INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

ANTIOXIDANT ACTIVITY OF *POLYGALA ROSMARINIFOLIA* WIGHT& ARN WHOLE PLANT IN ALLOXAN INDUCED DIABETIC RATS

Nishanthini A, Mohan V.R.*

Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu, India

Article Received on: 17/07/12 Revised on: 04/08/12 Approved for publication: 10/09/12

*E-mail: vrmohanvoc@gmail.com

ABSTRACT

Administration of ethanol extract of *Polygala rosmarinifolia* whole plant (100 mg/kg and 200 mg/kg body weight) to alloxan induced diabetic rats for 14 days reduced the elevated level of lipid peroxidation (LPO). The treatment also resulted in significant increase in reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in serum, liver and kidney. The results confirm the antioxidant activity of *Polygala rosmarinifolia* whole plant and suggest that because of its antioxidant effects its administration may be useful in controlling the diabetic complications in experimental diabetic rats.

Keywords: Antioxidant, *Polygala rosmarinifolia*, Oxidative stress.

INTRODUCTION

Oxygen free radicals and lipid peroxidation have been implicated in the pathogenesis of a large number of diseases such as diabetes, cancer, infectious diseases, atherosclerosis and in aging[1-2]. Antioxidant defense systems are also distributed in diabetes mellitus[3]. There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals[4]. A free radical is any species capable of independent existence that contains one or more unpaired electrons. Thus, free radicals result in the consumption of antioxidant defences which may lead to disruption of cellular functions and oxidative damage to membrane and enhance susceptibility to lipid peroxidation. Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown etiology and in the toxic actions of many compounds[5]. Antioxidants thus play an important role to protect the human body against damage caused by reactive oxygen species[6]. The endogenous antioxidant enzymes (e.g SOD, CAT, GSH and GPx) are responsible for the detoxification of deleterious oxygen radicals[7].

Many plant extracts and plant products have been shown to have significant antioxidant activity, which may be an important property of plant medicines associated with the treatment of several its fated diseases including diabetes. Thus, herbal plants are considered useful means to prevent and/ or ameliorate certain disorder, such as diabetes, atherosclerosis and other complications[8].

Polygala was traditionally used by Americans to treat snake bites [9] and as an expectorant to treat cough and bronchitis. *Polygala* is considered as a powerful tonic herb[10] that can help to develop the mind and aid in creative thinking. However, inspite of traditional use, pharmacology of its whole part has not yet been explored scientifically. To our knowledge no report on the *in vivo* antioxidant activity of effect of *Polygala rosmarinifolia* whole plant on alloxan induced diabetic rats. This study was therefore undertaken to evaluate the effect of ethanol extract of whole plant of *Polygala rosmarinifolia* on *in vivo* antioxidant activity in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *Polygala rosmarinifolia* were freshly collected from the well grown healthy plants inhabiting the natural forests of Maruthamalai, Coimbatore district, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The *Polygala rosmarinifolia* whole plant were shade dried at room temperature and the dried whole plant were powdered in a Wiley mill. Hundred grams of powdered *Polygala rosmarinifolia* whole plant was packed in a Soxhlet apparatus and extracted with ethanol.The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [11-12]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [13]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Induction of Diabetes in Experimental animal

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) [14]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Polygala rosmarinifolia* whole plant (100mg/kg body weight)

Group IV: Diabetic rats given ethanol extract of *Polygala rosmarinifolia* whole plant (200mg/kg body weight)

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg body weight).

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes and serum was stored at -4^{o}C until analyses completed. The liver and kidney tissues were excised, rinsed in ice cold saline, cut into small pieces and homogenized with homogenizer in Tris-HCl buffer (PH 7.4). The homogenate was centrifuged at 10,000 rpm for 10 min. Supernatant was used for enzyme assays for the estimation of non enzymatic and enzymatic antioxidants such as lipid peroxidation (LPO)[15]; superoxide dismutase (SOD) [16]; catalase (CAT)[17], glutathione peroxidase (GPx) [18]and reduced glutathione (GSH) [19].

Table 1. Effect of *Polygala rosmarinifolia* extracts on serum LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	1.63±0.08	621.14±34.19	31.96±1.36	428.64±31.44	73.94±2.48
II	3.09±0.03**	298.24±26.33***	20.33±1.03*	278.08±23.11***	60.14±1.94*
III	2.56±0.13	521.06±14.31	23.14±0.96	312.14±13.16	62.14±1.04
IV	1.83±0.02	591.56±19.36	28.64±1.12	389.27±21.64	66.39±1.03
V	1.54±0.11	593.94±23.08	29.29±1.08	391.56±19.36	78.23±1.47

Each value is SEM ± 5 individual observations * $P < 0.05$; ** $P<0.01$; $P<0.001$ Compared normal control vs -Diabetic rats

Table 2. Effect of *Polygala rosmarinifolia* extract on liver LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	0.094±0.013	8.14±0.12	49.11±1.37	5.27±0.93	81.96±2.04
II	0.173±0.021**	3.91±0.09*	12.94±1.14*	2.09±0.05**	62.22±1.86**
III	0.448±0.004	5.28±0.14	35.21±1.08	3.98±0.07	72.16±1.04
IV	0.112±0.011	6.81±0.12	47.59±1.22	4.96±0.12	80.08±1.09
V	0.081±0.001	6.93±0.16	48.53±1.76	4.97±0.13	83.66±1.22

Each Value is SEM ± 5 individual observations * $P < 0.05$; ** $P<0.01$ Compared normal control vs -Diabetic rats

Table3. Effect of *P. rosmarinifolia* extracts on Kidney LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	0.078±0.004	5.26±0.19	31.56±1.24	15.13±1.03	43.11±1.74
II	1.843±0.014*	2.13±0.12**	12.92±1.07**	7.96±0.36*	13.96±1.03**
III	1.516±0.016	3.54±0.18	19.56±1.11	11.22±0.56	28.11±1.21
IV	1.116±0.026	3.96±0.20	27.63±1.32	14.11±0.73	39.36±1.72
V	0.086±0.004	5.64±0.24	28.11±1.09	18.39±0.36	40.56±1.11

Each Value is SEM ± 5 individual observations * $P < 0.05$; ** $P<0.01$ Compared normal control vs -Diabetic rats

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *Polygala rosmarinifolia* whole plant revealed the presence of alkaloid, catechin, coumanin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *P. rosmarinifolia* whole plant.The results (Table 1, 2 &3) showed increased lipid peroxidation (LPO) in serum, liver and kidney of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats [20-21]. This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and there levels were significantly reduced after the supplementation of the ethanol extract of *P. rosmarinifolia* and glibenclamide (Table 1,2&3). This indicate that plant extract inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extract of *P. rosmarinifolia*. This should be correlated with previous study reported that Cassia auriculata flower, Syzigium cumini, Tinospora cordifolia, Scoparia dulcis and Nigella sativa [22-26] has antiperoxidative and antihyperlipidaemic effect of diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids [27].

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) (Table 1, 2&3) were significantly reduced in serum, liver and kidney of alloxan induced diabetic rats. These adverse changes were reversed to near normal values in ethanol extract of *Polygala rosmarinifolia* whole plant treated. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation of tissues[28]. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen[29], hence

diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes[30]. The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide[8]. Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase.

Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[31]. The decrease in CAT activity could result from inactivation by glycation of enzyme[32]. Reduced activity of SOD and CAT in the serum, liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides[33]. The reductions of hepatic SOD and CAT activities in alloxan induced diabetic rats when compared with normal rats were reported[2]. Whereas, the extract treated groups showed a significant increase in the hepatic SOD and CAT activities of the diabetic rats. This means that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.

GSH is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphate (GSSG) and the reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan induced rats and attainment of normally in *Polygala rosmarinifolia* whole plant extract treated rats indicate that oxidative stress elicited by alloxan was significantly reduced by this extract .

The present study reveals that the *Polygala rosmarinifolia* whole plant extract had antioxidant activity. The bioactive components, responsible for the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the whole plant extracts. In the present study, phytochemical screening reported that the presence of phenolics and flavonoids in extracts which might be the constituents responsible for the antioxidant activities. Further identification and isolation of three compounds may be fruitful.

ACKNOWLEDGEMENT

The Authors wishes to thank Dr. R. Sampatharaj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur, for their assistance in animal studies.

REFERENCES

1. Akkus I, Kalak S, Vural H et al. Leukocyte lipid peroxidation, Superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type 2 diabetes mellitus. Clin Chim Aeta 1996; 244:221-227.
2. Kaleem M, Sheema H, Sarmad H , Bano B. Protective effects of *Piper nigrum* and *Vinca rosea* in alloxan induced diabetic rats. Indian J Physiol Pharmacol 2005; 9:65-71.
3. Jones AF, Winkles JW, Jennings PE, Florkowski CM, Lunec J ,Barnett AH. Serum antioxidant activity in diabetes mellitus. Diabetes Res 1988; 7:89-92.
4. Garg MC, Ojha S, Bansal OD. Antioxidant status of Streptozotocin-diabetes rats. Indian J Exp Biol 1996; 34:264-6.
5. Andallu B, Varadacharyulu NCH. Antioxidant role of Mulberry leaves in Streptozotocin- diabetes rats. Clin Chim Acta 2003; 338:3-10.
6. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40:405-12.
7. Jacob RA. The integrated antioxidant system. Nutr Res 1995; 15:755-766.
8. Sathishsekar D, Subramanian S. Antioxidant properties of *Momordica charantia* (bitter gourd) seeds on Streptozotocin induced diabetes rats. Asian Pac J Clin Nutr 2005; 14:153-158.
9. Mc Guffin M, Hobbs C, Upton R (eds). American Herbal Products Association's Botanical Safety Handbook. Boca Raton, FL: CRC Press; 1997. p.89.
10. Teeguarden R. Radiant Health: The Ancient Wisdom of the Chinese Tonic Herbs. New York: Warner Books; 1998. p.194-95.
11. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on Merugan kizhangu. Bull Med Ethnobot Res 1981; 3: 84-96.
12. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers. Kolkata 1993.
13. OECD (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris 2002.
14. Nagappa AN, Thakurdesai PA, Venkat Rao N, Sing J. Antidiabetic activity of *Terminalia catappa* Linn. fruits. J Ethnopharmacol 2003; 88: 45-50.
15. Ohkawa H, Ohishi N, Yaki K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
16. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of Superoxide dismutase. Int Biochem Biophysics 1984; 21: 130-132.
17. Sinha AK . Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
18. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-590.
19. Sedlak J, Lindsay RH. Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellmans reagent. Anal Biochem 1968; 25: 192-205.
20. Latha M, Pari L. Prevention effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Molec cell and Biochem 2003a; 243: 23-28.
21. Ananthan R, Latha M, Ramkumar KM *et al*.,. Antidiabetic effect of *Gymnema montanum* leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. Nutri 2004; 6: 379-386.
22. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J 2002; 43: 617-621.
23. Prince PSM, Menon VP. Hypoglycemic activity of *Syzigium cumini* seeds; effect on lipid peroxidation in alloxan diabetic rats. Journal of Ethnopharmacology 1998; 61:1-7.
24. Prince PSM, Menon VP, Gunasekaran G. Hypolipidemic action on *Tinospora cordifolia* root extract in alloxan diabetic rats. Journal of Ethnopharmacology1999; 14: 4-16.
25. Latha M, Pari L. Modulatory effect of *Scoparia dulucis* in oxidative stress induced lipid peroxidation in streptozotocin diabetic rats. J Med Food 2003b; 6: 379-386.
26. Kaleem M, Kirmani D, Asif M, Ahmed Q, Bano B. Biochemical effects of *Nigella sativa* L seeds in diabetic rats. Indian J Exp Biol 2006; 44: 745-748.
27. Loci AS, Shaabha M, Khazraji AL, *et al*. Hypoglycemic effect of a valuable extract of *Artemisia herba alba* II. Effect of a valuable extract on some blood parameters in diabetic animals. Journal of Ethnopharmacology. 1994; 43: 167-171.
28. Oberly WR, Buettner RG. Role of superoxide dismutase in cancer. Cancer Res 1974; 35: 1141-1149.
29. Mc Crod JM, Keele BB, Fridovich I. An enzyme based theory of obigate anaerobiosis, the physiological functions of superoxide dismutase. Prod NaH Acad Sci USA1976; 68: 1024-1027.
30. Sozmen BY, Sozmen B, Delen Y, Onat T. Catalyse/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poorglycemic control. Ara Med Res 2001; 32: 283-287.
31. Searle AJ, Wilson RL. Glutothione peroxide: effect of superoxide, hydroxyl and bromine free radicals on enzymatic activity. Int J Radi Biol 1980; 37: 213-217.
32. Yan H, Harding JJ. Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J 1997; 328: 599-605.
33. Wohaieb SA, Godin DV. Alterations in free radical tissue defense mechanisms in streptozotocin diabetes in rats: effect of insulin treatment. Diabetes1987; 36: 1014-1018.