ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACOLOGY

HEPATOPROTECTIVE AND ANTI-OXIDANT EFFECT OF ROOT AND ROOT CALLUS EXTRACT OF *PREMNA SERRATIFOLIA* L. IN PARACETAMOL INDUCED LIVER DAMAGE IN MALE ALBINO RATS.

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ABSTRACT

Ethanolic extract of root and root derived callus extracts of *Premna serratifolia* L were investigated against paracetamol induced oxidative stress and hepatotoxicity in blood and liver of male albino rats. The root explants were cultured on MS medium supplemented with various combination and concentration of plant growth regulators. The best callus biomass was observed from the MS medium supplemented with 5.0mg/l of IAA. In the present study, hepatotoxicity induced by paracetamol (800mg/kg) administration was studied by assessing biochemical parameters such as serum glutamate phosphatase (SGOT), serum glutamate pyruvate transanaminase (SGPT), Lactate dehydrogenase (LDH), Lipid peroxidase (LPO), reduced glutathione (GSH), super oxide desmutase (SOD), catalase (CAT), Glutamyl transpeptidase (GT) ascorbic acid (vitamin-C) and α-Tocopherol (Vitamin E). The significant hepatic damage elicited by the levels of hepato specific enzymes such as SGOT, SGPT, LDH and CAT were observed in group one treated with root callus extracts (250 and 500mg) compared with other groups were treated with field grown root extracts (250 and 500mg/ kg). The effects of root callus extracts were comparable to that of standard drug, silymarine. Histopathological findings also suggest that the root callus extracts of *Premna serratifolia* L, prevents the development of chronic damage. From this present investigation, it can be concluded that the ethanolic extract of root callus is not only an effective hepatoprotective agent but also possess significant antioxidant activity can be attributed to the flavanoid Luteolin and other alkaloids like Premnin, ganikarin and kaniarin which helps to overcome the paracetamol – induced toxicity.

KEYWORDS

Premna serratifolia L., PGR-Plant growth regulators, silymarine, flavanoid and alkaloids.

INTRODUCTION

In recent years there has been renewed interest in natural medicines that are obtained from plants. Many ayurvedic medicinal plants have a long history of traditional use in revitalizing the liver and treating liver dysfunction and disease. About 40 families of Indian medicinal plants were investigated as liver protective drug[1] *Premna seratifolia* L. (Verbenaceae) is an important medicinal shrub having tremendous medicinal values like cardiac stimulant activity[2] anticoagulant activity[3] decoction of *P.serratifolia* exhibited anti inflammatory and anti arthritic activity[4] antibacterial activity from root extracts [5] antimicrobial activity from Bark and Wood[6] Hepatoprotective and activity of leaf of *P.serratifolia* L.[7]have also been reported, but the Hepatoprotective and antioxidant studies from root and *in vitro* cultured callus have not been studied so far. In the present study was carried out to evaluate the effect of ethanolic extract of root and root derived callus of *Premna serratifolia* L on paracetamol induced oxidative stress and hyper toxicity in blood and liver of male albino rats.

MATERIALS AND METHODS

Collection of plant material and authentication:

The plant *Premna serratifolia* L were collected from Keelathaniyam, Pudukottai district Tamil Nadu, India and scientifically authenticated by Fr John britto, Director, Rabinath Herbarium, Trichirappalli, Tamil Nadu, India.

Surface Sterilization and Callus Induction:

The root explants of *Premna serratifolia* L. collected and washed in running tap water for 30 minutes, and then the explants washed in 0.1% mercuric chloride for 1.5min, followed by 70% ethanol wash for 2mins. Finally washed in Hydrogen peroxide for 1 minute. Then the surface sterilized explants were exercised into 0.5-1.5cm long segments and small incision was made on the surface of each explants using sterile surgical blade. Then the surface sterilized explants were cultured on MS basal medium fortified with various concentrations of auxins like 2, 4-D NAA, IAA and IBA ranging from 1.0-7.0 mg/l. twenty-eight days old callus was collected and aseptically transferred to fresh medium in the same concentration and combinations of plant growth regulators for further proliferation. The callus was sub cultured twice in 4 weeks. All the cultures were incubated at 25±1°C under relative humidity of 50-60% and 12/12- hour photoperiod.

Plant material preparation:

The *in vivo* root materials of *Premna serratifolia* L. were collected and washed in running tap water and then chopped into small fragments. The materials were dried under sun sade condition for 30 days drying operation were carried out under controlled environment to avoid the chemical changes. The dried samples were powdered roughly with hands and stored in polythene container at room temperature. And the *in vitro* derived root callus materials were dried in hot air oven at 50°C for 48 hr. Then the dried callus materials were powdered using mortar and pestle and stored in polythene container at room temperature.

Preparation of Extracts:

The organic constituents from dried plant tissues were obtained by continuous extraction

of powdered materials in saxhlet apparatus with ethanol as solvent. The extracts were concentrated to one third of their original volume. After completion of extraction, extracts are filtered and concentrated to dryness under hot air oven at 55°C. The residue appeared as dark brown powder. Hepatoprotective and antioxidant activity were carried out by using this root and root derived callus extracts.

Experimental animals:

36 healthy male adult albino rats of Wister strain weighing 100±20g was procured from National Institute of Nutrition Hyderabad India. They were housed in clean sterile polypropylene cages with proper aeration and lighting (12 1h day/night rhythm) throughout the experiment period and temperature maintained between 27± 2°C and animals were fed with standard laboratory feed and water *ad* libitum. The usage and handling of experimental rats were followed as per the rules and regulations of institutional ethical committee for the purpose.

Experimental design:

The rats were divided into 6 groups, each group containing of six animals.

Group I : Control rats

Group II : Paracetamol (800 mg/kg body weight intraperitoneally) dissolved by keeping saline solution in a boiling water bath used after cooling at 37°C.

Group III : Orally pre-treated with the extract of field grown root at a dose of 250mg/kg body weight daily for a period of 15 days through intragastric intubation prior to paracetamol induction (800 mg/kg body weight intraperitoneally)

Group IV : Orally pre-treated with the extract of field grown root at a dose of 500mg/kg body weight daily for a period of 15 days through intragastric intubation prior to paracetamol induction (800 mg/kg body weight intraperitoneally)

Group V : Orally pre-treated with the extract of root callus at a dose of 250mg/kg body weight daily for a period of 15 days through intragastric intubation prior to paracetamol induction (800 mg/kg body weight intraperitoneally)

Group VI : Orally pre-treated with the extract of root callus at a dose of 250mg/kg body weight daily for a period of 15 days through intragastric incubation prior to paracetamol induction (800 mg/kg body weight intraperitoneally)

Measurement of Biochemical parameters

SGOT and SGPT were done through the method of [8]LDH

Collection of samples:

At the end of the experiments, three days after the induction of paracetamol the rats were made fasted for over night and killed by cervical decapitation under mild ether anesthesia and blood was collected to separate the serum. Liver were perfused *in situ* with cold 0.15M NaCl at 37° C.

Preparation of samples:

The liver tissues were homogenized with a motor-driven Teflon-coated homogenizer in ice-cold 0.1M Tris-HCl buffer (pH 7.4) to obtain a 10% homogenate. The homogenates of liver and aorta were used for the estimation.

Histology:

The liver samples were excised from the animals of each group after draining the blood and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hours. They were processed for paraffin embedding. The sections were taken at 5 µm thickness using microtome, processed in alcohol- xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Determination of marker enzymes:

The Following biochemical markers have been analyzed through standard methods. For Aspartate amionotransferase (2-oxyglutrate amino transferase), activity of aspartate aminotransferase, Alanine aminotransferase (L-Alanine: 2-oxyglumate amino transferase), activity of alanine aminotransferase, Lactate Dehydrogenase (LDH), activity of lactate dehydrogenase[8], γ-Glutamyl transpeptidase (γ GT), activity of γ-Glutamyl transpeptidase,[9&10] Lipid peroxidation (LPO),[11] Whereas reduced glutathione (GSH), reduced glutathione also determined,[12] Superoxide Dismutase (SOD), [13] Catalase (CAT), activity of the catalase,[14] ascorbic acid (Vitamin C), [15] and vitamin E (α - Tocopherol) have been analysed[16].

RESULT AND DISCUSSION

Effect of different auxins on biomass production from root explants:

When the root explants were cultured on medium supplemented with various auxins (IAA, IBA, 2,4-D and NAA 1.0 – 7.0 mg/l each) the best biomass (80mg) was obtained from 5.0 mg/l of IAA and 60 % callusing response was observed. The least biomass production (22mg) was obtained from 7.0 mg/l of IBA. But there was no callusing response observed in 1.0 to 3.0 mg/l of IBA and 1.0 mg/l of 2,4-D.

Hepatoprotective activity of *Premna serratifolia* L. :

A study was carried out to evaluate the effect of ethanolic extract of root and root derived callus of *Premna serratifolia* on paracetamol induced oxidative stress and hepatotoxicity in blood and liver of male albino rats. Extent of hepatic damage was assessed by histopathological examination and the levels of various biochemical parameters such as SGOT, SGPT, LDH, GGT, LPO, GSH, Vitamin-C, Vitamin-E, SOD and Catalase in the circulation. In animals treated with paracetamol a significant hepatic damage as elicited by the levels of hepatospecific serum markers (SGOT, SGPT, LDH and GGT) was observed. These markers are cytoplasmic in origin and released into the circulation after cellular damage[17]. The elevated levels in the activities of the hepatospecific marker enzymes observed in the present study are well correlated with the alteration in the hepatic cellular integrity. The rise in the SGOT is usually accompanied by the elevation in the levels of SGPT, which plays a vital role in the conversion of amino acids to keto acids. The extent of cellular disintegration is reflected with the elevated level of the LDH, a hydrogen transfer enzyme, which catalyzes the oxidation of lactate to pyruvate with the mediation of NAD^+. The destruction of the hepatic cells results in more hepatic release and hepatic dysfunction which causes an elevation in serum levels of LDH[18] which is in accordance with the results of our study. Treatment with the extracts of *Premna serratifolia* attenuated the elevated levels of serum marker enzymes. The normalization of serum marker enzymes was observed in the group III animals of our study which suggests that the extract of root and root callus of *Premna serratifolia* is able to condition the hepatocytes so as to protect the membrane integrity against paracetamol.

Table - 1

Hepatoprotective activity of root and root derived callus extracts of Premna serratifolia against paracetamol induced hepatic damage in albino rats.

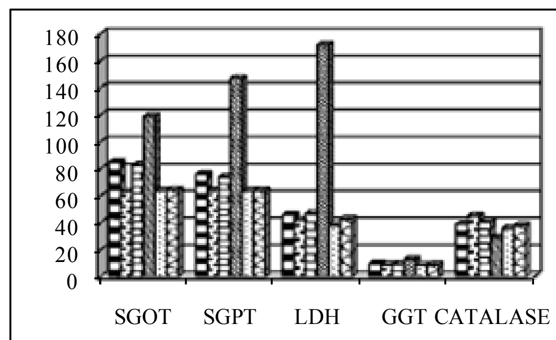
Enzyme	Normal	Control	Para+D1 (250 mg)	Para+D1 (500 mg)	Para+D2 (250 mg)	Para+D2 (500 mg)
SGOT	85.07 ± 8.8	62.9 ± 6.8	83.00 ± 8.8	119.2** ± 11.2	64.4** ± 9.2	64.33** ± 8.6
SGPT	76.77±10.6	64.32 ± 8.2	74.7 ± 9.2	147.32**±12.4	64.32** ± 9.8	64.32** ± 9.6
LDH	46.95 ± 5.2	42.68 ± 4.1	46.95 ± 4.9	172.16** ± 6.2	38.41** ± 4.8	42.68** ± 4.4
GGT	9.7 ± 0.96	8.9 ± 0.8	9.4 ± 0.98	13.0* ± 1.3	8.8* ± 0.84	8.9* ± 0.92
LPO	0.72 ± 0.06	0.69 ± 0.06	0.70 ± 0.06	3.49* ± 0.22	0.83* ± 0.07	0.79*± 0.06
GSH	0.40 ± 0.03	0.44±0.04	0.41 ± 0.04	0.152* ± 0.01	0.36*± 0.02	0.40*± 0.03
Vitamin-C	0.98 ± 0.06	1.76±0.14	1.01±0.09	0.62*±0.04	0.92* ± 0.06	0.96*±0.07
Vitamin-E	3.46 ± 0.33	4.06±0.36	3.61±0.40	2.01*±0.23	3.18*±0.30	3.29*±0.41
SOD	3.21 ± 0.31	3.92±0.19	3.40±0.32	2.40*±0.21	2.96*±0.22	3.01*±0.28
Catalase	39.98±3.68	45.34±3.32	41.01±4.08	29.86*±2.28	35.91*±3.20	38.18*±3.16

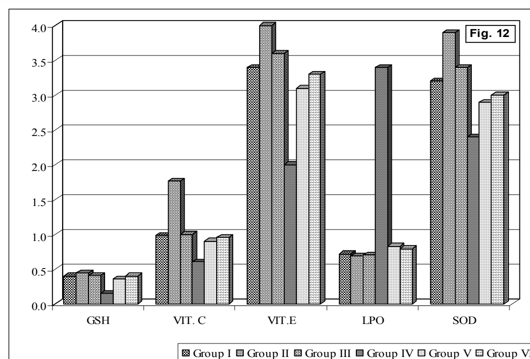
Group-1 Normal; Group-2 Paracetamol control; Group-3 Paracetamol + P.serratifolia root (250mg); Group-4 Paracetamol + P.serratifolia root extract (500mg); Group-5 Paracetamol + P.serratifolia root derived callus extract extracts (250mg); Group-6 Paracetamol + P.serratifolia root derived callus extract (500mg).

A significant increase in the levels of liver enzymes and hydroperoxides, markers of lipid peroxidation and iron, which are indicators of oxidative stress in paracetamol intoxicated animals observed in our study supports the concept that paracetamol overdose is a precursor of pro-oxidants, which mediate oxidative stress and cell death[19&20]. Lipid peroxidation arises mainly from damaged kupffer cells. Iron overload has also been found to play a crucial role in the generation of free radicals through Fenton reaction[21]. Paracetamol induced elevation in the lipid peroxidation and iron in the liver tissues observed in our study suggests the loss of iron hemostasis and increased accumulation of free radicals[22&23]. In addition, the extensive lipid peroxidation results in the membrane disorganization by peroxidizing the unsaturated fatty acids, which in turn alters the ratio of poly unsaturated fatty acids, leading to decrease in the membrane fluidity, which may be sufficient to cause cell death[24]. On treatment with *P. serratifolia* root and root callus extract, a significant decrease in the levels of MDA, hydroproxidase were observed. The results are in suggestive that *P. serratifolia* has the ability to protect the liver from paracetamol induced free radical injury and thus prove to be a good inhibitor of the oxidative stress and cell death. The increased level of hydroperoxidases in the liver indicates the enhanced lipid peroxidation due to the tissue injury caused by paracetamol in rats. Further, the increased tissue injury due to lipid peroxidation is reported to be due to the failure of antioxidant defense mechanisms to prevent the formation of free radicals.

Fig. 2&3

Hepatoprotective activity of root and root derived callus extracts of P. serratifolia against paracetamol induced liver damage in albino rats





Depletion in non-enzymic (GSH, Vitamins C and E) and enzymic (SOD and catalase) antioxidant observed in the liver of paracetamol intoxicated animals of our study are in consonance with the earlier reports of[25]. Thiols such as GSH plays a pivotal role in protecting hepatocytes by the elimination of reactive intermediates of acetaminophen[26] by the GST catalyzed conjugation and thus prevent their covalent binding to the liver proteins.

The depletion in the levels of Vitamin C and E observed in the paracetamol intoxicated animals in our study could be correlated with the excessive utilization of the non-enzymic antioxidants which quench the enormous free radicals[27]. On treatment with *P. serratifolia* extracts the animals showed a minimal loss in the levels of GSH, vitamin C and E. The levels of GSH was also found to have some positive relation with these vitamins i.e., GSH may maintain the vitamin E level either by reduction of α - tocopherolxyl radical to Vitamin E against antioxidant induced stress[28]. SOD and catalase are the most important antioxidant enzymes against toxic events of oxygen metabolites. SOD accelerates the dismutation of superoxide (O_2^-) radicals to hydrogen peroxide (H_2O_2), which can be said as a primary defense, as it prevents further generation of free radicals. Catalase catalyzes the removal of H_2O_2 formed during the reaction catalyzed by SOD. A decrease in the activity of SOD can be attributed to an enhanced superoxide production. The superoxide radicals also inhibit the activity of catalse[29].

The animals treated with the extracts of *P.serratifolia* showed a significant restoration in the activities of SOD and catalase. The results of our study reveal that *P.serratifolia* extract significantly attenuated the oxidative stress by modulating the non-enzymic and enzymic antioxidants in the liver of paracetamol intoxicated male albino rats. Further, the decreased rate of lipid peroxidation observed in the rats treated with *P.serratifolia* extract is well correlated with the induction of antioxidant defenses. On the basis of the above results, it could be concluded that *P.serratifolia* posse significant hepatoprotective activity (Table-1) and this activity can be attributed to the flavonoid, luteolin and other alkaloids like premnin, ganikarin and kaniarin which helps to overcome the paracetamol – induced toxicity(Fig-2&3).

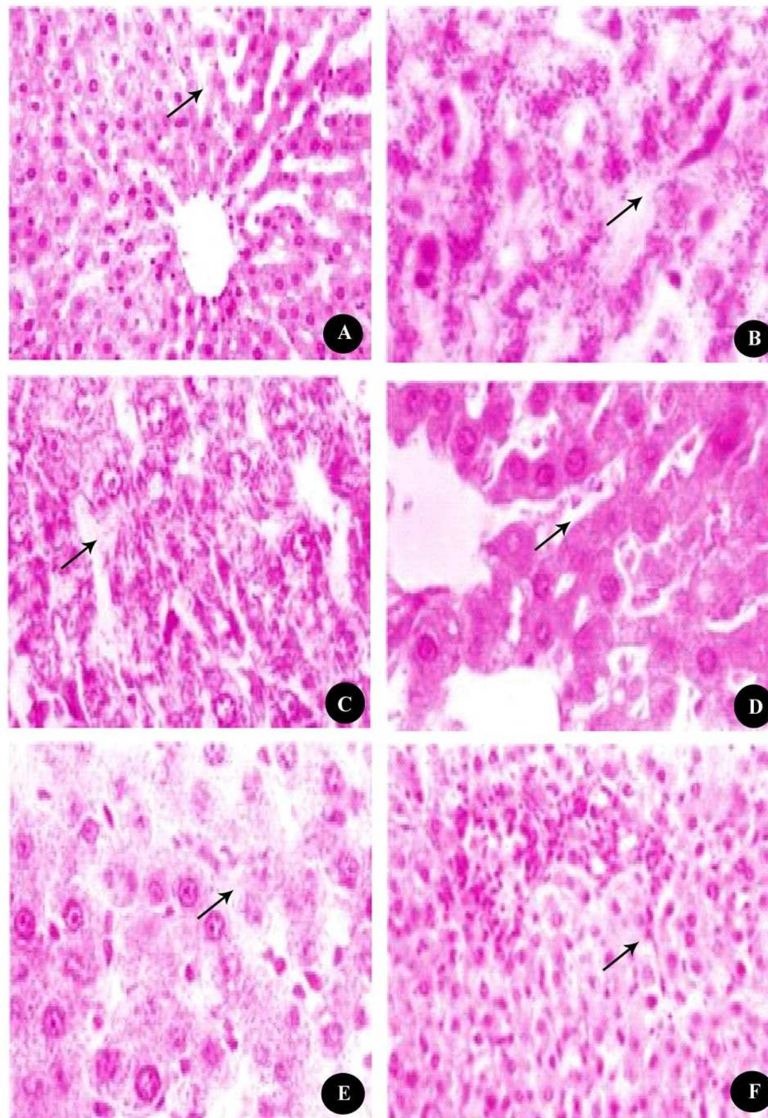


Fig-1 **Effect of root and root derived callus extracts of *Premna serratifolia* L. on paracetamol induced hepatic liver damage in Albino rats.**

→ Fatty vacuoles

Histological studies:

The histological examination of the liver sections revealed that the normal liver architecture was disturbed by hepatotoxin (paracetamol) intoxication. The control animals showed normal hepatic architecture with fatty vacuoles (Fig. 1A). The section of the liver of animals treated with paracetamol exhibited intense centrilobular necrosis, vacuolization and macro vesicular fatty changes (Fig. 1B). Moderate accumulation of fatty lobules and cellular necrosis was observed in liver sections of animals treated with natural root extract of *P.serratifolia* (Fig. 1C & D). The liver sections of animals treated with root derived callus extracts exhibited significant liver protection against paracetamol induced liver damage. This was evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Fig. 1E). The liver sections of silymarin treated animals showed normal hepatic architecture (Fig. 1F).

The present study indicated that the extracts of *P. serratifolia* were able to protect and restore the liver from the hepatotoxin. The effect was

comparable to the standard drug silymarin. Hence, it could be hypothesized that luteolin present in *P. serratifolia* could be considered responsible for this hepatoprotective activity against paracetamol induced liver damage in rodants when given before the metabolic activation of hepatic damage.

ACKNOWLEDGEMENT

Author acknowledgement the valuable help rendered by Dr Jhon Britto, Director, Rhabinath herbarium, Thiruchirapalli, Tamil nadu, India for identification of plant specimen. And Department of Biotechnology, J.J. College of Arts and Science, Pudukottai, Tamil Nadu, India for provided Lab facilities to carried out the present findings.

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