Open Access Journal

International Journal of Medical Research and Pharmaceutical Sciences

January 2015, Volume 2(Issue 1) ISSN: XXX-XXX

HYPOGLYCEMIC AND BIOCHEMICAL REMEDIES OF CATHARANTHUS ROSEUS (LINN) ON ALLOXAN-INDUCED DIABETIC RAT AND ITS ANTI OXIDANT STATUS IN RAT LENSES

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Keywords:

Hypoglycemic, Biochemical remedies , Catharanthus roseus, Alloxan-induced diabetic rat etc.

Abstract

Diabetes mellitus is one of the most recognized and clinically significant disorders of the endocrine system. This study was elucidated in order to evaluate the effects of aqueous leaf extract of Madagascar periwinkle (Catharanthus roseus) on the activities of glucose-6-phosphate dehydrogenase body wt,eye lens wt ,lens protein,fluid intake,sorbitol and fructose of adult Wistar rats. Thirty-two male rats (Albino, Wistar) were used. Animals weighed 211-234 g and were about 7-13 weeks old. The animals were divided into four groups of eight animals in each. Groups were administered with 400 mg kg-1, 300 mg kg-1 and 200 mg kg-1 b.wt. of the plant extract respectively while the control group was administered with equal volume of phosphate buffered saline orally for 21 days. It was observed that there was significant increase in glucose-6-phosphate dehydrogenase and decrease in fructose and sorbitol in the administered groups. The results obtained from this study suggested that the leaf extract of Madagascar periwinkle (Catharanthus roseus) has hypoglycemic and anti oxidative effects. Further studies should be done in order to know if such effects seen in Wistar rats may be seen in man.

INTRODUCTION

A number of plants shows anti-diabetic properties and are being studied in different laboratories throughout the world, and many of these are suggested for diabetes and diabetic complications (Joseph and Jini, 2011). The hypoglycemic properties of plants used in management of diabetes are reported as they contain flavonoids, glycosides, alkaloids ,terpenoids, plant polysaccharides and other bioactive compounds (Iweala and Oludare, 2011). According to Jaya et al. (2010). In recent years, the use of traditional and complementary medicine has been of great relevance as it is cheap and readily available with little or no toxic effects and are effective (Patrick-Iwuanyanwu and Wegwu, 2008). According to Hasani-Ranjbar et al. (2010) many pharmacists are not adequately prepared educationally to meet the patient's request for information about herbal medications and products.

Catharanthus roseus (Vinca rosea) is known as Madagascar periwinkle. It is a perennial herb of the Apocynaceae family originally native to Madagascar (Hall, 1978). It measures about two feet in height and has dark green glossy leaves and pale pink or white flowers. The organic extracts of C. roseus is used in the folklore treatment of diabetes, malaria, leukemia, wasp stings, sore throat, eye irritation, infections (Gaston and Spicer, 2004). It is also used a as an astringent, diuretic and expectorant. The plant contains about seventy alkaloids some of which include catharanthine, lochnenine, vindoline vindolinenine, vincristine, vinblastine, tetrahydroalstonine, reserpine, serpentine, etc. (Chabner and Longo, 2005).

The inhibition of glucose-6-phosphate dehydrogenase activity decreases NADPH, which is the principal intracellular reductant ,a coenzyme essential for the protection against **oxidative stress** (Zhang et al., 2000). It is involved in the repair of oxidative damage and also plays a crucial role in maintaining the normal 3-dimensional integrity of proteins in the cell membrane (Zhang et al., 2000). The integrity of the cells as well as the antioxidant system and other processes requiring reduction rely on the adequate supply of NADPH. The functional integrity of glucose-6-phosphate dehydrogenase will ultimately helps the supply of energy to the cells (Zhang et al., 2000). Any change in the potentials of G6PDH will always results into a deviation from the normal functional capacity of NADPH. This may lead various cells regulating vital organs of the body to **oxidative stress**. Catharanthus roseus leaves are famous for use in diabetes mellitus (Mostofa et al., 2007; Chattopadhyay, 1999; Iweala and Okeke, 2005; Hsia et al., 2004). The present study was undertaken to elucidate the effects of C. roseus on the activities of glucose-6-phosphate dehydrogenase, body wt, urine sugar, eye lens wt , lens protein, fluid intake, sorbitol and fructose in the eye lens of adult Wistar rats.

MATERIALS AND METHODS

Fresh leaves of C. roseus were collected from the premises of the college. The leaves of Catharanthus roseus plant were air-dried and the dried plant material was weighed using electronic weighing balance and grinded with Blender. Five hundred grams of the dried powdered sample was soaked in five liters of 70% ethanol for 24 h at room temperature with constant shaking on a shaker and then filtered through silk cloth .The filtrate was then concentrated using a rotary evaporator to obtain alcohol-free residual extract of Catharanthus roseus.

Wistar rats were housed in clean cages in well ventilated standard housing conditions .Their cages were cleaned every day.

Adult albino rats (Winstar strain) weighing between 200-220 g and are free from any kind of infections were used. The animals were maintained as per the guidelines. For experimental purposes, the rats were kept fasting overnight but were allowed free access to water. The standard protocol for laboratory animal care was followed. Animals were maintained under the laboratory conditions with standard stock diet and water. In the present experiment, a total of 32 rats (16 diabetic surviving rats; 16 normal rats) were used and were housed individually in wire mesh cages.. The rats were divided into four groups of eight each: control (C); control rats treated with C. roseus (C + CR); diabetic (D), and diabetic animals treated with C. roseus (D + CR).

Diabetes was introduced by intra- peritoneal administration of 150 mg/kg body weight of ice cold aqueous alloxan monohydrate (Alberti KG et.al.1998) to two groups of rats served on diabetic control and diabetic experimental, respectively. After a fortnight, hyperglycaemia was observed in both the groups of rats. The other two groups were kept as non- diabetic control and non- diabetic experimental, respectively. The rats were given high fibre and high protein diet. 20 g diets were fed and distilled water was provided adlibitum. The leftover food residues were collected to calculate the actual food intake. The rats were weighed every week up to 30days of experimental period to record the body weight changes. The initial and final blood glucose levels were measured from the tail veins with the help of glucometer. The results were compared with the control groups of non- diabetic and diabetic rats with the initial values of the same groups. Urine sugar was checked by uristrix strips from Bayer. The rats were observed continuously for gross behavioral changes.

The data was analysed statistically using variances' test. All the animals were sacrificed, the muscle were excised, and were homogenized in 0.25 M cold sucrose solution, the homogenized tissue samples were centrifuged at 5000 rpm for 10 min (David et al., 2009). The supernatants were carefully and immediately stored in a deep freezer at -20°C. These were assayed within 48 h. The activities of G6PDH were estimated by the methods of Baquer et.al(1973) .Eye lens were excised, instantaneously after sacrifice, washed with normal saline, blotted dry, weighed and was used for studing protein assay by the method of lowry et.al 1951 using BSA as standard. Sorbitol and fructose was estimated as described by Bergmeyer et.al (1974) .Data were statistically evaluated using the student's t-test with SPSS/14.0 software (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as Mean± Standard Error of Mean (SEM). A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS AND DISCUSSION

The behaviour of diabetic rats appeared sluggish and abnormally active initially but returned to normal after a week of treatment. The consumption of food increased initially which became normal in the treated rats. Fluid intake increased six times in the diabetic untreated rats while the intake of water was twice in Cathranthus roseus treated rats. . The leaf extracts of Cathranthus roseus appears to be useful in inhibiting glucose- 6- phosphate dehydrogenase, hepatic glucose output and controlling the elevated blood glucose levels.table1. Cathranthus roseus changed the insulin action in tissues. It is an insulin sensitizer which can be used in the treatment of diabetes. It improves the glycaemia control by enhancing the insulin sensitivity in liver and muscle. Improved metabolic control with Cathranthus roseus did not cause much weight gain.

The impact of leaf extracts of Catharanthus roseus on the body weight of the animals in the treatment groups when compared with that in the control group was as presented in table1. After 30 days of treatment, the body weights of the treated animals were observed to increase significantly ($p<0.05$) in comparison with the control. The activity of G6PDH was increased significantly in the treated animals. The activity of G6PDH was statistically significant in the treated group when compared with the control group. The animals treated with plant extract shows that the plant extract has modulating effects on the enzyme of carbohydrate metabolism (Table 1) The pentose phosphate pathway is a cytosolic process that serves to generate NADPH and the synthesis of pentose sugars. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated and the second is the non-oxidative synthesis of 5-carbon sugars. This pathway is an alternative to glycolysis. According to Beutler et al. (1996) the primary functions of the pathway is to generate reducing equivalents in the form of NADPH for reductive biosynthesis reactions within cells and provide the cells with ribose-5-phosphate for the synthesis of nucleotides and nucleic acids. the weight of the animals in all the treatment groups began to increase and this was observed throughout the remaining days of study. A similar study by Mostofa et al. (2007) showed a statistically significant increase in the body weight of rats treated with leaf extract of C. roseus.

Glucose-6-phosphate dehydrogenase is a cytoplasmic enzyme that affects the production of reduced form of cytosolic coenzyme (NADPH) by controlling the step from glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate pathway (Zhang et al., 2000; Kletzien et al., 1994). Until recently, the role of this housekeeping enzyme in cell responses to oxidative stress was limited to human erythrocytes that lack any other NADPH producing route (Xu et al., 2005). According to Salvemini et al. (1999), it was suggested that G6PDH also plays a protective role against ROS in eukaryotic cells and that G6PDH expression is unregulated by oxidants. The search of available literature(s) revealed no published report on the activities of C. roseus on enzymes of carbohydrate metabolism, G6PDH. In this study, oral administration of Catharanthus roseus to rats produced significant alterations in the enzymatic activities after 30 days of administration.

It was observed during the course of this study that the plant extract has a modulating effect of on the body weight of rats administered with the extract. The significance of the significant increase and decrease in the activities of G6PDH observed in this study may suggest the antidiabetogenic properties of C. roseus as reported by Singh et al. (2001). This characteristic activity of the plant extract could be as a result of the phytochemical constituents of the plant. The outcome of this study is in support of the claims of Chattopadhyay (1999), Chattopadhyay et al. (1991), Chattopadhyay et al. (1992) and Elgorashi et al. (2003).

Lens is one such tissue which is worst affected in diabetes. Due to over elevated glucose concenteration excessive glucose is fluxed into the lenses and enters into polylol pathway converting excessive glucose into sorbitol and fructose. Enormous increase in sorbitol and fructose was observed in diabetic lens during the present study. Normally glucose hardly utilizes polyol pathway because of low affinity of aldose reductase for glucose. In the tissues such as lens which do not require insulin for the intracellular transport of glucose, aldose reductase activity has been shown to be increased in hyper glycemic environment with concomitant sorbitol and fructose accumulation in tissues. This effect may lead to sorbitol induced osmotic stress, decrease in Na/K ATPase activity, increase in cytosolic NADH and decrease in cytosolic NADPH as well as depletion of other outer oxidant defenses. These metabolic changes cultivated tissue damages in the retinal vasculature. Diabetic untreated rats showed retinal ischemia.

CONCLUSION

Observations and data obtained from this study showed that the leaf extract of C. roseus alters carbohydrate metabolism. Also the plant extract altered the activities of Glucose-6-phosphate dehydrogenase (G-6PDH).From the experimental study it is observed that accumulation of sorbitol and fructose were reduced in diabetic induced rat ,when treated with C. roseus . Damages caused to eye lenses were almost negligible as compared to untreated diabetic rats. Leaves extract were found to effective in controlling blood sugar. Rat showed normal behaviour through out the experimental period when treated with C.roseus. The present study concludes that C.roseus leaves helps in regulating and maintain homeostatic metabolism in the body. Leaves extract were found to be effective as antidiabetic agent.

TABLES

1. Effect on general parameters of diabetic rats after 30 day treatment with Cathranthus roseus

Sr. No.	Parameters	Control	control+CR	Experimental (D)	Experimental D+CR
1	**Urine Sugar**	-ve	-ve	+5	-ve
2	G6PDH unit/mg protein	0.248±0.03	0.250±0.05	0.164±0.08	0.218±0.02
3	Bodywt(intial) gm	200±5.0	200±4.5	205±3.9	200±3.2
4	Bodywt(final)gm	215±4.3	216±3.9	175±4.1	199±2.6
5	Lens wt(mg)	88±1.3	87±1.8	66±2.3	78±2.9
6	Lens protein mg/g	358±2.9	354±3.6	301±2.0	350±4.2
7	Fluid intake ml/day/wt	35±6.2	37±4.9	220±2.0	50±4.2
5	Fructose µmol/g	1.57±0.02	1.7±0.10	18.12±0.13	1.62±0.21
9	sorbitol µ mol/g	1.30±0.04	1.8±0.07	18.04±0.18	1.71±0.18

2. Effect on PPG & FBG levels in blood glucose of diabetic rats after 30 days treatment with *Cathranthus roseus* .

No.	**Group**	**FBG (Initial) mg/ dl**	**FBG (Final) mg/ dl**	**PPG (Initial) mg/dl**	**PPG (Final) mg/dl**
1	**Control**	69 ± 8.6	70 ± 5.4	100 ± 9.8	102 ± 7.6
2	**Control +CR**	68 ± 7.8	69 ± 5.8	102 ± 6.7	102 ± 9.8
3	**Experimental (D+CR)**	288± 8.4	141±6.3	258 ± 3.6	155 ± 5.2
4	**Experimental (D)**	279 ± 9.4	438 ± 4.6	287 ± 5.7	523 ± 7.8

REFERENCES

1. *Alberti KG, Zimmet PZ. New diagnostic criteria and classification of diabetes-again. Diabet Med.1998;15:535–[Baquer N Z,M Clean,Green baich A L (1973) Biochem Bio Phy .Res 52,1282-88.*
2. *Beutler, E., T. Vulliamy and L. Luzzatto, 1996. Hematologically important mutations: Glucose-6-phosphate dehydrogenase. Blood Cells Mol. Dis., 22: 49-56. PubMed | Direct Link | .*
3. *Bergmeyer H.U, Bernt E,Gchmidt F and Stork F 1974 Assay for hexoses N Y Acedemic press.*
4. *Chabner, B.A. and D.L. Longo, 2005. Cancer Chemotherapy and Biotherapy: Principles and Practice. 4th Edn., Lippincott Williams and Wilkins, Philadelphia, PA., USA., ISBN: 9780781756280, pp: 879.*
5. *Chattopadhyay, R.R., 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. J. Ethnopharmacol., 67: 367-372.*
6. *Chattopadhyay, R.R., R.N. Banerjee, S.K. Sarkar, S. Ganguly and T.K. Basu, 1992. Antiinflammatory and acute toxicity studies with leaves of Vinca rosea Linn in experimental animals. Indian J. Physiol., Pharmacol., 36: 291-292.*
7. *Chattopadhyay, R.R., S.K. Sarkar, S. Ganguly, R.N. Banerjee and T.K. Basu, 1991. Hypoglycemic and antihyperglycemic effects of leaves of Vinca rosea Linn. Indian J. Physiol. Pharmacol., 35: 145-151.*
8. *David, A.J., A.G. Adefolaju, B.U. Enaibe, M.S. Ajao and E.A. Caxton-Martins, 2009. LDH and G-6PDH activities in the ovaries of adult female Wistar rats following the administration of aqueous extracts of neem (Azadirachta indica) leaves. Afr. J. Biotechnol., 8: 1310-1313.*
9. *Elgorashi, E.E., J.L.S. Taylor, A. Maes, J. Van Staden, N. De Kimpe and L. Verschaeve, 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicol. Lett., 143: 195-207.*
10. *Gaston, K.J. and J.I. Spicer, 2004. Biodiversity: An Introduction. 2nd Edn., Wiley-Blackwell Science, Oxford, UK., ISBN: 9781405118576, pp: 191.*
11. *Hall, N., 1978. Botanists of the Eucalypts. Commonwealth Scientific and Industrial Research Organization, Melbourne, ISBN: 9780643002715, pp: 160.*
12. *Hasani-Ranjbar, S., N. Nayebi, B. Larijani and M. Abdollahi, 2010. A systematic review of the efficacy and safety of Teucrium species; from anti-oxidant to anti-diabetic effects. Int. J. Pharmacol., 6: 315-325.*
13. *Hsia, S.H., M. Bazargan and M.B. Davidson, 2004. Effect of pancreas tonic (an ayurvedic herbal supplement) in type 2 diabetes mellitus. Metabolism, 53: 1166-1173.*
14. *Iweala, E.E.J. and C.U. Okeke, 2005. Comparative study of the hypoglycemic and biochemical effects of Catharanthus roseus (Linn) g. apocynaceae (Madagascar periwinkle) and chlorpropamide (diabenese) on alloxan-induced diabetic rats. Biokemistri, 17: 149-156.*
15. *Iweala, E.E.J. and F.D. Oludare, 2011. Hypoglycemic effect, biochemical and histological changes of Spondias mombin Linn. and Parinari polyandra benth. Seeds ethanolic extracts in alloxan-induced diabetic rats. J. Pharmacol. Toxicol., 6: 101-112.*
16. *Jaya, A., P. Shanthi and P. Sachdanandam, 2010. Hypoglycemic effect of Semecarpus anacardium in streptozotocin induced diabetic rats. Int. J. Pharmacol., 6: 435-443.*
17. *Joseph, B. and D. Jini, 2011. Insight into the hypoglycaemic effect of traditional indian herbs used in the treatment of diabetes. Res. J. Med. Plant, 5: 352-376.*
18. *Kletzien, R.F., P.K. Harris and L.A. Foellmi, 1994. Glucose-6-phosphate dehydrogenase: A "housekeeping" enzyme subject to tissue specific regulation by hormones, nutrients and oxidant stress. FASEB J., 8: 174-181.*
19. *Lowry O H, Bose Brough N J,Farr A L and Randali R J (1951) Protein measurement with the folin phenol reagent.J.Bio Chem 193, 265-275 .*
20. *Mostofa, M., M.E. Choudhury, M.A. Hossain, M.Z. Islam, M.S. Islam and M.H. Sumon, 2007. Antidiabetic effects of Catharanthus roseus,Azadirachta indica, Allium sativum and Glibenclamide in experimentally diabetic rats. Bangladesh J. Vet. Med., 5: 99-102.*
21. *Patrick-Iwuanyanwu, K.C. and M.O. Wegwu, 2008. Prevention of carbon tetrachloride (CCl4)-induced liver damage in rats by Acanthus montanus. Asian J. Biochem., 3: 213-220.*
22. *Salvemini, F., A. Franze, A. Iervolino, S. Filosa, S. Salzano and M.V. Ursini, 1999. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. J. Biol. Chem., 274: 2750-2757.*

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23. *Singh, S.N., P. Vats, S. Suri, R. Shyam, M.M.L. Kumria, S. Ranganathan and K. Sridharan, 2001. Effect of an antidiabetic extract ofCatharanthus roseus on enzymic activities in streptozotocin induced diabetic rats. J. Ethnopharmacol., 76: 269-277.*
24. *Xu, Y., B.W. Osborne and R.C. Stanton, 2005. Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. Am. J. Physiol. Renal Physiol., 289: F1040-F1047.*
25. *Zhang, Z., K. Apse, J. Pang and R.C. Stanton, 2000. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. J. Biol. Chem., 275: 40042-40047.*