed, the livers of the rats in each group were immediately removed, rinsed with chilled saline, and then homogenised in ice-cold sucrose (20% w/v) using a mortar and pestle. The homogenate was centrifuged at 10,000 g for 20 mins at 4 °C [23] and the supernatant obtained was used to measure the activity of the enzymes catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH).

Reduced glutathione concentration

The reduced GSH content was measured using the Jollow *et al*. [24] technique. 150 µL of the liver homogenate and 150 µL of sulfosalicylic acid (SSA) are combined to form the reaction mixture, which is centrifuged at 5000 g for 10 minutes at 4 °C. By mixing 66 µL of supernatant with 66 µL of 0.01 M 5,5-dithiobis2-nitrobenzoic acid (DTNB) and 865 µL of potassium phosphate buffer, the amount of GSH was calculated (0.1 M, pH 7.4). The absorbance was measured at 412 nm after 5 minutes against SSA as a blank, and the concentration was estimated as follows:

GSH concentration (U⁄mL) = $(Abs / 0.416)\ X\ 2$

Where, Abs is the sample absorbance.

Superoxide dismutase enzyme activity

Superoxide dismutase (SOD) enzyme activity was assayed following the method reported by Misra and Fridovich [25]. The reaction mixture consists of 20 µL of liver homogenate, 960 µL of sodium carbonate buffer (50 mM, pH 10.2) and 0.1 mM EDTA. The reaction was initiated by adding 20 µL of 30 mM epinephrine (dissolved in 0.05% v/v acetic acid) to the mixture. The control contained 20 µL of distilled water instead of the sample while sodium carbonate buffer (0.05 M, pH 10.2) was used as the blank. The increase in absorbance was measured at 480 nm for 4 mins and activity calculated as follows:

% inhibition =100 – [(ΔAbs control − ΔAbs sample)/ΔAbs control]X100

SOD Activity (U⁄mL) = %inhibition X 3.75

where ΔAbs control is the difference in control absorbance at different times and ΔAbs sample is the difference in sample absorbance at different times.

Catalase enzyme activity

Catalase (CAT) enzyme activity was determined according to the method of Awad et al. [23]. Add 50 µL of liver homogenate to 2.5 mL of 30% H_2O_2 buffer, vortex and measure absorbance of the mixture at 240 nm after 30 secs and 90 secs. A 30% H2O2 solution was used as blank in place of the sample. The CAT activity was measured using the formula below:

$CAT\ activity\ (U⁄mins\ per\ mL\ of\ serum) = (\Delta Abs/0.0008)\ X\ 1\ min$

Where, ΔAbs is the difference in sample absorbance after 30 and 90 secs.

STATISTICAL ANALYSIS

In each group, all data was reported as mean ± standard deviation (S.D.). Using SPSS-20 statistical software, a one-way analysis variance was performed. The POST-Hoc test was run at a 5% level of significance, and the results are shown as bar graphs. When the p-value was less than 0.05, the results were deemed statistically different.

RESULTS

Table 1 demonstrates that, when compared to the control group, all tested doses of the RT extract significantly lowered the glucose level. As evidenced by raised blood glucose levels (460±2.11 mg/dL) at day 5, it was evident that intraperitoneal administration of alloxan at a dose of 90 mg/kg body weight induced a significant diabetogenic response in rats. In diabetic rats, administration of RT leaf extract resulted in a statistically significant decrease in mean blood glucose levels (p 0.05). The blood glucose level of untreated diabetic rats was considerably greater than that of treated diabetic rats (p0.05) and all the untreated rats died within 10 days of experiment due to high blood glucose levels (Table-1). The lowest glucose level obtained on the 21st day of the experiment (109.00±2.33 mg/dL) was observed at 150 mg/kgbwt which was significantly higher ($p<0.05$) than the glibenclamide-treated group (78.91±1.90 mg/dL). Due to their elevated blood glucose levels, the negative control group was unable to survive for more than 10 days (Table-1). Blood glucose levels were relatively same in experimental rats given plant extracts (150 mg/kgbwt) and glibenclamide medication, with small variations. These levels were found to be equivalent to those of normal rats after oral treatment of plant extract of RT, as well as the synthetic antidiabetic medication glibenclamide. The extracts, as well as the glibemclamide therapy, significantly reduced blood sugar levels in diabetic rats.

Table-1. Effect of *Rauvolfia tetraphylla* leaf extract on blood glucose concentration of alloxan-induced diabetic rats.

Day	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6
5	81±1.21	460±2.11	166±1.32	368±2.33	324±2.11	266±1.39
10	86±3.65	492±3.61	142±2.87	356±1.34	316±2.44	245±1.23
15	85±2.41	*	141±1.39	248±1.51	259±2.13	205±1.34
20	86±3.16	*	100±52	239±2.36	224±2.32	146±1.54
21	89±.88	*	78.91±1.90	218±1.87	16±2.15	109.00±2.33

* All animals died before the end of the study

The results of the effect of RT leaf extract on the body weight of alloxan-induced diabetic rats are shown in Table 2. When compared to the group treated with glibenclamide (2.34%), the group treated with 150 mg/kgbwt of the leaf extract recorded the highest bodyweight growth (3.77%) on day 21.

Table-2. Effect of *Rauvolfia tetraphylla* leaf extract on the body weight of alloxan-induced diabetic rats

Dosage	Initial	Final	% Change
50 mg/kgbwt.	118.67 ± 2.78	110.75 ± 1.21	−7.15
100 mg/kgbwt.	123.45 ± 3.12	127.09 ± 1.89	2.86
150 mg/kgbwt.	116.09 ± 2.89	120.64 ± 2.10	3.77
Normal	117.86 ± 4.02	136.09 ± 1.02	13.40
6-G	124.12 ± 1.74	127.09 ± 0.50	2.34
Untreated	121.13 ± 3.90	all animals died before the end of the study	

When compared to the untreated group, the RT extract significantly decreased (p 0.05) the concentrations of total cholesterol, LDL cholesterol, and triglycerides at various doses of the fraction (50, 150, and 300 mg/kgbwt). The RT extract at different doses not only decreased the levels of the aforementioned parameters but also increased HDL cholesterol levels, which were considerably greater than those of the glibenclamide-treated group (66.832.35 mg/dL) but lower than those of the control group (187.321.69 mg/dL) (Table 3).

Table-3. Effect of *Rauvolfia tetraphylla* leaf extract on lipid profile of alloxan-induced diabetic rats.

	Lipid profile (mg/dL)			
Dosage	Total cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triglycerides
50 mg/kgbwt.	439.11 ± 1.24	148.90 ± 3.12	55.90 ± 1.09	321.15 ± 2.34
100 mg/kgbwt.	310.32 ± 1.90	90.50 ± 3.22	108.40 ± 2.38	230.32 ± 1.98
150 mg/kgbwt.	270.89 ± 1.57	93.75 ± 4.10	97.92 ± 1.11	234.17 ± 3.10
Normal	371.39 ± 2.22	187.32 ± 1.69	148.39 ± 3.87	222.85 ± 0.50
6-G	257.87 ± 1.38	66.83 ± 2.35	78.70 ± 2.12	213.80 ± 2.37

The administration of 150 mg/kgbwt of the RT extract, as shown in Table 4, resulted in the greatest elevation in the liver's antioxidant enzymes, with catalase (33.360.30 U/mL), superoxide dismutase (71.660.56 U/mL), and reduced glutathione (100.643.25 g/mL) significantly different from the untreated, control, and glibenclamide-treated groups.

Table-4. Effect of *Rauvolfia tetraphylla* leaf extract on liver antioxidant enzymes in alloxan-induced diabetic rats.

	Antioxidant enzymes		
Dosage	Catalase (U/mL)	Superoxide dismutase (U/mL)	Reduced glutathione (µg/mL)
50 mg/kgbwt.	22.44±0.44	40.05±0.54	84.87±1.26
100 mg/kgbwt.	26.89±0.21	63.49±0.39	57.87±2.30
150 mg/kgbwt.	34.36±0.30	70.66±0.56	100.64±3.25
Normal	24.86±0.76	33.32±0.73	63.82±2.10
6-G	23.54±0.42	48.91±0.42	76.41±3.38

The pancreas' histopathology showed that Group-2 had a reduction in beta cells, while Groups 1 through 6 had no histological abnormalities. When compared to Group-3, which received the normal medicine, Groups 4 to 6 showed a significant increase in cells (Fig.I). In the groups that received RT extract plus glibenclamide treatment, there were no histological abnormalities in the rats' kidneys. Mild multifocal tubular vacuolation was seen in Group 2. Comparing treatment groups to the glibenclamide-treated group, a significant decrease in tubular vacuolation was seen.

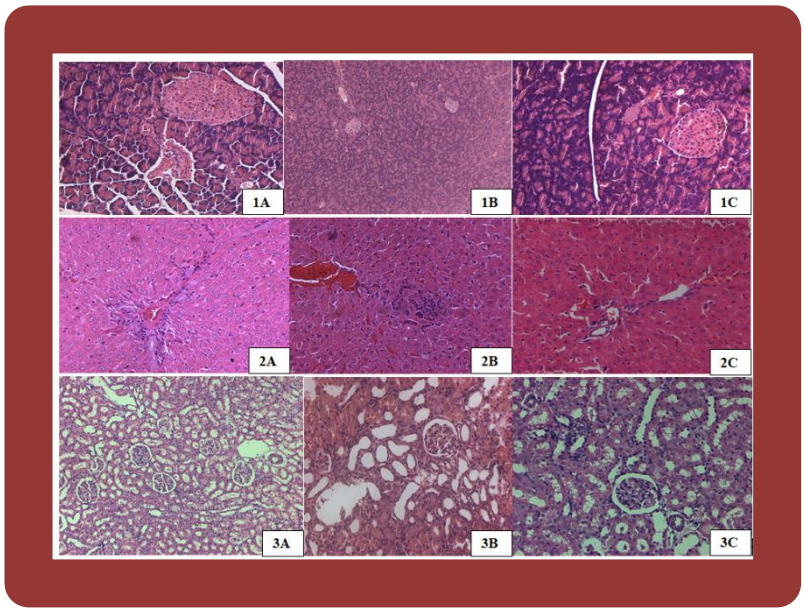

Fig I. Microphotograph of Pancreas, Liver and Kidney: 1 A Microphotograph of normal Pancreas (negative control) showing normal architecture (X 230), 1B Microphotograph of alloxan (positive control) treated rat pancreas showing mild to moderate degeneration of beta cells noticed in the islets of the pancreas(X 320),1C Microphotograph of the pancreas from RT extract treated diabetic rat showing the beta cells population proliferate, indicative of reactive changes to insulin production(X 230) ; 2A Microphotograph of normal liver (negative control) showing normal architecture (X 230), 2B Microphotograph of alloxan (positive control) treated rat liver showing moderate foci of inflammation noticed in vascular region of the liver (X 230), 2C Microphotograph of liver from RT extract treated diabetic rat showing most of the hepatocytes in the portal, periportal, and centrilobular region appeared normal(X230) ; 3A. Microphotograph of normal kidney showing normal architecture (X 230), 3B Microphotograph of alloxan (positive control) treated rat kidney showing dilatation of tubules, the tubular region is inflamed along with infiltration of inflammatory cells (X 230), 3C Microphotograph of kidney from RTextract (high dose) treated diabetic rat showing Glomerulus and Tubular region appeared normal (X 230).

In the DM group, the liver's histological imaging showed cellular shrinkage, an irregular nucleus, enlarged mitochondria with an accumulation phenomena, and lipid droplets vacuoles that were overly fatty. However, RT leaf extract showed dose-dependence in Fig. and could greatly alleviate the pathogenic traits seen on DM. The pancreatic histopathology imaging in Figure demonstrated that RT leaf extract could greatly alleviate the pathogenic features including uneven pancreatic islets, lipid droplets vacuoles, and aberrant islet cells with accumulation phenomenon seen on DM. Animal pancreatic, liver, and kidney histopathological analyses are shown in Figures 1, 2, and 3, respectively. According to scientific assessments, the livers of diabetic animals severely deteriorated [26, 27]. Microscopic analysis of the pancreatic sections from the alloxan-induced diabetic rats in the current study revealed that the pancreatic acini had less islet cells, the peripancreatic adipose tissue had swollen, and the blood vessels were overfilled (Fig. 1B). A slight structural modification, periportal inflammation, enlargement of the sinusoids, and normal kupfer cell activity were seen in the liver tissue (Fig.I 2B), whereas hypercellular glomeruli, thick-walled jammed blood vessels, overfilled proximal and distal convoluted tubules, and overfilled proximal convoluted tubules were seen in the kidney tissues (Fig. 3B). Control animals, in contrast, showed a well-ordered hepatic design with typical pancreatic acini with expanded ducts, a minor stratification, thick-walled overfilled blood arteries, and uncommon islet cells (Fig.I 1A). Liver tissue had the typical portal triad and minor central vein jamming of a normal liver (Fig.I 2A). Normal proximal convoluted tubules, distal convoluted tubules, and regular glomeruli were observed in the kidney tissue (Fig.I 3A). The pancreas, liver, and kidney, respectively, in animals treated with RT extraction, nearly recovered to normal, as seen in Figs.I 1C, 2C, and 3C. The pancreas architecture was enriched by RT extract and glibenclamide therapy in a manner identical to the control with no change. The pancreatic acini were hyperplastic at 50 mg/kgbwt of RT extract, along with the islet cell pool, overfilled blood vessels, and thick-walled ducts; the pancreatic acini were hyperplastic at 100 mg of

RT extract, along with the overfilled blood vessels and decreased islet cell pool. With the use of RT extract (150 mg), the liver's histopathology significantly improved, showing liver tissue with little central vein congestion, dilated sinusoids, periportal inflammation, kupfer cell activity, the proliferation of bile ducts, and mild ballooning hepatocyte degeneration with little necrosis.

DISCUSSION

In this work, rats with diabetes induced by alloxan were used as test subjects to determine the antidiabetic effect of *Rauvolfia tetraphylla* leaf extract. For the antidiabetic investigation, three different dosages of RT leaf extract (50, 100, and 150 mg/kgbwt) were assessed. Alloxan causes diabetes by destroying pancreatic beta cells with reactive oxygen species (ROS), which causes hyperglycemia since the injured pancreas either partially or completely stops secreting insulin [28]. The primary sign of diabetes has been determined to be hyperglycemia [28]. Therefore, the significant glucose-lowering capability displayed by the RT extract at all test doses may be attributable to both its antioxidant activity, which reduces ROS produced by glucose auto-oxidation in the hyperglycemic condition, and its capacity to promote pancreatic insulin secretion [29]. Alkaloids, phenols, flavonoids, saponins, and other secondary metabolites have all been found to have antidiabetic properties [30–31]. Therefore, the existence of these secondary metabolites could be responsible for the antidiabetic effect displayed by the RT extract. Once more, the higher concentration of these secondary metabolites at this dose may be the reason for the fraction's better glucose reducing power at 150 mg/kgbwt compared to previous test levels. Although the fraction did not lower blood glucose levels as much as or as much as the glibenclamide-treated group, it did so significantly (p 0.05) compared to the untreated group, and this may be a beneficial property that prevents hypoglycemia in long-term use of the fraction. Hypoglycemia is a physiological condition that is more dangerous than hyperglycemia when caused by glibenclamide. Possible methods of action include increased glycogenesis, decreased glycogenolysis or gluconeogenesis, and/or the insulin secretogogue effect of RT extract, which encourages higher glucose absorption and usage by cells.

Loss of body weight is a side effect of alloxan-induced diabetes in experimental animals, which may be caused by cells' failure to absorb glucose from the blood stream for various physiological functions, leading to lipolysis in adipose tissue and protein breakdown in skeletal muscles [32, 33]. It's interesting to note that the RT leaf extract's capacity to stimulate glucose uptake by cells, resulting in increased glucose utilisation and preventing lipolysis and proteolysis in adipose tissue and skeletal muscle, respectively, may explain the rats' gain in body weight when compared to the control and glibenclamide-treated groups (Table 3). This suggests that the fraction's antidiabetic effect is dose-dependent and was activated at higher dosages. Diabetes' unusual fatty acid metabolism can lead to dyslipidemia, a chain of heart illnesses, and other related problems [34]. High differences in serum lipid profiles are often indicative of diabetic dyslipidemia [35]. According to certain publications, HDL cholesterol plays a crucial function in the transfer of cholesterol and lowers the risk of cardiovascular diseases. In order to better understand these aberrations, all experimental groups' serum fatty acid levels were examined. As compared to a normal control, Table 3 showed a notable increase in serum lipid levels and a conspicuously lower level of HDL cholesterol. In diabetic rats over the trial period, the commercial medication glibenclamide and the RT extract caused a dose-dependent decrease in TC, TG, and LDL-C levels (significance of P 0.01). HDL-C values, however, increased in relation to Group 2 (p 0.001). The results demonstrated that by limiting the lipid abnormalities in experimental animals, commercial drugs and RT extract might reduce the risk of heart problems. Other than hyperglycemia, oxidative stress has been found to be the primary cause of death in diabetic patients. Vascular problems in diabetic patients are brought on by oxidative stress [36]. This is due to the body's antioxidant reserves being depleted, which causes free radicals produced by diabetes to start the damage of key organs. A variety of cells become vulnerable to free radicals, for instance, when the levels of

catalase, superoxide dismutase, and glutathione are reduced in diabetes, which has an elevated level of ROS . When compared to the untreated group, the rats given 100 and 150 mg/kgbwt had elevated levels of these enzymes, which may be related to the RT extract's antioxidant activity, which was demonstrated by in vitro antioxidant assays (Table 4). The potential of the bioactive components in the RT extract to upregulate the transcription of CAT, SOD, and GPx genes, which are translated into these antioxidants, may contribute to the elevated levels of these antioxidants in addition to the fraction's antioxidant activity.

CONCLUSION

In diabetes care, adequate glycemic control is critical. In the present study, crude extracts of *Rauvolfia tetraphylla* leaves were beneficial in controlling the blood glucose level, limits the lipid peroxidation by radical scavenging activity as proved with increased SOD, glutathione and CAT antioxidant enzymatic defenses in experimental alloxan-induced diabetic rats. Diabetic management is a mix of antihyperglycemic medication therapy and monitoring of liver and kidney function. Thus, RT leaf extracts studied in our investigation demonstrated promising antidiabetic effects equivalent to the synthetic medication glibenclamide, could be employed in the treatment of diabetes. Since plant components act more slowly than manufactured drugs and higher dosages may have a plateau effect, which would be harmful to the treatment of diabetes, further experimental research is needed to pinpoint the mode of action in the protective effect of these extracts on diabetic animals. To extract and pinpoint the precise active ingredients that give the studied plant materials their antidiabetic properties, more research needs to be done.

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