Biospectra : ISSN: 0973-7057, Vol. 19(1), March, 2024, pp. 95-100
An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Effect of methanolic extract of *Catharanthus roseus* leaf in alloxan induced diabetic albino mice through biochemical and metabolic changes assessment

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Received : 12th January, 2024 ; Revised : 12th February, 2024
DOI:-https://doi.org/10.5281/zenodo.14263389

Abstract- *Catharanthus roseus,* a medicinal plant with historical use in diabetes management, has shown potential in reducing oxidative stress and lowering glucose levels in preclinical studies. This study investigates the antidiabetic efficacy of *Catharanthus roseus* leaf extract in alloxan-induced diabetic mice. Results indicate that *Catharanthus roseus* extract effectively lowers blood glucose levels and reduces markers of kidney dysfunction, including blood urea, creatinine, SGOT, and SGPT, with effects comparable to rosiglitazone. Thus, *Catharanthus roseus* has significant rule in therapy for the long-term management of diabetes mellitus, emphasizing its therapeutic benefits and broader applicability in diabetes care. It improve the function of pancreatic tissue by insulin secretion.

Key words: *Catharanthus roseus*, alloxan, albino mice, Diabetes

INTRODUCTION

Diabetes mellitus (DM) is recognized as one of commonest emerging endocrine disorder. Its high prevalence, poor clinical treatment outcomes resulted into reduced patient's life quality. It appears due to abnormalities in metabolism of carbohydrate, lipid and lipoprotein.[1] It is estimated that by 2025 approximately more than 350 million people worldwide will suffer from this disease and annual cost of treatment could be reach more than trillion dollars annually. Whereas, in India alone number of patient suffering from diabetes attains 109 million by 2035.[2] It will create public health crisis.

It is caused by defects in insulin secretion, insulin action, or both.[3] Insulin is produced in beta cells of pancreas, due to several pathogenic processes involved in development of diabetes such as autoimmune destructions of beta cells lead to increased or decreased concentration of blood glucose. Chronic abnormalities of hyperglycaemia affect many organs or organ system like blood vessels, kidney, heart, nerves and especially the eyes.

Catharanthus roseus is easily available, reputed medicinal plant and widely utilized by people as folk medicine to treat diabetes mellitus in the ayurvedic system of medicine. It has been reported secondary metabolite of this plant utilized to treat effectively so many diseases. These evidences from the literature demonstrated that *Catharanthus roseus* extract is a reliable natural source of bioactive chemicals and that it has positive health effects when consumed. Extract of *Catharanthus roseus* leaf

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adequately lowered the oxidative stress induced by alloxan, and lowered glucose levels.

Diabetes contributes to the development of heart failure, retinopathy, nephropathy etc by metabolic and biochemical changes. Diabetes contributes to increased aldose reductase activity and O-Glc Nacylation that leads to gluco-toxicity.

MATERIALS & METHODS

This study includes leaf of *Catharanthus roseus* was collected from B N College Campus of Patna University, Patna, Bihar, India. It was further identified and botanically authenticated according to the relevant monographs of Indian Pharmacopoeia.[3] For animal studies, the research study was carried out at the Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India. The albino mice of weighing around 16-20 gram having 6.4 ± 0.5 cm lengths were taken. The mice were housed in polypropylene cages and fed on normal lab made chow and maintained under standard environmental conditions (21±2°C, 55±5% humidity, 12 hr Light: Dark cycle).

To study the induction of diabetes, the Alloxan is purchase from Loba chemical pvt. Ltd. was dissolved in 1m Molar citrate buffer at the pH 4.5 and always prepared fresh for immediate use (within 5 minutes). Diabetes was induced by multiple intra-peritoneal injection of freshly prepared alloxan solution in 0.05 M sodium citrate (pH 4.5) at the dose of 35 mg/kg body weight followed by fasting.[4]

To study the animal groupings and experimental design, the mice were divided into five groups having six mice in each group:

Group I- Alloxan induced diabetic control mice receiving citrate buffer only

Group II- Non-diabetic control mice receiving only citrate buffer solution

Group III- Diabetic treated (DT_{100}) receiving of 100 mg/ kg of body wt. extract

Group IV- Diabetic treated (DT_{200}) Receiving 200 mg/ kg of body wt. extract

Group V- Diabetic treated (DTRGZ) Receiving 2mg/ kg of body wt. Rosiglitazone

For the extract preparation, freshly harvested leaves samples were washed under running tap water, blotted with filter paper and was dried in the shade at room temperature. The dried plant sample (2.6 kg) was then soaked with absolute methanol under reflux condition for the methanolic extract preparation. The sample was then homogenized with extraction buffer and the supernatant collected after three rounds of extraction. The solvent was evaporated under reduced pressure in a rotary evaporator at 40°C. To this thick paste colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained plant extract was stored in freezer at -20°C until further test.

For the biochemical estimation, the desired biochemical parameters were accessed to monitor the metabolic activity of the mice in the respective groups. Fasting Plasma Glucose by GOD/POD method, serum Cholesterol CHOD/POD, triglyceride using GPO method, HDL by Phosphotungestic method. Serum LDL and VLDL were calculated using Frieldwald formula, serum creatinine by alkaline picrate method, Serum urea by Nitro prussic method, Alanine aminotransferase (ALT) Reitman and frankel method and Aspartate aminotransferases (AST) Modified IFCC method.

The statistical analysis, data were expressed as the mean ± S.E.M. for statistical analysis of the data; group means were compared by one-way ANOVA (analysis of variance) with Post Hoc analysis. The Tukey-Karmer Post Hoc test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Mathworks Inc. 2009.

RESULT & DISCUSSION

Acute toxicity and lethal test (LD_{50}) estimation of *Catharanthus roseus*.

The acute toxicity test in albino mice was recorded in table. 1 the acute toxicity testing of *Catharanthus roseus* extract in albino mice gave oral LD_{50} 500 mg/ kg

Table 1: LD_{50} Estimation

Stage I Treatment	**Number used/ number dead**
Group 1 (10 mg/kg)	3/0
Group 2 (100 mg/kg)	3/0
Group 3 (1000 mg/kg)	3/1
Stage II Treatment	**Number used/ number dead**
Group 1 (120 mg/kg)	3/0
Group 2 (200 mg/kg)	3/0
Group 3 (390 mg/kg)	3/0
Group 4 (500 mg/kg)	3/1

Effect of *Catharanthus roseus* extract on biochemical and metabolic changes in body weight, blood glucose levels, SGPT, SGOT, serum creatine and blood urea are markers for assessment of biochemical and metabolic changes.

Effect of *Catharanthus roseus* extract on body weight:

The *Catharanthus roseus* plant, a reputed medicinal plant, has been traditionally used to treat diabetes mellitus. The extract of *Catharanthus roseus* leaf has been shown to have positive health effects when consumed, lowering oxidative stress induced by alloxan the body weight of mice gradually gain that lossed due to diabetes.

Table 2: Body weight changes in mice.

Groups	Day 0	Day 7	Day 15
Normal control (NC)	18.85 ± 1.66	21.01 ± 1.29	22.79 ± 1.20
Diabetic control (DC)	12.67 ± 1.08	11.04 ± 0.85	9.47 ± 1.13
Catharanthus roseus extract (100 mg/kg) (DT_{100})	12.65 ± 2.03*	13.84 ± 1.46*	14.16 ± 1.46*
Catharanthus roseus extract (200 mg/kg) (DT_{200})	11.88 ± 1.86*	13.36 ± 2.03*	15.23 ± 0.84*
Rosiglitazone (2 mg/kg) (DTRGZ)	11.68 ± 2.64*	16.65 ± 1.33*	17.86 ± 2.04*

Values expressed as Mean ± S.E.M. (Standard Error of the Mean) n=3 in each group: Significant as Compared to Control.

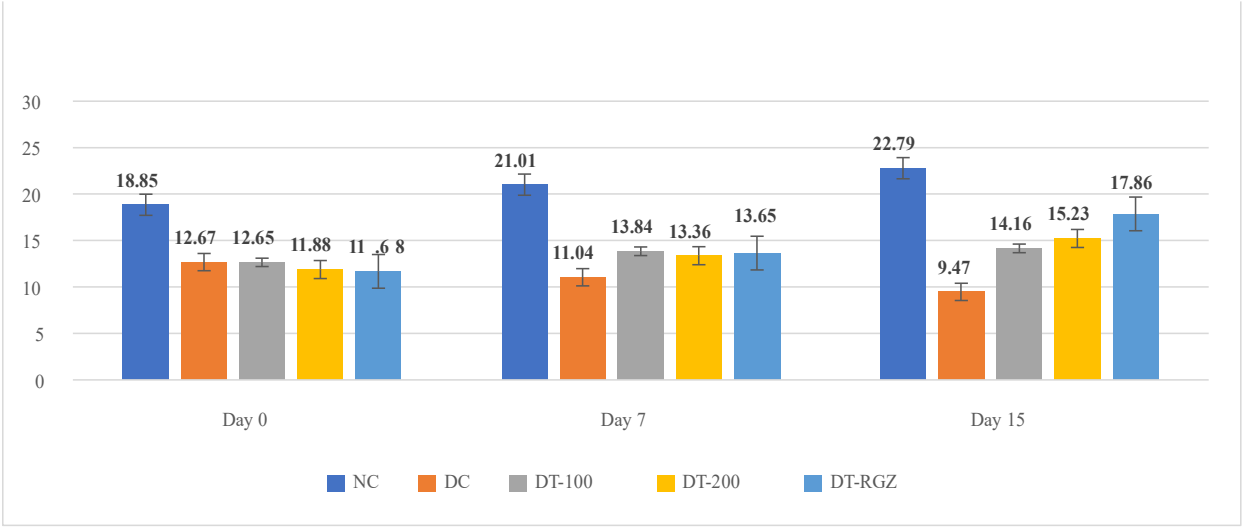

Fig. 1: Effect of treatment with *Catharanthus roseus* extract on body weight of different mice groups. NC, untreated normal control animals; DC, diabetic control animals; DT_{100}, diabetic animals treated with a low *Catharanthus roseus* extract dose (100 mg / kg b.w.); DT_{200}, diabetic animals' treatment with a high *Catharanthus roseus* extract dose (200 mg / kg b.w.). Each bar represents the mean ± SE (n=3).

Table 3: Effects of different doses of *Catharanthus roseus* extract and rosiglitazone on blood glucose level in mice.

GROUPS	Blood glucose levels (mmol/L) in week				
	Pretreatment	Post-treatment			
	0	1	2	3	4
Normal control (NC)	3.85 ± 1.03**	4.06 ± 0.84**	4.03 ± 0.79**	4.02 ± 0.76**	4.01 ± 0.46**
Diabetic control (DC)	14.76 ± 1.45*	14.71 ± 0.85*	14.02 ± 0.68*	13.98 ± 0.78*	13.96 ± 0.31*
***Catharanthus roseus* extract (100 mg/kg) (DT_{100})**	15.01 ± 1.36**	14.05 ± 1.13*	10.78 ± 1.08**	9.78 ± 1.36**	9.16 ± 1.16**
***Catharanthus roseus* extract (200 mg/kg) (DT_{200})**	15.23 ± 1.48**	14.06 ± 1.08**	11.76 ± 1.28**	8.06 ± 1.16**	8.16 ± 1.25**
***Rosiglitazone* (2 mg/kg) (DT_{RGZ})**	16.02 ± 0.85**	10.16 ± 1.37**	9.18 ±1.36**	5.47 ± 0.78**	4.98 ± 0.88**

* P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.

Effect of *Catharanthus roseus* extract on blood glucose level:

The changes in the blood glucose levels before and after receiving the treatment in normal and diabetic mice are listed in Table 3. As expected, the DC mice showed significantly ($p<0.001$) higher level of glucose (+278%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT_{100} and DT_{200}) showed a reduction in glucose levels when compared to the DC ones; nevertheless, the reduction was particularly evident in the DT_{200} mice (-50%; $p<0.001$).

When compared, the glucose levels of the DT_{200} versus the DC group mice during the 4-week treatment program, a significantly lower value in the first was also found (-68%; $p<0.001$). Nevertheless, this drop in the glucose levels was more evident in the DT_{100} rats (-57%) than in the DT_{200} mice. In contrast to this, DTRGZ group mice showed almost 100% drop in glucose level after 4-weeks of the treatment program (Table 3). These findings of the botanical extract of *Catharanthus roseus* may be indebted to their blood glucose-lowering properties to inhibition of glucose absorption and enhancement of glucose storage and utilization. The findings are in line with the previous study.[5,6]

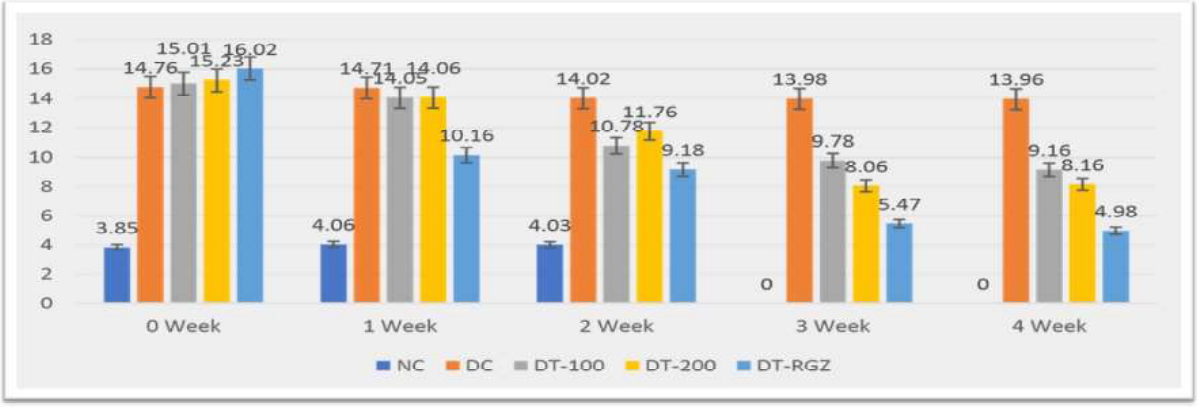

Fig. 2: Effect of treatment with *Catharanthus roseus* extract on body glucose levels in different mice groups. NC, untreated normal control animals; DC, diabetic control animals; DT_{100}, diabetic animals treated with a low *Catharanthus roseus* extract dose (100 mg / kg b.w.); DT_{200}, diabetic animals' treatment with a high *Catharanthus roseus* extract dose (200 mg / kg b.w.). Each bar represents the mean ± SE (n=3). * P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.

Study of Kidney &Liver markers:

Diabetic mice have higher levels (approximately twice) of blood urea, creatinine, SGOT, SGPT. All four markers decrease considerably in Rosiglitazone treated diabetic mice (DTRGZ) when compared to diabetic control mice (DC). In DTRGZ mice the parameters, serum urea, serum creatinine, serum SGPT, and serum SGOT were reduced by 134%, 52%, 106%, and 53% respectively (Table 4) Treatment with *Catharanthus roseus* extract decreases the values of all the four markers in a dose-dependent manner when compared to diabetic control mice.

The maximum efficacious dose was found to be 200 mg/kg body weight of mice (Table 4). Thus, the result showed that the *Catharanthus roseus* extract is also as effective as rosiglitazone in improving kidney function. The extract of *Catharanthus roseus* helps to preserve kidney function towards normal by ameliorating histopathological changes through reduction of, inflammation, fibrosis, and apoptosis in diabetic mice. The present results are supported with the previous findings.[7]

Table 4: Blood Urea, Creatinine, SGPT & SGOT in all groups before and after treatment with *Catharanthus roseus*

Group	Urea (mg/dl)	Creatinine (mg/dl)	SGPT (IU/L)	SGOT (IU/L)
Normal control (NC)	38.87 ± 0.41**	0.87 ± 0.36**	28.59 ± 0.33**	68.08 ± 1.38**
Diabetic control (DC)	89.08 ± 1.68*	1.32 ± 0.36*	66.68 ± 1.68*	116.30 ± 2.05**
***Catharanthus roseus* extract (100 mg/kg) (DT_{100})**	38.32 ± 0.77**	1.01 ± 0.57 **	32.34 ± 0.66**	72.31 ± 0.67**
***Catharanthus roseus* extract (200 mg/kg) (DT_{200})**	37.79 ± 2.31**	0.94 ± 0.21**	25.74 ± 0.57**	63.61 ± 1.36*
***Rosiglitazone* (2 mg/kg) (DT_{RGZ})**	37.57 ± 0.14	0.96 ± 0.24	29.68 ± 0.54	71.41 ± 1.62

*** P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.**

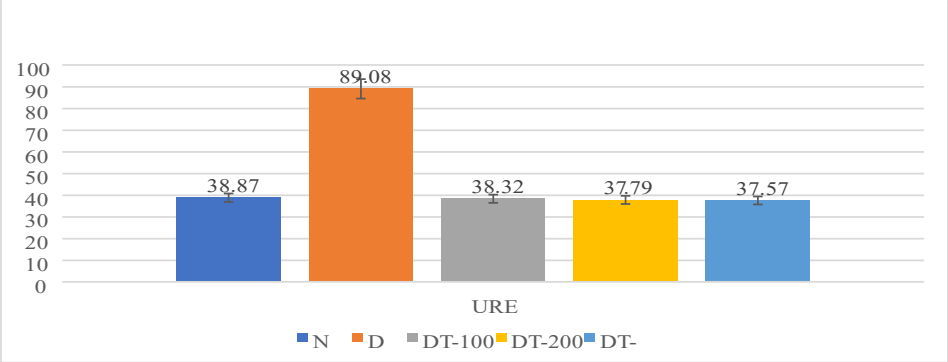

Fig. 3: Effect of treatment with *Catharanthus roseus* extract blood urea levels in different mice groups. NC, untreated normal control animals; DC, diabetic control animals; DT_{100}, diabetic animals treated with a low *Catharanthus roseus* extract dose (100 mg / kg b.w.); DT_{200}, diabetic animals' treatment with a high *Catharanthus roseus* extract dose (200 mg / kg b.w.). Each bar represents the mean ± SE (n=3). * P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.

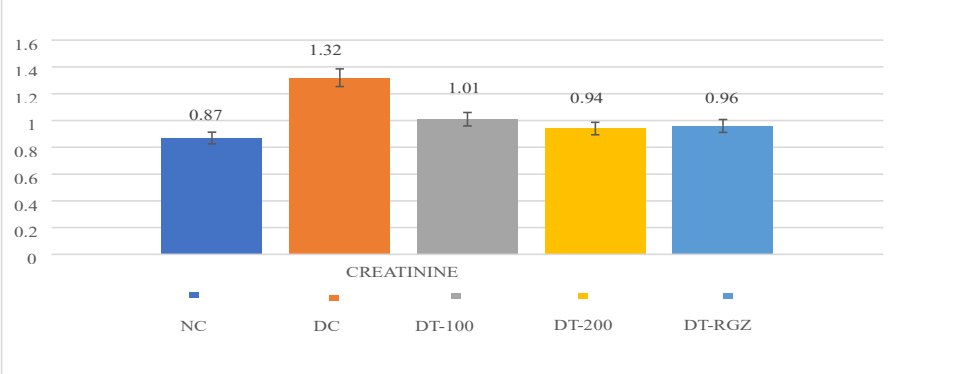

Fig. 4: Effect of treatment with *Catharanthus roseus* extract blood creatinine levels in different mice groups. NC, untreated normal control animals; DC, diabetic control animals; DT_{100}, diabetic animals treated with a low *Catharanthus roseus* extract dose (100 mg / kg b.w.); DT_{200}, diabetic animals' treatment with a high *Catharanthus roseus* extract dose (200 mg / kg b.w.). Each bar represents the mean ± SE (n=3). * P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.

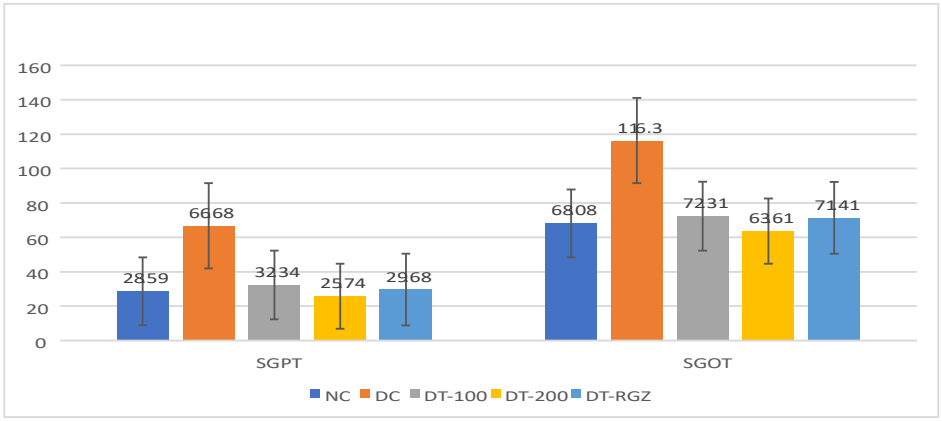

Fig. 5: Effect of treatment with *Catharanthus roseus* extract on liver function tests (SGPT & SGOT) in different mice groups. NC, untreated normal control animals; DC, diabetic control animals; DT_{100}, diabetic animals treated with a low *Catharanthus roseus* extract dose (100 mg / kg b.w.); DT_{200}, diabetic animals' treatment with a high *Catharanthus roseus* extract dose (200 mg / kg b.w.). Each bar represents the mean ± SE (n=3). * P< 0.05 as Compare with Normal Control. ** P< 0.01 as Compare with Diabetes Control.

CONCLUSION

The plant under investigation, *Catharanthus roseus* leaves extract is anti-diabetic due to the presence of different types of active components, which may have different mechanisms of action which reflects with the restored concentration of glucose, organ function enzymes, and lipid level. The DC mice showed significantly ($p<0.001$) higher level of glucose (+278%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT_{100} and DT_{200}) showed a reduction in glucose levels when compared to the DC ones; nevertheless, the reduction was particularly evident in the DT_{200} mice (-50%; $p<0.001$ Treatment of diabetic albino mice with the two different doses of *Catharanthus roseus* extracts showed a dose-dependent differential protective effect on liver function tests and kidney function tests. Therefore, relying on botanical phytochemicals as an anti-diabetic therapy may be beneficial and this could be considered as a safe supplementary therapy for long-term and effective management of diabetic patients.

ACKNOWLEDGEMENT

Authors are very grateful to the CSIR for providing necessary financial help in the form of research fellowship file no.- 09/0138(15586)2022-EMR-1 and also to the Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India for providing necessary equipment, chemicals and other research facilities.

ETHICAL CLEARANCE STATEMENT

The Current Research Work Was Ethically Approved by the ethical committee of the Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India.

CONFLICT OF INTEREST

Authors declares no conflict of interest regarding publication or any other activity related to this article.

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