



INVESTIGATION OF *IN VIVO* ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *POLYGONUM GLABRUM*

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Article Received on 16/06/2017

Article Revised on 06/07/2017

Article Accepted on 26/07/2017

ABSTRACT

The aim of present study was to assess the *in vivo* antioxidant property of methanol extract of whole plant of *Polygonum glabrum* (polygonaceae). The rats were administered with methanol extract of *Polygonum glabrum* at two different doses (200mg/kg, 400mg/kg) for a period of 21 days to estimate the oxidative stress parameters including ferric reducing ability of plasma (FRAP), lipid peroxidation, reduced glutathione (GSH) and antioxidant enzymes (catalase and superoxide dismutase). FRAP analysis showed that both the doses of methanol extract possessed better reducing power in comparison to control group. The extract (400mg/kg) treatment group exhibited a significant ($p < 0.05$) increase in serum levels of catalase and superoxide dismutase when compared with other groups. Similarly, pretreatment with methanol extract of *Polygonum glabrum* at the dose level of 400 mg/kg significantly restored the levels of catalase, superoxide dismutase and reduced glutathione in tissue (liver, heart and kidneys) homogenates. In addition, the tested doses of methanol extract of *Polygonum glabrum* (200mg/kg and 400mg/kg) possessed significant ($p < 0.05$) antioxidant activity by effectively preventing lipid peroxidation levels in serum and tissues. Thus the overall efficacy indicates that methanol extract of *Polygonum glabrum* could be used as a bioactive source of natural antioxidants.

KEYWORDS: *Polygonum glabrum*, Oxidative stress, Antioxidant enzymes, Catalase, Lipid peroxidation.

INTRODUCTION

Biological oxidants such as superoxides and hydroxyl radicals are produced endogeneously by mitochondria during oxidative metabolism. Phagocytic cells also produce hydrogen peroxide during their oxidative burst.^[1] Excess production of these reactive species such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) and nitric oxide ($NO^{\cdot}$) often results in oxidative stress in the human body.^[2] Mounting evidence has indicated the key role played by reactive oxygen species (ROS) and other oxidants in triggering numerous disorders and diseases by targeting vulnerable sites in proteins and lipids.^[3] To encounter the potential damage inflicted by oxidants in the body, a network of enzymatic (superoxide dismutase (SOD), catalase, glutathione, NADH peroxidase) and non enzymatic antioxidants (glutathione, vitamin C, α -tocopherol) act in every link of the chain of oxidation to ensure safety from free radicals.^[4] Contemporary treatment with synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propylgallate may be effective but are viewed as a threat to human health owing to their undesired side effects. Quenching of reactive oxygen species by the use of medicinal plants has gained interest due to the plants

presumed safety, nutritional value and therapeutic value. Infact, most of the beneficial health effects of phytoconstituents like flavones, isoflavones, phenols, anthocyanins, terpenes etc occurring in fruits and vegetables are attributed to their antioxidant and chelating abilities.^[5] Several plants such as *Ocimum gratissimum*, *Terminalia chebula* and *Oroxylum indicum* are highly proposed by researchers as their credential in prevention of immune deficiency diseases and proliferative disorders due to oxidative stress are widely reported.^[6]

Polygonum glabrum (PG) is a semi aquatic perennial plant. Traditionally, the root stock is used for the treatment of jaundice and piles. A decoction of the leaves and seeds are used as cardiostonic, astringent and anthelmintic.^[7] Peels from stem are used for treating rheumatism. Pharmacologically, the plant has been effectively screened for several studies namely, anticancer, nephroprotective, hepatoprotective, antimicrobial and antipyretic.^[8-10] Substantial literature survey suggests that genus *Polygonum* are rich source of flavonoids. Flavonoids like avicularin, rhamnetin, diosmetin, cyanidin 3 5-diglucoside and delphinidin 3, 5, diglucoside were isolated from the methanol extract of

leaves and flowers of *Polygonum glabrum*.^[11] Other, compounds such as (2)-2-methoxy-2-butenolide-3-cinnamate, β -hydroxyfriedalanol, 3-hydroxy-5-methoxystilbene, pinocembrin, sitosterol -(6 α -O-palmitoyl) -3-O- β -Dglucopyranoside, pinocembrin-5-methyl ether and sitosterol-3-O- β -Dglucopyranoside were also isolated from *Polygonum glabrum*.^[12] Sesquiterpene diesters were isolated through reverse phase chromatography of methanol extracts of leaves of *Polygonum glabrum*.^[13] All the above works imply that methanol extract of *Polygonum glabrum* consisted of different types of phytoconstituents. The present study was aimed to investigate the antioxidant activity of methanol extract of whole plant of *Polygonum glabrum* by *in vivo* method.

MATERIALS AND METHODS

Plant collection

Polygonum glabrum was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. The plant was preserved in the herbarium of GITAM Institute of Pharmacy, GITAM University, for future reference (Voucher specimen No -1916). The whole plant was washed, air-dried, homogenized to fine powder and stored under ambient conditions.

Extraction

About 500 gm of plant powder was extracted with methanol in a Soxhlet apparatus. The extraction was done continuously till a clear solvent was observed in the thimble. The excess solvent was removed from methanol extract using a rotary vacuum evaporator and later on concentrated on a water bath. The percentage yield of the extract was calculated. Finally, dried extract was stored in desiccators for antioxidant study.

In vivo antioxidant activity

Experimental animals

Albino rats of wistar strain of either sex (200-250gm) were procured from Ghosh enterprises. The animals were maintained in standard laboratory conditions. They were fed with standard pellet diet and water ad libitum. The study protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted as per the guidelines of CPCSEA. Reg. Number: 1287/PO/Re/S/09/CPCSEA.

Experimental design

A total of eighteen rats were divided into 3 groups of 6 rats each. Group I animals were treated as normal control and received only saline (1ml/kg/day p.o) for 21 days. Group II animals were treated with methanol extract of *Polygonum glabrum* (200 mg/kg, p.o.) daily for 21 days. Group III animals were treated with methanol extract of *Polygonum glabrum* (400 mg/kg, p.o.) daily for 21 days. Twenty-four hours after the last treatment, all rats were weighed, blood was collected through direct cardiac puncture and then sacrificed.

Effect of *Polygonum glabrum* on the body weight of treated rats.

The results of effect of methanol extract of *Polygonum glabrum* on the body weight of the treated rats was presented in Table 1.

Ferric reducing ability of plasma (FRAP) assay

Total plasma antioxidant capacity was measured according to the FRAP method described by Benzie and Strain.^[14] Briefly, the working FRAP reagent was prepared by adding 1 mL of 10 mM 2,4,6 tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, 1 mL of 20 mM FeCl₂·6H₂O, 10 mL of 0.3 M/L acetate buffer (pH 3.6)] and 0.375 mL of distilled water. The blood samples were collected from the rat retro-orbital venous plexus into heparinized glass tubes at 0, 7th, 14th and 21st days of treatment. To 0.025 mL of plasma samples, 3 mL of freshly prepared FRAP reagent was added. The absorbance of developed colour in organic layer was measured at 593 nm. Ferrous sulphate (FeSO₄·7H₂O) was used as a standard for calibration. The results of effect of methanol extract of *Polygonum glabrum* on FRAP assay was presented in Figure 1.

Serum preparation

The serum was prepared using standard method as described by Yesufu et al.^[15] Following mild chloroform anaesthesia of the rats, blood was collected by direct cardiac puncture and subjected to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and used for estimation of serum catalase, superoxide dismutase and lipid peroxidation.

Tissue homogenate Preparation

The heart, kidneys and liver were isolated following the sacrifice of animals. The removed organs were washed with ice cold saline and weighed. A 10% homogenate of heart, liver and kidney tissue were prepared separately in phosphate buffer (pH 7.4) (100 mM KH₂PO₄ buffer having 1 mM EDTA). The tissue homogenates were centrifuged at 12,000 × g for 30 min at 4°C. The supernatant obtained was used for the estimation of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation (LPO).

Estimation of catalase in serum

The catalase activity in serum was determined using the method of Atawodi.^[16] To a test tube, add 5 μ L of serum and 1.40 ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.05ml of fresh 30mM hydrogen peroxide. The rate at which hydrogen peroxide decomposes was measured at 240nm for 5min on a spectrophotometer.

Estimation of superoxide dismutase (SOD) in serum

Superoxide dismutase activity was assayed according to the method of Marklund and Marklund.^[17] To 2.8 ml of Tris buffer (containing 50 mM of Tris buffer and 1 mM of EDTA), 0.1 ml of serum sample was added and mixed

thoroughly. The reaction was started by adding 0.1 ml of pyrogallol solution. Absorbance was read at 420 nm.

Estimation of lipid peroxidation in Serum

Thiobarbituric acid reactive substance (TBARS) is the breakdown product of lipid peroxidation. The level of TBARS and malondialdehyde (MDA) production was measured in serum by Draper and Hadley method.^[18] Briefly, 1ml of 14% trichloroacetic acid and 1ml of 0.6% thiobarbituric acid was added to 50 μ L of serum. The mixture was heated in a water bath for 30 min to deproteinize the serum. The reaction was then cooled on ice for 5min. After cooling the precipitate was removed by centrifugation at 2,000 g for 15 min and absorbance of the supernatant was measured at 535 nm.

Estimation of catalase in tissue

The principle of this assay was based on the scavenging of hydrogen peroxide radicals by catalase. The technique was carried out according to the method of Aebi.^[19] The reaction mixture contained 0.1ml of supernatant and 1.8ml of 50mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0ml of 30mM of hydrogen peroxide. The rate of the decrease in absorbance of hydrogen peroxide was measured spectrophotometrically at 240 nm. Activity of catalase was expressed as Umg^{-1} of protein.

Estimation of superoxide dismutase in tissue

This was analyzed by the method described by Rai et al.^[20] To 0.1ml of supernatant, 1.2ml of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1ml of phenazine methosulfate (186 mM), 0.3ml of nitroblue tetrazolium (300 mM), and 0.2ml of NADH (750 mM) were added. Reaction was initiated by adding NADH. After incubation at 30°C for 90s, the reaction was stopped by the addition of 0.1ml of glacial acetic acid. 4.0mL of n-butanol was added to the reaction mixture. Absorbance was measured spectrophotometrically at 560nm and the concentration of SOD was expressed as Ug^{-1} of protein.

Estimation of lipid peroxidation in tissue

Lipid peroxidation can be monitored and detected by thiobarbituric acid reactive substances (TBARS) assay.^[21] To 0.25 ml of 10% homogenate, 0.25 ml of trichloroacetic acid 20% (m/V) and 0.25 ml of thiobarbituric acid (0.67%) were added and vortexed. It was incubated for 15 min in a boiling water bath at 100°C. Later, 2 ml of n-butanol was added and the solution was centrifuged at 3000 rpm for 15 min. Further it was cooled and absorbance was recorded for the supernatant solution at 530 nm using a UV-Visible spectrophotometer.

Estimation of reduced glutathione in tissue

Reduced glutathione (GSH) was measured according to the method of Ellman.^[22] To 0.01 ml of supernatant, 2ml of phosphate buffer (pH 8.4), 0.5 ml of Ellman reagent [5''5-dithio, bis (2-nitrobenzoic acid)] and 0.4ml double

distilled water were added. Mixture was stirred vigorously and the absorbance was read at 412nm. The concentration of reduced glutathione was expressed as $\mu\text{g}/\text{mg}$ of protein.

Estimation of protein

The total protein content of the serum was assayed by using Folin -lowry method.^[23] To 0.2ml of sample solution, 4.5 ml of reagent I (48 ml of 2% sodium carbonate in 0.1 N NaOH, 1ml of 1% sodium potassium tartarate and 1ml of 0.5% copper sulphate.5 H_2O) were mixed. After 10 minutes of incubation at room temperature, 0.5 ml of reagent II (Folins reagent- a mixture of sodium tungstate, sodium molybdate, 85% phosphoric acid and 10 ml of concentrated hydrochloric acid.) were added and vortexed. Bovine serum albumin (BSA) served as standard protein. After 30 min of incubation, the absorbance was measured at 660 nm.

RESULTS

Percentage yield

The percentage yield of methanol extract of *Polygonum glabrum* was found to be 7.1%w/w.

Effect of *Polygonum glabrum* on the body weight of treated rats

Table 1 illustrates the effect of methanol extract of *Polygonum glabrum* on body weight of treated rats. The body weight of control rats increased during the duration of treatment. Methanol extract of *Polygonum glabrum* at a dose of 200mg/kg produced a slight increase in body weight of treated rats. Similarly, administration of methanol extract of *Polygonum glabrum* with a dose of 400mg/kg for a period of 21 days created a minor increase in the body weight of the rats.

Ferric reducing ability of plasma (FRAP) assay

The total antioxidant capacity after oral administration of methanol extract of *Polygonum glabrum* over a period of 21 days was shown in Figure1. In control group (group-I), there was no significant change in FRAP value on days 7 (1014 nM Fe^{2+}/L), day 14 (1026 nM Fe^{2+}/L) and day 21 (1032 nM Fe^{2+}/L) when compared to day zero (998 nM Fe^{2+}/L). In group II and group III, there was a mild increase $p < (0.05)$ in FRAP value on day 7 (1214 nM Fe^{2+}/L and 1386 nM Fe^{2+}/L respectively) when compared to control group. However, group II and group III exhibited a significant change $p < 0.001$ in FRAP values on day 14 (1389 nM Fe^{2+}/L , 1484 nM Fe^{2+}/L) and day 21 (1406 nM Fe^{2+}/L , 1524 nM Fe^{2+}/L) as compared to control group.

In vivo antioxidant effects of *Polygonum glabrum* in rat serum

The in vivo antioxidant effect of methanol extract of *Polygonum glabrum* on rat serum was given in Table 2. The rats treated with 200mg/kg of methanol extract of *Polygonum glabrum* exhibited higher levels of catalase (16.51 ± 18.90 unit/mg protein) in serum. However, the serum catalase level of rats administered with 400mg/kg

of methanol extract of *Polygonum glabrum* was found to be significantly higher (17.72 ± 11.29 unit/mg protein) when compared to low dose group (Group II) and control group. Thus the extract produced a dose dependent increase in the catalase levels in serum. Additionally, the methanol extract produced a dose dependent increase in the serum level of superoxide dismutase enzyme. The group treated with 400mg/kg of methanol extract of *Polygonum glabrum* showed significantly higher level (12.33 ± 0.68 unit/mg protein) of SOD in serum when compared to rats treated with 200mg/kg of methanol extract of *Polygonum glabrum* (11.00 ± 0.54 unit/mg protein) and control group. A decrease in serum lipid peroxide level (2.466 ± 0.02 nmol/mg protein) was observed in rats treated with a dose of 200mg/kg of methanol extract of *Polygonum glabrum* when compared to control animals. Efficient control of lipid peroxidation and lowered serum malondialdehyde level (2.219 ± 0.03 nmol/mg protein) was exhibited by methanol extract of *Polygonum glabrum* at a dose of 400mg/kg when compared to group I and group II animals.

In vivo antioxidant effects of *Polygonum glabrum* in rat tissue

The *in vivo* antioxidant effect of methanol extract of *Polygonum glabrum* on rat tissue homogenate (liver, heart and kidney) are given in Table 3, 4 and 5. Treatment with methanol extract of *Polygonum glabrum* augments the activity of antioxidant enzymes and may help to control free radicals. In rats treated with

400mg/kg of methanol extract of *Polygonum glabrum*, the level of catalase (194.7 ± 0.50 , 128.7 ± 0.5 , 154.7 ± 0.5) and superoxide dismutase (12.9 ± 1.18 , 3.5 ± 1.1 , 7.1 ± 1.1) in different tissues (liver, heart and kidney) were found to be elevated when compared to levels found in group I and group II. Further, in rats treated with 200mg/kg of methanol extract of *Polygonum glabrum*, the level of catalase (192.5 ± 0.37 , 128.5 ± 0.3 , 152.5 ± 0.3) and superoxide dismutase (10.56 ± 1.05 , 3.5 ± 1.05 , 6.5 ± 1.05) in different tissues (liver, heart and kidney) were found to be lesser than the rats treated in group III and nearer to the levels found in control animals. Hence, it is obvious that the plant extract produced a dose dependent increase in antioxidant activity. Similarly, the concentration of glutathione in tissues of liver, heart and kidneys (39.2 ± 2.7 , 27.2 ± 2.7 , 32.2 ± 2.7) of rats treated with 200mg/kg of methanol extract of *Polygonum glabrum* were found to be nearer to normal levels seen in control group animals. With an increase in dose (400mg/kg) of methanol extract of *Polygonum glabrum*, the glutathione concentration in liver, heart and kidney (40.9 ± 1.2 , 27.9 ± 5.2 , 32.9 ± 1.3) increased in group III rats when compared to group II and group I. By administration of 400mg/kg of methanol extract of *Polygonum glabrum* a significant decreased levels of lipid peroxidation in liver, heart and kidney (2.1 ± 0.4 , 2.2 ± 0.5 , 4.1 ± 0.4) were observed when compared with rats administered with 200mg/kg of methanol extract of *Polygonum glabrum* and control group animals.

Table 1: Effects of methanol extract of *Polygonum glabrum* on body weight of treated rats

Groups	Day 0	Day 7	Day 14	Day 21
Group I (control)	222.80 ± 10.82	220.00 ± 1.38	224.50 ± 0.08	224.46 ± 3.48
Group II <i>Polygonum glabrum</i> 200mg/kg	228.51 ± 19.90	$228.30 \pm 0.54^*$	$230.26 \pm 0.02^*$	$231.16 \pm 0.05^*$
Group III <i>Polygonum glabrum</i> 400mg/kg	225.72 ± 11.29	$226.76 \pm 0.68^*$	$229.69 \pm 0.03^{**}$	$229.61 \pm 1.03^{**}$

Data are given as mean SD of six animals. * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) when normal compared with PG-group II and PG-group III extracts.

Table 2: In vivo antioxidant effects of methanol extract of *Polygonum glabrum* in rat serum

Groups	Catalase (unit/mg protein)	SOD (unit/mg protein)	LPO (nmol/mg protein)
Group I (control)	14.80 ± 10.82	10.00 ± 0.38	2.501 ± 0.08
Group II <i>Polygonum glabrum</i> 200mg/kg	$16.51 \pm 18.90^{**}$	$11.00 \pm 0.54^{**}$	$2.466 \pm 0.02^*$
Group III <i>Polygonum glabrum</i> 400mg/kg	$17.72 \pm 11.29^{***}$	$12.33 \pm 0.68^{***}$	$2.219 \pm 0.03^{**}$

Data are given as mean SD of six animals. * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) when normal compared with PG-group II and PG-group III extracts.

Table 3: In vivo antioxidant effects of methanol extract of *Polygonum glabrum* in rat liver

Groups	CAT (U/mg of protein)	SOD (U/g of protein)	GSH (µg/mg of protein)	LPO (nmole of MDA/ mg of protein)
Group-I(Control)	188.6±0.06	9.1± 0.63	39.1±07	4.9±03
Group-II <i>Polygonum glabrum</i> 200mg/kg	192.5±0.37 **	10.56±1.05**	39.2±2.7*	3.5±1.05*
Group-III <i>Polygonum glabrum</i> 400mg/kg	194.7±0.50***	12.9±1.18***	40.9±1.2**	2.1±04***

Data are given as mean SD of six animals. * ($P<0.05$), ** ($P<0.01$), *** ($P<0.001$) when normal compared with PG - group II and PG- group III.extracts.

Table 4: In vivo antioxidant effects of methanol extract of *Polygonum glabrum* in rat heart

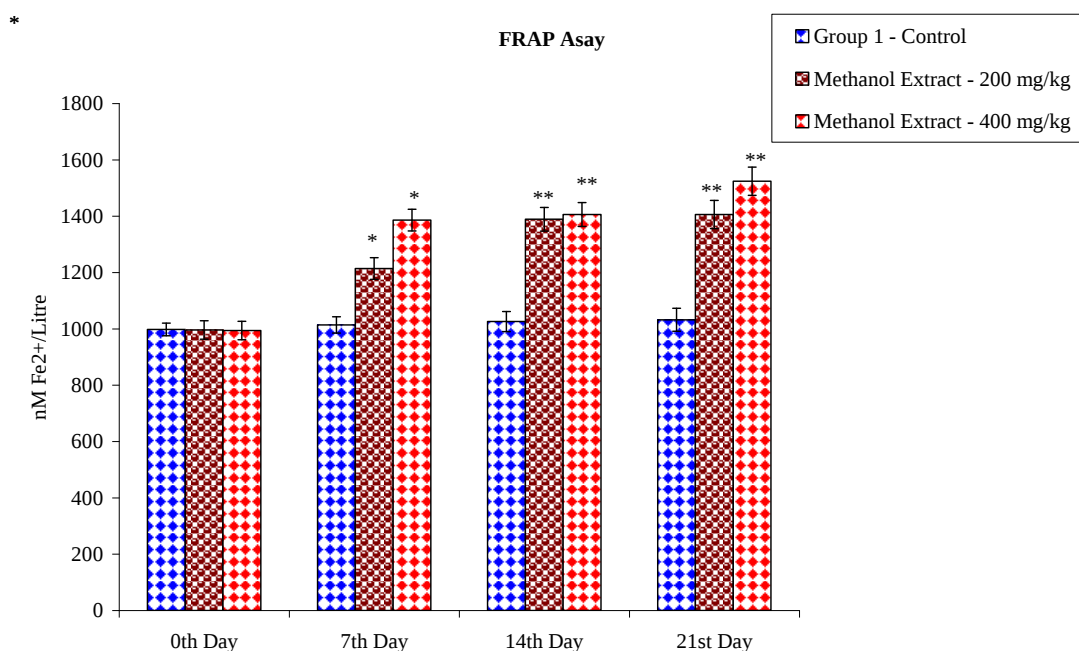
Groups	CAT(U/mg of protein)	SOD (U/g of protein)	GSH (µg/mg of protein)	LPO (nmol of MDA/ mg of protein)
Group-I(Control)	128.6±0.06	3.4±6.5	27.1±07	4.5±03
Group-II <i>Polygonum glabrum</i> 200mg/kg	128.5±0.3 *	3.5±1.05*	27.2±2.7*	3.8±1.05*
Group-III <i>Polygonum glabrum</i> 400mg/kg	128.7±0.5 **	3.5±1.1**	27.9±5.2**	2.2±05**

Data are given as mean SD of six animals. * ($P<0.05$), ** ($P<0.01$), *** ($P<0.001$) when normal compared with PG - group II and PG -group III.extracts.

Table 5: In vivo antioxidant effects of methanol extract of *Polygonum glabrum* in rat kidneys

Groups	CAT(U/mg of protein)	SOD (U/g of protein)	GSH (µg/mg of protein)	LPO (nmol of MDA/ mg of protein)
Group-I(Control)	148.6±0.06	5.4±6.5	33.1±07	5.4±03
Group-II <i>Polygonum glabrum</i> 200mg/kg	152.5±0.3 **	6.5±1.05**	32.2±2.7*	4.8±1.05*
Group-III <i>Polygonum glabrum</i> 400mg/kg	154.7±0.5 ***	7.1±1.1***	32.9±1.3**	4.1±04**

Data are given as mean SD of six animals. * ($P<0.05$), ** ($P<0.01$), *** ($P<0.001$) when normal compared with PG- group II and PG- group III extracts.

**Figure-1 Effect of methanol extract of *Polygonum glabrum* on FRAP assay**

DISCUSSION

Mammalian cells are comprised of both enzymatic and non enzymatic antioxidant defense system to minimize the pathogenesis arising from the interaction between cellular components and reactive oxygen species. Despite the presence of these, an unbalanced production of ROS leads to a number of clinical disorders. This clearly emphasizes the importance of antioxidant system in maintaining the normal physiology.^[24] Three distinct isomers of superoxide dismutase are present in the mammalian cells. The copper-zn SOD is present in the cytosol, the manganese-SOD is expressed in mitochondria of the cell and extracellular SOD is present in fluids such as plasma, lymph and synovial fluid. Similarly reduced glutathione is believed to play an important role in the antioxidant defense. Lipid peroxidation is a free radical related process associated with cellular damage due to oxidative stress. As a result of this a wide variety of aldehydes are formed out of which malondialdehyde and 4-hydroxyl nonenal are important. In this study, we attempted to demonstrate the *in vivo* antioxidant role of methanol extract of *Polygonum glabrum*.

In the study, total antioxidant capacity of plasma was measured by FRAP method. The assay is based on the reduction of a colourless ferric-tripyridyltriazine (Fe^{III} -TPTZ) to blue coloured ferrous-tripyridyltriazine (Fe^{II} -TPTZ). The values expressed from the FRAP assay is directly proportional to the concentration of electron-donating antioxidants/reductants in the extract.^[25] Maximum reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was obtained in group III, which corresponds to animals administered with 400 mg/kg body weight of methanol extract of *Polygonum glabrum*. The elevation of plasma FRAP values after pre treatment with methanol extract of *Polygonum glabrum* may be associated with the decreased lipid peroxidation by plant antioxidants.

Catalase is a peroxisomal enzyme present chiefly in liver, kidney and erythrocytes. In organs like heart and brain, catalase is present in a lesser quantity.^[26] It is one of the antioxidant defense enzymes which plays a indispensable role in the oxidation of hydrogen peroxide to oxygen and water. Hydrogen peroxide naturally gets decomposed to oxygen and water. Nevertheless, in presence of certain metal ions it may react to form cell sensitive hydroxyl radicals which are toxic to biological system. Administration of 400mg/kg of methanol extract of *Polygonum glabrum* to rats maintained the catalase enzyme levels to normalcy. An increase in the concentration of methanol extract could scavenge hydrogen peroxide free radicals through which the level of catalase in serum and tissue were maintained. Ozyurek et al reported that phenolic compounds are better scavengers of hydrogen peroxide since they can donate electrons to H_2O_2 , thus neutralizing it to water.^[27] Since different phenol compounds were reported in whole plant methanol extract of *Polygonum glabrum*, these

compounds may be capable of ameliorating the effect of free radicals in biological system thereby balancing the endogenous antioxidant enzyme level in serum and blood.^[28]

Superoxide dismutase is another antioxidant defense enzyme present exclusively in the mitochondrial matrix and its prime function is to assist in dismutation of superoxide radicals.^[29] It scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen. In present study, rats treated with 400mg/kg of *Polygonum glabrum* methanol extract showed significant increase in the level of SOD enzyme than rats treated with 200mg/kg of *Polygonum glabrum* methanol extract. Since flavonoids like delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside and quercetin were reported to be present in methanol extract of *Polygonum glabrum* they might inactivate the super oxide free radicals there by maintaining the levels of superoxide dismutase in serum and tissue. Hence, pretreatment of rats with *Polygonum glabrum* could significantly protect the level of SOD in serum and tissues in a dose dependent manner.

The reduced glutathione antioxidant system plays a crucial role in cellular defense against oxidative stress by upholding a cascade of reactions. In general the reduction in non enzymatic GSH and enzymatic glutathione peroxidase and glutathione reductase in rats might be due to the influence of free radicals on antioxidant system.^[30] Glutathione is found in liver at high concentration and the level of GSH determines the susceptibility of tissues to oxidative damage. In present study, reduced glutathione levels were significantly elevated by administration of 400mg/kg of *Polygonum glabrum* methanol extract when compared to 200mg/kg of methanol extract of *Polygonum glabrum*. It was understood that increased levels of GSH in liver, heart and kidney may be because of presence of flavonoids like pinocembrin and pinocembrin-5-methyl ether in methanol extract of *Polygonum glabrum* which indirectly helped in maintaining intracellular glutathione levels in tissues.

The level of lipid peroxide in tissue is an indication of cellular membrane damage and any changes in structure and function of macro molecules. Thus an elevation in LPO levels in liver, heart and kidney suggested enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms. Hydroxyl radical attacks the unsaturated fatty acids of tissue causing lipid peroxidation.^[31] This result in generation of carbonyl fragments called MDA (malondialdehyde) which react with thiobarbituric acid to form a pink coloured compound which is absorbed at 532 nm. The methanol extract of *Polygonum glabrum* exhibited a steady scavenging impact of hydroxyl group at different concentrations (200mg/kg and 400mg/kg). The marked inhibition of lipid peroxidation by the methanol extract

may be due to the presence of reductants like phenols and steroids in the extract.

CONCLUSION

According to the results obtained from the present study, methanol extract of *Polygonum glabrum* was found to possess effective *in vivo* antioxidant activity. It offers a promising therapeutic value in prevention of oxidative stress by enhancement of antioxidant defense enzymes in serum and in all the tissues selected. The methanol extract was also efficient in decreasing lipid peroxidation in tissues of liver, heart and kidney. The antioxidant effects could be attributed to different phytoconstituents present in the extract. In future, further studies on active ingredient in the extracts responsible for antioxidant effect should also be assessed.

ACKNOWLEDGEMENT

The authors are thankful to the management of GITAM University, Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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