

***In-vivo* antioxidant activity of *Premna serratifolia* Linn. in high fat diet fed rabbits**

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ABSTRACT

Antioxidant activity of the ethanol extract of stem-bark & stem-wood of *Premna serratifolia* Linn., (Verbenaceae) was determined by high fat diet (HFD) induced oxidative stress in rabbits. Preliminary phytochemical analysis of the ethanol extract revealed the presence of phytoconstituents such as alkaloids, flavonoids & phenolic compounds. The ethanol extract of stem-bark & stem-wood of *P. serratifolia* Linn., showed significant antioxidant activity by lowering the enzyme levels of (TBARS) thiobarbituric acid reactive substance & by increasing the enzyme levels of catalase (CAT), glutathione (GSH) & super oxide dismutase (SOD), which is comparable with the standard Atorvastatin in a dose dependent manner & remarkable activities to scavenge reactive oxygen species (ROS) may be attributed to the high amount of hydrophilic phenolic compounds. It is confirmed that the ethanol extract of *P. serratifolia* Linn., possesses the antioxidant substance, which may be potentially responsible for the cardio protective activity & chemo protective mechanism as well as using this plants extract in folkloric remedies.

Key words: *Premna serratifolia*, ethanol extract, atorvastatin, antioxidant activity.

INTRODUCTION

In living system, free radicals are generated as part of the body's normal metabolic process & the free radical chain reactions are usually produced in the mitochondrial, chain liver mixed function oxidases, through xanthine oxidase activity, atmosphere pollutants & from transitional metal catalyst, drugs & xenobiotics. In addition, chemical modification fat stores under condition such as lactation, exercise, fever, infection & even fasting, can results in increased radical activity & damage. Free radical or oxidative injury now appears the fundamental mechanisms underlying a number of human neurological & other disorders. For instance in diabetes, increased oxidative stress which coexists with reduction in the antioxidant status has been postulated. Oxygen free radical can initiate peroxidation of lipids, Which turn to Stimulation of glycation protein, inactivation of enzymes &

alteration in the substance & function of collagen role in the long term complication of diabetes¹. Although synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) & tetra butyl hydro guanine (TBHG) have been commonly used as antioxidant in foods for years, their safety has long been questioned². This has lead to an increased interest in natural antioxidant³ speices & herbs are recognized as sources of natural antioxidants that can protect from oxidative stress & thus play an important role in the chemoprevention of diseases that has their etiology & pathophysiology in (ROS) reactive oxygen speices. The medicinal properties of folk plants are mainly attributed to the presence of flavonoids, but may also be influenced by other organic & inorganic compounds such as coumarins, phenolic acid & antioxidant micronutrients such as copper, manganese & zinc⁴.

P. serratifolia Linn., (Verbenaceae) is a large shrub wide spread throughout micronesia⁵ & tropical Asia. *P. serratifolia* Linn., stem-bark & stem-wood showed cardio protective effect⁶, anticoagulant activity⁷ & decoction exhibited anti-inflammatory activity⁸. Literature review did not revealed any information on the antioxidant study of stem-bark & stem-wood of *P. serratifolia* Linn., A high fat diet induces oxidative stress in the cells by producing reactive oxygen species. Hence in the present study, the influence of the *P. serratifolia* Linn., ethanol extract on high fat diet (HFD) induced oxidative stress in rabbits have been screened.

MATERIAL AND METHODS

Plant material

Stem-bark & stem-wood of *Premna serratifolia* Linn., were collected from (IMPCOPS) Indian Medicinal Practitioners Co-Operative Pharmacy Stores Garden, Chennai, Tamil Nadu, in August 2007 & identified by Botanist Dr. P. Jayaraman, Chennai & a voucher specimen (PARC/2007/71) have been retained in Pharmacognosy department, Madras Medical College, Chennai, Tamil Nadu, India.

Phytochemical screening

The freshly prepared extract of *P. serratifolia* Linn., was quantitatively tested for the presence of various chemical constituents. Phytochemical screening of the extract was performed using the following reagent & chemicals. Alkaloids with Dragendorff's reagent, flavonoids with the use of magnesium & hydrochloric acid, tannins with ferric chloride & potassium dichromate, saponins with ability to produce suds & gums was tested using Molisch's reagent & concentrated sulphuric acid. These were identified by characteristic colour changes using standard procedure⁹.

EXPERIMENTAL

Animals

New Zealand white rabbits, weighing 900-1050 g were procured from Mohamed Sathak A.J. College of pharmacy, Chennai-119 & the experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for

experimental clearance (Registration No: 991/C/06/CPCSEA). The animals were kept in cages 2 per cage with 12:12 light/dark cycle at 25±2° C. The animals were maintained on the respective diets & water *ad libitum*.

Acute toxicity studies (LD₅₀)

Acute toxicity studies was carried out according to OECD guidelines 423. The ethanol extract of *P. serratifolia* Linn., was administered orally, the signs of toxicity & mortality at a dose of 2000mg/kg, were observed for a period of 14 days.

Evaluation of anti-oxidant activity:

Rabbits were divided into five groups (N=6) of six animals each group. Group I is considered as control, which received a standard chow diet for 11 weeks. Group II is considered as high fat diet (HFD) group, which received high fat diet for 11 weeks.

The composition of 2 diets

Control diet

| | |
|----------------------------|--------|
| Wheat flour | -22.5% |
| Roasted Bengal gram powder | -60% |
| Shimmed milk powder | -5% |
| Casein | -4% |
| Refined oil | -4% |
| Salt mixture with starch | -4% |
| Vitamin & choline mixture | -0.5% |

High fat diet

| | |
|----------------------------|--------|
| Wheat flour | -20.5% |
| Roasted Bengal gram powder | -52.6% |
| Shimmed milk powder | -5% |
| Casein | -4% |
| Refined oil | -4% |
| Coconut oil | -5% |
| Salt mixture with starch | -4% |
| Vitamin & choline mixture | -0.5% |
| Cholesterol | -0.4% |

Group III & group IV were considered as test group which received HFD plus ethanol extract of *P. serratifolia* Linn., at a dose of 200 & 400 mg/kg body weight per oral for 11 weeks which is suspended in 5% gum acacia. Group V is considered as standard, which received HFD plus Atorvastatin at a dose of 1.2 mg/kg body weight by oral intubation for 11 weeks. At the end of the 11th

week, all the animals were sacrificed by cervical decapitation after over night fasting.

Portions of the tissue from the liver, heart & aorta were blotted, weighed & homogenized with 3 volumes methanol. The lipid extract obtained by the method of Folch *et al*¹⁰, was used for the estimation of thiobarbituric acid reactive substances¹¹ (TBARS). Another portions of the tissues was homogenized with phosphate buffer saline & used for estimation of reduced glutathione¹² (GSH), catalase¹³ (CAT), & super oxide dismutase¹⁴ (SOD).

Statistical analysis

Results were expressed as mean \pm SEM of six rabbits in each group. One way analysis of variance (ANOVA) using Scheffe's multiple comparison test was performed to determine the statistical significance. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

The results of the study assessing the toxicological effect of ethanol extract have shown no mortality and morbidity effect up to the dose of 2000mg/kg body weight for a period of 14 days, and hence the ethanol extract was considered as safe and non toxic.

The effect of ethanol extract of *P. serratifolia* Linn., on average body weight changes in rabbits were shown in the table-1. The average

body weight was found to be increased in group-II (HFD fed rabbits) when compared with that of control group-I. After the administration of the two doses of *P. serratifolia* Linn., i.e., in the group-III & IV, the average body weight was found to be decreased & this is comparable with the group-V standard Atorvastatin. TBRS levels (table-2) was found to be increased in the high fat diet fed rabbit group-II, and whereas in group III & IV (*P. serratifolia* Linn., 200 & 400 mg /kg body weight) these levels was significantly lowered & thus decline in the level of TBRS, unveils the antioxidant potential of *Premna serratifolia* Linn., extract.

GSH levels were significantly decreased in liver, heart & aorta of rabbits fed HFD (Group-II) & GSH levels (table-2) were found to be decreased in liver, heart & aorta of group-III & IV animals. Both doses of *P. serratifolia* Linn., extract & restored to near normal. This increase in GSH concentration in animals treated with the plant extract may be due to the increased activity of the enzymes, glutathione reductase, which catalyses the conversion of oxidized glutathione to reduced glutathione in liver & it may also due to the enhanced synthesis & transport GSH. Higher doses of the plant extract was found to be more effective & showed comparable results with standard drug Atorvastatin on these two parameter.

The activities of antioxidant enzymes such as SOD & CAT in all the groups were shown in table 3. These two enzymes showed a measured

Table 1: Effect of ethanol extract of *P. serratifolia* on average body weight changes

| Treatment | Body weight (g) | |
|---|---------------------|------------------------------------|
| | Initial | Final |
| Group-I (Control) | 1016.66 \pm 29.85 | 1149.16 \pm 32.15 ^{b**} |
| Group-II (HFD) | 1070.18 \pm 47.25 | 1281.66 \pm 49.15 ^{a**} |
| Group-III (HFD+ ethanol extract 200mg/kg/day) | 1100.16 \pm 34.54 | 1247.00 \pm 26.38 |
| Group-IV (HFD+ ethanol extract 400mg/kg/day) | 0970.33 \pm 28.16 | 1106.66 \pm 20.27 ^{b**} |
| Group-V (Standard Atorvastatin 1.2 mg/kg/day) | 1041.66 \pm 30.48 | 1103.33 \pm 27.64 ^{b**} |
| One-way ANOVA | F | 6.53 |
| | P | <0.001 |

N=6, values are expressed as mean \pm SEM, $p < 0.001$, $**p < 0.05$; df=4, 18.

a-Group I compared with group II, III, IV & V.

b- Group II compared with group I, III, IV & V.

Table 2: Effect of ethanol extract of *P. serratifolia* on tissue TBARS & GSH in rabbit.

| Treatment | TBARS (n moles of MDA formed/g tissue) | | | Glutathione (mg/g tissue) | | |
|--|--|----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| | Liver | Heart | Aorta | Liver | Heart | Aorta |
| Group-I(Control) | 42.19±0.88 ^b | 40.40±0.79 ^b | 41.64±1.07 ^b | 18.65±0.78 ^b | 20.52±1.26 ^b | 14.64±0.52 ^b |
| Group-II(HFD) | 62.70±1.01 ^a | 63.09±0.83 ^a | 61.30±0.86 ^a | 07.21±0.66 ^a | 10.65±0.91 | 07.56±0.31 ^a |
| Group-III (HFD + ethanol extract 200mg/kg/day) | 47.63±1.05 ^{a,b} | 41.78±0.94 ^b | 41.43±1.05 ^b | 13.22±1.19 ^{a,b} | 16.29±0.95 ^{a,b} | 11.66±0.32 ^{a,b} |
| Group-IV (HFD + ethanol extract 400mg/kg/day) | 07.48±0.98 ^{a, b} | 34.22±1.15 ^{a, b} | 34.56±1.60 ^{a, b} | 16.42±0.99 ^b | 21.54±0.92 ^b | 12.7±0.36 ^{a, b} |
| Group-V (Standard Atorvastatin 1.2mg/kg/day) | 41.80±1.07 ^b | 40.37±1.18 ^b | 35.78±1.18 ^{a, b} | 13.60±0.54 ^{a, b} | 17.61±0.94 ^b | 13.21±0.43 ^b |
| One-way F | 318.92 | 203.38 | 81.18 | 31.92 | 38.39 | 58.57 |
| ANOVA P | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

N=6, values are expressed as mean±SEM, p* < 0.001, **p < 0.05, df=4, 18.

a-Group I compared with group II, III, IV & V. b- Group II compared with group I, III, IV & V.

Table 3: Effect of ethanol extract of *P. serratifolia* on tissue SOD & CAT in rabbit

| Treatment | SOD (min/mg/protein) | | | CAT (µmoles of H ₂ O ₂ consumed min/mg/protein) | | |
|--|----------------------|------------------|-------------------|---|-------------------|------------------|
| | Liver | Heart | Aorta | Liver | Heart | Aorta |
| Group-I(Control) | 12.55±0.53 b** | 15.58±0.54 b* | 22.09±0.60 b* | 24.07±0.65 b* | 14.56±0.51b* | 13.76±0.60 b* |
| Group-II (HFD) | 07.91±0.50 a** | 06.94±0.38 a* | 11.69±0.58 a* | 11.30±0.55a* | 07.91±0.37a* | 05.72±0.35a* |
| Group-III (HFD + ethanol extract 200mg/kg/day) | 10.26±0.43a**,b** | 09.75±0.57a*, b* | 19.50±0.64a*, b* | 17.64±0.24a*, b* | 10.61±0.16a*, b* | 09.50±0.15a*, b* |
| Group-IV (HFD + ethanol extract 400mg/kg/day) | 11.74±0.35 b* | 11.65±0.91 b* | 22.48±0.29 b* | 22.84±0.37a**,b* | 12.75±0.4 a**, b* | 12.58±0.32a*, b* |
| Group-V (Standard Atorvastatin 1.2mg/kg/day) | 12.55±0.17 b* | 14.43±0.28 b* | 23.67±0.16a**, b* | 22.47±0.17b* | 13.46±0.17 b* | 12.44±0.12a*, b* |
| One-way F | 27.36 | 59.35 | 98.70 | 144.60 | 63.88 | 74.37 |
| ANOVA P | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

N=6, values are expressed as mean±SEM, p* < 0.001, **p < 0.05, df=4, 18.

a-Group I compared with group II, III, IV & V. b- Group II compared with group I, III, IV & V.

reduction in activity in heart, liver & aorta of rabbits in group-II (high fat diet group). Those animals which received the plant extract plus HFD significantly improved the above tissues levels of antioxidant enzymes of rabbits in groups III & IV when compared with group-II. Restoration of the activities of SOD & CAT to near normal observed in the tissues of rabbit supplemented with *P. serratifolia* Linn., ethanol extract may be due to the removal of toxic intermediates by the plant extract in HFD fed animals & it is comparable with the standard Atorvastatin group. It was concluded that the administration of ethanol extract of stem-bark & stem-wood of *P. serratifolia* Linn., manifests a protective effect against HFD induced oxidative

stress in different tissues in rabbits. Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids & phenolic compounds, which may also be directly responsible for the observed antioxidant activity. The higher dose of the plant extract was found to be more effective & showed comparable results with standard drug Atorvastatin & these results confirm the traditional use of *P. serratifolia* Linn., against high fat diet induced oxidative stress, such as cardiovascular & autoimmune disorders. However further studies are needed to isolate the active principle, elucidate their structure & determine their pharmacological activities.

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