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Efficacy of astragalin on lead acetate induced nephrotoxicity in male albino rats

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Abstract

The nephroprotective efficacy of astragalin (kaempferol-3-O-glucoside) isolated from flowers of *Pongamia pinnata* against lead acetate induced nephrotoxicity was studied in male albino rats. Administration of 160 mg/kg body weight/day of lead acetate for 90 days to male albino rats, resulted a significant elevation in the level of serum urea, creatinine and uric acid, and decreased levels of kidney superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), reduced glutathione (GSH), vitamin C and vitamin E also in the kidney of treated rats when compared to control. Astragalin at a dose of 20 mg/kg / b.w when combinely administered with 160 mg /kg /b.w of lead acetate, significantly brought the levels of serum urea, uric acid, creatinine, and activities of SOD, CAT, GPx, GR, GST, GSH, vitamin C and vitamin E in the kidney comparably similar to that of untreated (control) rats. The present results indicated that astragalin treatment might protect the kidney tissues against free radical – mediated oxidative stress induced by lead acetate.

Keywords: antioxidants, astragalin, kidney, lead acetate, *Pongamia pinnata*

INTRODUCTION

Pongamia pinnata (L) Pierre (Leguminosae, Papilionoidae, Syn. *P. glabra* Vent), commonly called *Karanj* in Hindi, is a medium sized glabrous tree. Different parts of the plant have been recommended in Ayurvedic literature as a remedy for various ailments. *P. pinnata* roots have been described as a useful remedy for foul ulcers, fistulous sores, gonorrhoea, urethritis etc., (Kirtikar and Basu, 1933) . The seeds and seed oil have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago and rheumatism (Nadkarni, 1989). The ulcer protective and healing effects and anti-inflammatory activity alcoholic extract of *P. pinnata* seeds (Singh *et al.,* 1997; Srinivasan *et al.,* 2001 and Prabha *et al.,* 2003) and the anti-oxidative and cognition enhancing properties of *P. pinnata* (Raghavendra *et al.,* 2007) have also been well documented. Flowers of the plant are rich in bioflavonoids and are extensively used to treat various skin diseases, diabetes and renal disorders(Vedavati *et al.,* 1997) .

Lead is an ubiquitous environmental contaminant due to its significant role in modern industry (Shalan *et al.,* 2005). As a result, both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yucebiligic *et al.,* 2003). It has many undesired effects, including neurological (Moreira *et al.,* 2001, Soltaninejad *et al.,* 2003) behavioural (Moreira *et al.,*2001; De Marco *et al.,* 2005) immunological (Razani-Boroujerdi *et al.,* 1999; Ercal *et al.,* 2000 and Bunn *et al.,* 2001), renal (Loghman, 1997; Patra, 2001 and Vargas *et al.,* 2003) hepatic (Vargas *et al.,* 2003), and especially haematological dysfunctions (Mousa *et al.,* 2002 and Sivaprasad *et al.* 2003). Lead toxicity is closely related to its accumulation in certain tissues and its interference with the bioelements, whose role is critical for several physiological processes. Therefore, in the present study an attempt was made to evaluate the chronic effects of exogenous administration of lead acetate and the protective effecacy of astragalin isolated from the plant *Pongamia pinnata* on various biochemical parameters. The aim of this study was to investigate nephroprotective and antioxidant properties of astragalin. The effect of the astragalin in lead acetate induced toxicity was evaluated by determining the levels of urea, uric acid and creatinine from the serum in different experimental groups of male albino rats. The activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and reduced glutathione (GSH), vitamin C and vitamin E were measured in kidney homogenates. Further, the level of lipid peroxidation were also estimated from renal samples and the efficacy of astralagin was estimated.

MATERIALS AND METHODS

The fresh flowers of *Pongamia pinnata* was collected from the local gardens of STET Women's College, Mannargudi, Tamilnadu, India and voucher specimens

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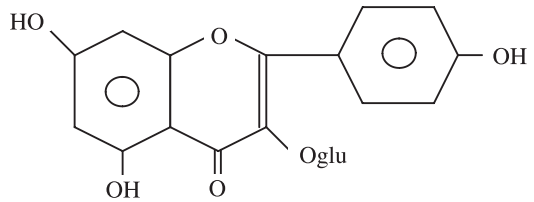
are deposited in the STET Herbarium at the Department of Botany and Microbiology, S.T.E.T. Women's College, Mannargudi, Tamil Nadu, India.

The flowers were extracted with 85% EtOH (4 x 500 ml) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was successively fractionated with benezene (3 x 250 ml), peroxide – free Et_2O (3 x 250 ml) and EtOAc (4x 250 ml).

By ^{1}H and ^{13}C – NMR, the identity of the pigments obtained from EtOAc fraction was found to be astragalin.



Kaempferol-3-0-glucoside (astragalin)

Male albino rats (body weight 140-200 g) bred in the Central Animal House, Rajah Muthaih Medical College, Annamalai University, were used for the study. The animals were fed with pelleted feed purchased from the market and water *ad libitum* in normal laboratory conditions (26 ± 1^{0}C).

All the animal used for the experimental purposes were approved by the Institutional Animal Ethics Committee, Annamalai University (Reg.No. 160/1999 / CPCESA) and animals were cared for in accordance with the principles and guidelines of Indian National Law on Animal care and use.

The animals were divided into four groups of 6 rats each. Groups 1 received only distilled water. Nephrotoxicity was induced in rats of groups 2, 3 and 4 by oral administering lead acetate at a dose of 160 mg/kg body weight / day (Banu *et al.*, 2006) as a freshly prepared solution daily for 90 days. Group 2 animals continued receive standard pellet diet and lead acetate daily. Group 3 animals continued to receive standard pellet diet, lead acetate and carvedilol (5 mg/kg body weight / day) (Ayman *et al.*, 2006) every day. Group 4 animals continued to receive standard pellet diet, lead acetate and Astragalin at a dose of 20 mg/kg body weight / day. The total experimental duration was 90 days.

At end of the experimental period, the animals were fasted overnight, anaesthetized with an intramuscular injection of ketamine hydrochloride (30 mg/kg body weight), and sacrificed by cervical dislocation. The blood samples were collected by retro-orbital puncture, in test tubes, left for 20 minutes and then centrifuged for the separation of serum. Kidney tissues were immediately processed and used for various biochemical estimations.

Assay of biochemical parameters

Level of serum urea was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960), serum creatinine by the method of Tietz (1987), using colour reaction (Jaffe's, 1886), serum uric acid by the enzymic method described by Caraway (1955), lipid peroxidation in tissues by the method of Ohkawa *et al.* (1979), CD in the tissues by the method of Recknagel and Glende (1984), Tissue LOOH by the method of Jiang *et al.*, (1992). SOD was assayed by the method of Kakkar *et al.*, (1984) and the activity of CAT by the method of Sinha (1972) and the activity of GPx by the method of Rotruck *et al.*,(1973), Glutathione reductase was assayed according to the method of Carlberg and Mannervik (1975). Glutathione S-transferase (EC 2.5.1.18; GST) activity was assayed according to the method of Habig *et al.*, (1974) and GSH by the method of Boyne and Ellman (1972). Vitamin C was measured according to the method of Omaye *et al.*, (1979), and Vitamin E in tissues by the method of Desai (1971).

Statistical analysis

Data were analyzed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using the statistics software package SPSS for Windows, v. 13.0; Chicago, IL, USA.

RESULTS

The level of serum urea, creatinine and uric acid were found to be significantly higher ($p<0.05$) in lead acetate treated rats than control (Table 1). But the levels of these renal function markers in the lead acetate and carvedilol combinely treated animals did not differ significantly ($p<0.05$) than that of the control. In lead acetate and astragalin administered rats the results similar to that of lead acetate and carvedilol treated rats were obtained (Table 1).

TBARS, LOOH and CD levels in the kidney of rats treated with lead acetate (group 2) were significantly higher compared to those of the control rats (group 1) (Table 2; $p > 0.05$). Carvedilol and lead acetate combinely treated rats (group 3) had significantly lowered levels of TBARS, LOOH and CD when compared with the lead acetate alone treated rats (group 2). Treatment of astragalin along with lead acetate (group 4) also resulted in similar levels of TBARS, LOOH and CD (Table 2).

SOD,CAT, GSH and glutathione related enzymes such as GPx, GR, GST and Vitamin C and E activities in the kidney tissue of rats treated with lead acetate were significantly ($p<0.05$) lesser than control rats (Table 3). The activities of these enzymes were brought to near normal levels in the kidney of animals when carvedilol/

Table 1. Levels of serum urea, creatinine and uric acid in the control and treated rats.

Experimental Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group I (Control)	15 23±1 46[a]	0.55 ± 0.05[a]	0.80 ± 0.07[a]
Group II (Lead acetate)	24.97 ± 2.40[b]	1.16 ± 0.11[b]	1.59 ± 0.15[b]
Group III (Lead acetate + Carvedilol)	16.52± 1.59[a]	0.52± 0.05[a]	0.85 ± 0.08[a]
Group IV (Lead acetate + Astragalin)	16 64 ± 1.60[a]	0.61 ± 0.05[a]	0.97 ± 0.09[a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at P < 0.05 (DMRT) for the respective parameter.

Table 2: Levels of lipid peroxidative markers of kidney in the control and treated rats.

Experimental Groups	TBARS (mmol /mg tissue)	LOOH (mmol/mg tissue)	CD (mmol/mg tissue)
Group I (Control)	0.52 ± 0.05[a]	53.67 ± 5.16[a]	11.07 ± 1.06[a]
Group II (Lead acetate)	2.02 ± 0.19[b]	153.42 ± 14.76[b]	22.27 ± 2.14[b]
Group III (Lead acetate + Carvedilol)	0.5 ± 0.04[a]	72.09 ± 6.94[a]	13.81 ± 1.32[a]
Group IV (Lead acetate + Astragalin)	0.59 ± 0.05[a]	72.15 ± 6.94[a]	14.01 ± 1.34[a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at P < 0.05 (DMRT) for the respective parameter.

astragalin was supplemented with lead acetate when compared to the control rats (p<0.05). The result of the present study indicated that the levels antioxidant enzymes got increased when the animals were administrated .with astragalin, and carvedilol along with lead acetate with significant elevations in the activities of SOD,CAT, GSH and GPx, GR, GST, Vitamin Cand E in the kidney tissue when compared to those of the unsupplemented lead acetate treated rats.

DISCUSSION

Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites and are especially vulnerable to damage. Markers such as urea,uric acid and creatinine are used to determine the kidney function. Urea is the nitrogen containing metabolic product of protein metabolism, uric acid is the major product of purine nucleotides and creatinine is endogenously produced and released into body fluids and its clearance is measured as an indicator of glomerular filtration rate (Burtis and Ashwood, 1996; Perone *et al.,* 1992). The present study showed that levels of renal function markers were much lower in case of lead acetate treated rats supplemented with astragalin than those given lead acetate alone. It might be due to the protective effect of astralagin against lead acetate induced renal toxicity.

Lead acetate administered rats showed an increased levels of lipid peroxidation markers such as TBARS, LOOH and CD in the kidney. The increased peroxidation might be due to functional changes in the kidney. Winrow *et al*., (1993) reported that accumulation of lipid peroxidation products in cells can result in cellular dehydration, whole cell deformity and cell death. Oxidation of polyunsaturated fattyacids (lipid peroxidation) of membrane is a common process in living organisms, since they are the target of oxygen derived free radicals produced during mitochondrial electron transport (Porter *et al.,* 1995).

Free radical mediated oxidative damage can contribute to acute hepatitis (Disilvestro *et al.,* 2002). High levels and/or inadequate removal of reactive oxygen species may cause severe metabolic imbalance and oxidative damage to biological macromolecules (Limaye *et al.,* 2003). To prevent oxidative damage in the cell, a variety of antioxidants scavenge free radicals. The primary defense against oxidative stress in the tissue rests with antioxidants. Therefore, these antioxidants are expected to be consumed by enhanced radical reactions (Sun *et al.,* 2003). The decreased activities of the antioxidant enzymes such as SOD, CAT, GPx in lead acetate administered rats is in accordance with earlier reports (Sreepriya and Devaki, 2001;Vimal and Devaki, 2004). The decreased activity of these enzymatic antioxidants may be due to the accumulation of H_2O_2 which in turn causes the inhibition of these enzymes (Rister and Banchner, 1976), and these enzymes were normalized upon treatment with astragalin. SOD which converts superoxide radicals to H_2O_2 is widely distributed in cells having oxidative metabolism and is believed to protect such cells against the toxic effects of superoxide anion (Fridovich, 1975). Superoxide anions are known to exert destructive effects on cellular components with lipid peroxidation being one such consequence. CAT is a heme protein, which catalyses the direct degradation of hydrogen peroxide to water. It protects the cellular constituents against oxidative damage. GPx catalyses the reduction of hydrogen peroxide and hydroperoxide to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membrane. GPx and the cellular NADPH-generating mechanisms together form a system for removing hydroperoxides from the cell (Halliwell, 1977). The decreased activity of GPx in lead acetate intoxicated group might be due to the decreased availability of its substrate GSH. Oral treatment with astragalin improved the GPx levels significantly to near normal.

GR helps to restore the levels of GSH by reducing the oxidized product of glutathione. The activity of GR was also lower upon lead acetate administration as compared to control rats which in turn may inactivate many enzymes containing 'SH' groups and inhibit protein synthesis (Hayes and Pulford, 1995).

The increase in tissue GSH content in astragalin treated group shows that the astragalin tends to prevent the tissue depletion of GSH. GSH are important endogenous

Table 3. Levels of the activities of SOD, CAT, GPx, GR,GST,GSH, vitamin-C and vitamin-E of control and treated rats.

Experimental Groups	SOD	CAT	GPx	GR	GST	GSH	Vitamin C	Vitamin E
Group I (Control)	1.77 ± 0.17[a]	29.19 ± 2.81[a]	7.58 ± 0.73[a]	20.31 ± 1.95[a]	5.26 ± 0.50[a]	10.77 ± 1.03[a]	0.42 ± 0.04[a]	2.40 ± 0.23[a]
Group II (Lead acetate)	0.97 ± 0.09[b]	21.13 ± 2.03[b]	5.26 ± 0.50[b]	13.19 ± 1.27[b]	2.57 ± 0.24[b]	8.09 ± 0.78[b]	0.14 ± 0.01[b]	0.89 ± 0.08[b]
Group III (Lead acetate + Carvedilol)	1.71 ± 0.16[a]	25.94 ± 2.49[a]	7.40 ± 0.71[a]	19.09 ± 1.83[a]	5.14 ± 0.49[a]	10.46 ± 1.00[a]	0.36 ± 0.03[a]	2.38 ± 0.23[a]
Group IV (Lead acetate + Astragalin)	1.46 ± 0.14[a]	25.15 ± 2.42[a]	7.09 ± 0.68[a]	18.32 ± 1.76[a]	4.95± 0.47[a]	10.28 ± 0.99[a]	0.31 ± 0.03[a]	2.16 ± 0.20[a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT) for the respective parameter.
SOD: Enzyme required for 50% inhibition of NBT reduction/min/mg Hb; **CAT:** µM of H_2O_2 utilized/min/mg Hb; **GSH:** m-g/dL; **GPx:** µmoles of GSH utilized/min/mg Hb; **GR**: µmoles of NADPH oxidized/min/mg Hb; **GST:** µmoles of CDNB-GSH conjugate formed/min/mg Hb; **Vit-C & Vit-E** : mg/dL

antioxidant system found in particularly high concentration in liver and is known to have key function in protective processes (James and Hrabison, 1982). The protective role of GSH against cellular lipid peroxidation has been well documented (Burk, 1983). Moreover, in addition to being a direct free radical scavenger, GSH is known to function as a substrate for GPx and GST. The activities of GPx and GST in this study were lowered on lead acetate treatment which may be attributed to the unavailability of GSH. Administration of astragalin to lead acetate treated rats increased the levels of GSH and the activities of GPx and GST. This may be due to their increased utilization to scavenge the significantly elevated levels of Reactive Oxygen Species (ROS) that are formed on lead acetate treatment.

Apart from enzymatic antioxidants, the non enzymatic antioxidant defence system protects the aerobic organisms from deleterious effects of the active oxygen metabolites. Vitamin C is important in cellular system in curtailing Reactive Oxygen Species (ROS). Ascorbic acid is reported to be associated with better scavenging activities *in vivo* than the antioxidant enzymes, because they are present both intracellularly as well as in the extra cellular fluid (Chatterjee and Nandi, 1991). As an antioxidant it is reported that ascorbate reacts with superoxide, hydrogen peroxide or the tocophroxyl radical to form monodehydro ascorbicacid and/ or didehydro ascorbic acid. The oxidized forms are recycled back to ascorbic acid. Kidney tissue levels of vitamin C and Vitamin E in our study were significantly reduced in lead acetate intoxicated rats (group 2) than in the experimental contro rats (group 1). Supplementation with astragalin to lead acetate intoxicated rats resulted in near normal levels of vitamin C and vitamin E.

In this study, animals treated with astragalin isolated from *Pongamia Pinnata* retained the activity of the antioxidant enzymes to near normal levels. Also, the potency of astragalin with respect to renal markers (urea, creatinine and uricacid) seems to be good. The above findings showed that astragalin was effective in amelioration of antioxidant levels in lead acetate induced toxicity. Further, studies are needed to examine the mechanism of action of astragalin in the lead acetate induced toxicity.

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