



Assessment of Different Oxidative, Endocrinological and Biochemical Parameters using *Catharanthus Roseus* on Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current study was planned to assess the different oxidative, Endocrinological and biochemical parameters using extract of *Catharanthus roseus* (*C. roseus*) on diabetic rats. Three groups of Diabetic rats were assigned viz. Group A have Negative Control which was fed basal diet; Group B have Positive Control and was non-supplemented; and Group C supplemented with *C. roseus* extract. The results showed that blood glucose level, ALT, TGs and urea concentration was elevated non-significantly ($P>0.05$), of Group C which was supplemented by *C. roseus* when compared with non-supplemented Group B animals. In Group C which was supplemented by *C. roseus*, AST, and Cholesterol results were decreased as showed by statistics analysis (non-significantly). When level of Blood cortisol was compared between Group C and B, there was increased in Group C, as per non-significant statistics measurements ($P>0.05$). It was observed that level of T^4 and T^3 hormones was decreased in Group C (supplemented by herbal extract) when compared with Group B. When catalase enzyme results were checked in animals comprised of

Group A, B and C, it showed that rate of catalase increased, respectively. In conclusion, *Catharanthus roseus* restore the levels of thyroid hormones, and cholesterol; and elevate catalase enzyme activity in animals which produce stress relieving effect.

Keywords: *Catharanthus roseus*; diabetes; alloxan; herbal therapy; catalase.

1. INTRODUCTION

Diabetes Mellitus (DM) is a complex metabolic syndrome which occurs due to insufficient release of the insulin from the pancreatic beta cells defect in insulin action or both [1]. It may usually occur due to a combination of hereditary and environmental effects [2] and occasionally due to reduced tissue sensitivity to insulin [3]. Diabetes can lead to some changes in biochemical parameters like cholesterol, urea and creatinine [4]. It is considered as one of the five causes of mortality worldwide [5] and has gained increasing attention as an emerging disease problem. The current estimates by World Health Organization reveal that near 2030, the number of diabetic patients will reach up to 370 million in the world. It would be one of the most common degenerative illnesses in human beings in future [6].

In advanced medication, there are still unsatisfactory and ineffective therapies to treat DM [7]. Therefore, the demand for using herbal products having antidiabetic effects has increased due to associated side effects of insulin and oral hypoglycemic drugs [5]. Extensive work is being reported from India [8] and Bangladesh [9-10] However, in Pakistan the same trend is yet to be deep rooted.

Catharanthus roseus also known as *Vinca rosea* (*C. roseus*) is a herb that belongs to the family Apocynaceae. The antidiabetic effects of *C. roseus* aqueous leaf extract has been discovered recently [8] [11-12]. *C. roseus* aqueous leaf extract has its effects on liver enzymes, blood glucose level and serum proteins in rabbits [12]. Its affects on blood glucose, cholesterol, protein, lipid peroxidation and liver enzymes have also been studied [13]. However, it is first time to check the Assessment of different oxidative, Endocrinological and biochemical Parameters using *Catharanthus Roseus* on rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Catharanthus roseus leaves were collected from Bahauddin Zakariya University, Multan and

authenticated by Botanist from University of the Punjab. Leaves were washed, dried and ground to the powder form. This powder was passed through a sieve and combination of Dichloromethane and methanol having ratio 1:1 was passed through extraction process in Soxhlet apparatus. Solid mass was attained after removal of the solvent [14]. The diabetic rats were fed solid extract at the dose rate of 500mg/kg bw once daily for 21 days.

2.2 Preparation of Diabetic Rats

Fifteen healthy adult rats (130–200g) were taken in the current study. They were acclimated for one week and maintained at $23 \pm 2^\circ\text{C}$, 40–50% relative humidity, 12 hours light and dark period in iron cages and had an open access to food and water.

2.3 Animal Grouping and Treatment

The animals were divided into three groups including A, B and C consisting of five rats in each group. All the animals were weighed and alloxan was injected intra-peritoneally with dose rate of 150 mg/kg body weight for induction of diabetes. After 3 days, animals showing blood glucose level higher than 150 mg/dl which indicate the development of hyperglycemia as one of the manifestations of diabetes mellitus.

Treatment started after induction of diabetes. Group-A was selected as negative control and fed with only basal diet; Group-B was selected as positive control (diabetic, non-supplemented) and given basal diet + normal saline and Group-C was positive control (diabetic, supplemented) given basal diet + *C. roseus*. The study was approved in full by the Office of Research, Innovation and Commercialization (ORIC) of University of Veterinary and Animal Sciences, Lahore, Pakistan.

2.4 Collection of Blood Sample and Serum Preparation

In order to anesthetized, chloroform was given to rats after 21 days and blood was collected directly from the heart and transferred to

heparinized coated tubes, after 21 days. Blood was centrifuged at 4°C for 15 minutes at 3000 rpm to isolate the serum. Serum samples were analyzed for biochemical {Glucose, Cholesterol, Triglycerides (TGs), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Urea} endocrinological {Triiodothyronine (T^3), Thyroxine (T^4) and Cortisol} and oxidative (catalase) parameters through commercially available kits using spectrophotometry.

2.5 Statistical Tests

Statistical tests were proceeded through SPSS version 12.0 (SPSS Inc., Chicago, USA). Data was expressed as mean \pm S.E. The data testing was done by using One-Way Analysis of Variance with conflict between groups measured through Duncan's Multiple Range Test. Variation was considered as significant at $P < 0.05$. [15- 16;]. The values were expressed in Mean \pm SE.

3. RESULTS AND DISCUSSION

Pakistan, just like India, Bangladesh and Sri Lanka, is witnessing a soaring trend in use of herbs as anti-hyperglycemic agents. Various recipes and extracts of different herbs are being given in inventories [17-18]. To the best of our knowledge, the only study reported for hypoglycemic effects of *C. roseus* in diabetic rats is by Palwasha et al. 2015 in which only glucose level has been ascertained.

Effect on certain biochemical parameters by *Catharanthus roseus* is illustrated in Table.1. The results show a non-significant statistical ($P>0.05$) lift in ALT, TGs, blood glucose levels of Group C which was supplemented by *C. roseus* when compared with Group B. An extensive work has already been reported on various biochemical attributes as affected by *C. roseus* extract which have clearly indicated its restorative effects in diabetic rats and rabbits [19,9,14,20]. The antidiabetic effects of this herb have been attributed to a restorative effect on glucose transport genes [21,20]. Our results of these parameters for *C. roseus* supplemented group, however, are not in accordance with any of the previously reported work as the plant extract did not show restoration of their elevated levels. This cannot lead to a negation in anti-hyperglycemic activity of *C. roseus* in diabetes. The difference in results could be attributed to geographical variance or a variance in breed-specific response of experimental animals.

For AST and Cholesterol, Group C (Table 1) showed that decrease in measurements observed through statistics analysis ($P>0.05$). Though a statistical significant could not be attained in these parameters, however, the previous literature has also indicated a decrease in these parameters after supplementation of *C. roseus* extract [22,14] or its juice [19]. This is attributed to the hypocholesterolemic property of the herb. Furthermore, a decreasing effect on AST indicates that the extract had no toxic effect on liver parenchyma as already shown by the work of Singh et al. [23].

Amongst the endocrinological parameters studied for the treatment of *C. roseus* in diabetic rats, blood cortisol level was more in group C (*C. roseus* supplemented) in contrast to group B (Table 2), though statistically non-significant ($P>0.05$). Cortisol hormone has extensively been studied both as a stress biomarker [24] and as its potential role in hyperglycemia [25]. Its elevated level in diabetic non-supplemented as well as in *C. roseus* supplemented groups as compared to negative control group in the present study indicates stressor effect of hyperglycemia in experimental animals. The harmonious pattern of cortisol in Group B and C animals could be attributed to anti-stress effect of the herbal extract.

When Group C was compared with Group B with respect to T^4 and T^3 hormones, it was observed that the level of hormones decreased non-significantly in Group C which was supplemented by herbal extract (Table 2). This is indicative of a corrective/restorative effect of *C. roseus* extract. The thyroid hormones have been studied extensively in diabetic humans as well in various other species of animals [26]. It has been well established that diabetes causes an elevated levels of thyroid hormones which in the present study is corrected by the *C. roseus* extract. Various studies have revealed a sequence of complex intertwining biochemical, genetic, and hormonal malfunctions associated with pathophysiology of hyperthyroidism and diabetes [27].

Catalase is one of the vital intra-cellular enzymes responsible for scavenging the damage caused by reactive oxygen species (ROS) [28]. The results of immediate study discovered a moderate but non-significant statistical ($P>0.05$) shift in groups A, B and C respectively (Fig 1). [29-30]. A plausible justification to this

trend could be a steady stress-free order to combat the ROS generated during environment provided by the *C. roseus* extract in diabetes.

Table 1. Effect on certain biochemical parameters by *Catharanthus roseus* in experimental rats. The values are Mean±SE

Parameters	Groups		
	Group A	Group B	Group C
Glucose (mmol/L)	5.1±1.0	16.8±1.8	21.3±1.8
Cholesterol (mmol/L)	2.4±0.8	4.9±0.9	3.8±0.8
Triglycerides (mmol/L)	1.6±0.5	2.6±0.6	3.0±0.5
Alanine Transaminase (u/L)	38.2±2.0	13.5±1.1	13.9±1.0
Aspartate Transaminase (u/L)	48±2.8	25.0±1.0	24.5±1.0
Urea (mmol/L)	17.8±1.0	16.7±1.9	36.0±2.8
Catalase (U/L)	80±2.0	90±1.7	120±2.5

Where Group A: Negative control, Group B: Positive control, and Group C: Supplement with *Catharanthus roseus*. It is the mean value (Mean±SE) of results (where n= 5), results were in triplicate, p < 0.05

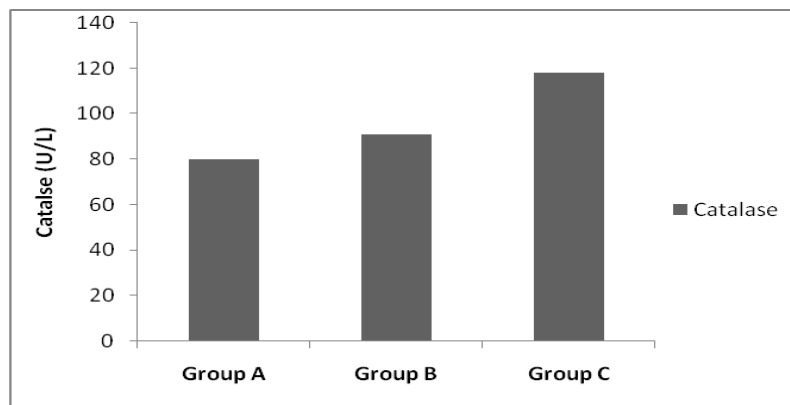


Fig. 1. Effect on catalase level by *Catharanthus roseus* in experimental rats. Where Group A: Negative control, Group B: Positive control, and Group C: Supplement with *Catharanthus roseus*. It is the mean value (Mean±SE) of results (where n= 5), results were in triplicate, p < 0.05

Table 2. Effect on certain endocrinological parameters by *Catharanthus roseus* in experimental rats. The values are Mean±SE

Parameters	Groups		
	Group A	Group B	Group C
Cortisol (nmol/L)	276±9.2	295.3±12.0	292.5±11.1
Triiodothyronine (nmol/L)	1.3±0.9	1.4±0.9	1.3±0.2
Tetraiodothyronine (nmol/L)	90.3±2.7	114.1±2.8	62.5±1.3

Where Group A: Negative control, Group B: Positive control, and Group C: Supplement with *Catharanthus roseus*. It is the mean value (Mean±SE) of results (where n= 5), results were in triplicate, p < 0.05.

4. CONCLUSION

In conclusion, *Catharanthus roseus* had a recuperative impression on thyroid hormones and cholesterol levels in alloxan-induced diabetic rats. Furthermore, it elevated catalase enzyme for potential scavenging of ROS. A statistically significant hypoglycemic effect of the extract could not be evident for glucose and certain other parameters under study. However, this

does not negate its importance as a potent anti-hypoglycemic agent. In future, assessment of activity of isolated compounds from the extract will be targeted to get promising results against other diseases in experimental animals.

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "herbal therapy" which is an ancient tradition, used in

some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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