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Evaluation of tissue level antioxidant activity of *Premna serratifolia* leaf in paracetamol intoxicated wistar albino rats

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Abstract

Antioxidant activity of methanolic extract of *Premna serratifolia* Leaf was determined in Paracetamol intoxicated Wistar albino rats. The experiment was comprised of five groups as Healthy control group, Disease control (paracetamol treated), Positive control group (paracetamol+ silymarin), Treatment groups (test drug + paracetamol) lower dose (100mg/kg b.wt.) and higher dose (200mg/kg b.wt.), having six animals in each group. The animals were maintained in the standard laboratory conditions and the test extract was administered p.o. The antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase were evaluated in the blood samples and as well as in the isolated tissue samples of liver, kidney and heart. The disease control group showed decreased status of antioxidant enzymes in blood and tissue samples. But the elevated levels of SOD, catalase, glutathione were observed significantly (*$p<0.05$, *$p<0.01$) in liver, kidney and heart tissue samples and blood samples of the extract administered groups. The activity was found to be dose dependent. The overall efficacy of the extract is comparable with the standard drug silymarin. The study revealed that the methanolic extract of *Premna serratifolia* was found to have potential antioxidant activity in the animal model system. The further research on characterization of functional molecules has to be initiated.

Key words: *Premna serratifolia*, Paracetamol, Antioxidant enzymes, SOD, catalase

Introduction

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Boyd *et al.*, 1996). Paracetamol toxicity is due to the formation of toxic metabolites when a part of its metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity.

Indian medicinal plants and many herbal formulations belonging to the traditional systems of medicine like Ayurveda, have been investigated on various aspects of disease curing drugs. Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs which are without the side effects, especially those belonging to the traditional systems of medicine (Jose and Kuttan, 2000). Research emphasis has been directed towards herbal drugs either in single or in combination having specific diagnostic and therapeutic principles. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (Kunchandy and Rado, 1990). Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the human diet (Nadeem *et al.*, 1997).

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Premna serratifolia is a large shrub or a small tree upto 9 m in height with yellowish lenticellate bark, spinous large branches and yellowish brown woody aromatic root, leaves simple, opposite, sometimes whorled, elliptic-ovate. Their flowers are small, greenish yellow. The medicinal properties are astringent, bitter, acrid, sweet, thermogenic, anodyne, anti-ionflammatory, ardiotonic, alternat, expectorant, depurative, digestive, carminative. The plant parts are used in neuralgia, inflammations, cardiac disorders, hepatopathy, cough, asthma, bronchitis, flatulence, tumours, haemorrhoids and general debility (Warrier *et al*., 1995).

The present study has been designed to evaluate the antioxidant activity of methanolic extract of *Premna serratifolia* Leaf in various tissues of Paracetamol intoxicated Wistar albino rats.

Materials and methods

Animals

Adult Wistar Albino Rats of either sex weighing between 150-200g were used for the present study. The animals were housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25± 2^0C) with dark and light cycle (14/10 hrs). They were allowed free access to standard dry pellet feed (Amrut, Bangalore) and water *ad libitium.* The rats were acclimatized to laboratory condition for 30 days before commencement of experiment. All procedure described were reviewed and approved by the Institute Animal Ethical committee (IAEC).

Chemicals

Methanol (Qualigens Fine chemicals limited, Mumbai), Paracetamol (GSK, Mumbai.), Silymarin (Serum Institute of India, Pune), Sodium chloride (Merck Specialties Private limited, Mumbai),Superoxidedismutase (Randox), Catalase (Randox), glutathione peroxidase (Randox), Total protein (Transasia Lab).

Experimental Set Up

The following experimental protocol was followed (Gupta and Mishra, 2006)

Healthy Wistar albino rats were divided in to five groups consisting of six animals each.

The first group (I) consisted of normal control rats which received single daily dose of distilled water throughout the experiment.

The paracetamol group (II) received single daily dose of distilled water for nine days and single dose of paracetamol on day 8 (2.5g/kg).

The third group (III) was treated with standard drug silymarin (100mg/kg) on all nine days and paracetamol (2.5g/kg) on day 8, two hour after administration of Silymarin.

The fourth group (IV) was treated with lower dose of methanolic extract of *P. serratifolia* (100mg/kg) throughout the experiment and single dose of paracetamol (2.5g/kg) on day 8, two hour after administration of test extract.

The fifth group (V) was treated with higher dose methanolic extract of *P. serratifolia* (200mg/ kg) on all nine days and paracetamol (2.5g/kg) on day 8, two hour after administration of test extract.

On tenth day of the experiment, rats were anaesthetized by light chloroform anesthesia and the blood was withdrawn through intra-cardiac puncture to the rats. The animals were fasted 12 hours before the collection of blood. After blood collection, the rats were sacrificed by cardiac dislocation and their liver, kidney and heart were excised, rinsed in ice-cold normal saline and stored in refrigerator.

Biochemical Studies

Serum was separated by centrifugation at 2500 rpm at 30^0C for 15 minutes and utilized for the estimation of antioxidant parameters like SOD (Suttle, 1986), Catalase (Sinha, 1972), GSH (Kraus and Ganther, 1980). The isolated organs liver, kidney and heart tissue samples were homogenized and made in to 10% in PBS.

Statistical Analysis

Each experimental value is expressed as the mean ± SEM. Statistical calculations of the data were performed using Student's t-test and ANOVA analysis. A probability of $P < 0.05$ $p<0.01$ was considered significant.

Results and discussion

The *in vivo* antioxidant activity of methanolic extract of *P. serratifolia* was evaluated in tissues of wistar albino rats intoxicated with paracetamol. The antioxidant enzymes superoxide dismutase, catalase, glutathione levels were assessed in the blood samples and tissue samples of different organs liver, kidney and heart in all the experimental groups.

The results showed the decreased levels of antioxidant enzymes in both blood and tissue samples of the disease control group. The extract treated group showed elevated levels of SOD, catalase, glutathione in tissues samples of

liver, kidney and heart of both lower dose and higher dose significantly (Table. 1- 3). Antioxidant enzymes in blood samples of treatment group also exhibited the increased levels than disease group significantly (Table.4). The efficacy of the extract was found to be dose dependent in the experiment. The overall improved antioxidant levels showed that the efficacy of the methanolic extract of *P. serratifolia* is comparable one with the standard drug silymarin.

Table No. 1. Effect of methanolic extract of *P. serratifolia* on tissue level SOD status in paracetamol treated rats.

Groups	Tissue level antioxidant status –SOD (u/mg protein)		
	Liver	Kidney	Heart
Healthy Control	9.11± 0.63	6.15±0.36	22.6±4.30
Disease Control	4.11±0.21*	3.86±0.12*	10.78±1.25*
Silymarin Treated	7.65±0.15*	5.80±0.74**	19.81±3.70**
P. serratifolia L.D (100mg/kg)	7.15 ± 0.22**	5.63 ± 0.36*	21.56 ± 2.89*
P. serratifolia H.D (200mg/kg)	6.53 ± 0.55*	5.74 ± 0.53*	20.61 ± 3.65*

(Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control)

Table No 2. Effect of *P. serratifolia* extract on tissue level catalase enzyme level in paracetamol treated rats.

Groups	Tissue level antioxidant status –Catalase (µm/min/mg protein)		
	Liver	Kidney	Heart
Healthy Control	197.58 ± 8.4	168.8 ± 4.50	149.89 ± 5.66
Disease Control	83.60 ± 3.17	73.16 ± 4.10	68.10 ± 4.89
Silymarin Treated	163.11 ± 6.50*	141.19 ± 4.68**	123.05 ± 7.28*
P. serratifolia L.D (100mg/kg)	106.40 ± 5.66*	90.80 ± 6.78*	90.47 ± 10.4**
P. serratifolia H.D (200mg/kg)	132.52 ± 6.20**	130.15 ± 7.49*	109.42 ± 6.58*

(Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control)

Table No. 3 Effect of *P. serratifolia* extract on tissue level SOD status in paracetamol treated rats.

Groups	Tissue level antioxidant status –SOD (u/mg protein)		
	Liver	Kidney	Heart
Healthy Control	9.11± 0.63	6.15±0.36	22.6±4.30
Disease Control	4.11±0.21*	3.86±0.12*	10.78±1.25*
Silymarin Treated	7.65±0.15*	5.80±0.74**	19.81±3.70**
P. serratifolia L.D (100mg/kg)	7.15 ± 0.22**	5.63 ± 0.36*	21.56 ± 2.89*
P. serratifolia H.D (200mg/kg)	6.53 ± 0.55*	5.74 ± 0.53*	20.61 ± 3.65*

(Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control)

Table No. 4 Effect of methanolic extract of *P. serratifolia* on blood antioxidant enzyme levels in rat subjected to paracetamol induced toxicity.

Experimental Groups	**SOD** (Units/mg protein)	**GPx** (n moles of glutathione oxidized/min/mg protein)	Catalase (nM H2O2 decomposed/min/mg protein)
Healthy Control	11.13 ± 0.69	12.47 ± 0.79	18.34 ± 2.48
Disease Control	5.41 ± 0.62*	5.86 ± 0.38**	6.68 ± 1.27*
Positive Control	9.68 ± 0.44*	11.40 ± 0.59*	16.16 ± 2.45*
P. serratifolia L.D (100mg/kg)	8.31 ± 0.91**	8.62 ± 0.48**	11.71 ± 2.48*
P. serratifolia H.D (200mg/kg)	9.76 ± 0.87*	10.27 ± 0.24*	14.90 ± 3.11*

(Values are mean ± SEM, n=6 animals in each group. *p<0.05, **p<0.01 when compared to disease control)

The antioxidant system comprises of different types of functional components classified as first line, second line, third line and fourth line defenses. The first line defense preventive antioxidants are which act by quenching of $O_2^{\cdot-}$, decomposition of H_2O_2 and sequestration of metal ions (Gupta *et al.*, 2006). The antioxidants belonging to this category are enzymes, like superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and non-enzymatic molecules like minerals and some proteins (Schmidt *et al.*, 1975). Superoxide dismutase mainly acts by quenching of superoxide radical, produced in different aerobic metabolism. Catalase is a tetrameric enzyme, present in most of the cells and acts by catalyzing the decomposition of H_2O_2 to water and oxygen. Glutathione peroxidase is a selenium containing enzyme which catalyses the reduction of H_2O_2 and lipid hydroperoxides, generated during lipid peroxidation, to water and oxygen (Singh *et al.*, 1998).

In the present study, the catalase, superoxide dismutase and glutathione levels were significantly increased in the test extract treated animal groups. The efficacy of the extract was found to be dose dependent. Recent studies on various plants and herbal formulations also having the similar effect (Dandagi *et al.*, 2008; Girish *et al.*, 2009). The phytochemical molecules that are present in the crude extract might be the reason for bringing up the level of antioxidant enzymes in the treatment group. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in a few well defined experimental systems (Vidyashankar *et al.*, 2010) and concluded that ROS and lipid peroxidation may play a role in pathogenesis of hepatic fibrosis with loss of normal liver architecture (Schmidt, 2005; Campion *et al.*, 2008). The results obtained thus indicate that the methanolic extracts of *P. serratifolia* has potent antioxidant activity in the *in vivo* system.

Conclusion

The present study demonstrates that the methanolic extract of *Premna serratifolia* leaves possess potential antioxidant activity in *in vivo* experimental model system. The antioxidant property may be due to the presence of phenols, flavanoids and other phytochemical molecules that are present in the crude extract. The further focused research is required for isolation and characterization of functional molecules.

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