International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1692-1696, Oct-Dec 2009

Evaluation of antioxidant and hepatoprotective efficacy of methanolic extract of Mesua ferrea linn leaves in experimentally challenged mice

Sandeep Garg[1,3]*, Kameshwar Sharma[1], Rajeev Ranjan[1] and Pankaj Attri[2]

[1]Department of Zoology, University of Delhi, Delhi, India-110007
[2]Department of Chemistry, University of Delhi, Delhi, India-110007
[3]Department of Zoology, Smt. C. H. M. College, Ulhasnagar, 421003, Thane, Maharashtra, India-110007

***Corres. Author: gsk185@gmail.com**
Ph.no.: 09764033917,Office Ph.no.-0251-2734940
Fax no. 0251-2731869

Abstract : The purpose of this study was to determine the hepatoprotective and antioxidative efficacy of methanolic extract of dried flowers of *Mesua ferrea* in *Staphylococcus aureus* infected Balb/c mice. For this, 50, 100, and 200 mg of extract/kg of BW of mice were prepared. Infection was artificially installed by injecting *S.aureus* intraperitoneally. Different extracts were injected twice a day (10.00 am and 6.00 pm) intraperitoneally up to 7th day of infection installation. Blood samples were collected at 0 and 7th day of infection. Enzymatic antioxidants, CAT, SOD, GPx, GSH and GR were determined at day 0 and 7 and found to be significantly ($p<0.05$) restored in *Mesua* extract treated mice than controls. Serum ALT, AST, CPK, Creatinine and Urea profiles were examined on day 0 and 7 of the infection in all the mice groups. The values for all the above mentioned serum biochemical parameters were ranged in normal limits in extract treated groups compared to infected untreated group. AAT and AST showed a significant ($p<0.05$) restoration in *Mesua* treated group compared to control. 100 mg of extract/kg of BW was concluded as appropriate among all the three doses. This investigation positively demonstrated the antioxidant potency of *M. ferrea* flower methanolic extract.
Keywords: *Mesua ferrea*, enzymatic antioxidants, Catalase, Superoxide dismutase, Glutathione reductase, Glutathione peroxidase.

Introduction

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine because they are potential sources of medicinal bioactive compounds laced with antibacterial, antioxidant and cytotoxic activities (Kirtikar et al. 1995). These plant derived natural products have received considerable attention in recent years due to their diverse pharmacological characteristics especially their antioxidative properties. Antioxidants are the key enzymes which play an important role in inhibiting and scavenging free oxygen radicals in stressful conditions and provide protection to animal against infection and degenerative diseases (Lee et al. 1995).

Mesua ferrea belonging to family Clusiaceae, commonly known as Ceylon ironwood, Indian rose chestnut, Cobra's saffron or Nagkesar is native to tropical Sri Lanka but also cultivated in Assam, Konkan region, southern Nepal, Indochina and the Malay Peninsula. This is widely used in

formulations of traditional medicines meant for treating rheumatism, cough, dysentery, vomiting, sore throat, fever, itch, fever, itching, nausea, leprosy, skin disorders, erysipelas, bleeding piles, metrorrhagea, excessive thirst, and sweating etc (Kirtikar et al. 1995). The seed oil of *Mesua* is found to be astringent, digestant, anti poisonous, antimicrobial, anti-inflammatory, antipyretic and anthelminthic in many cases (Kirtikar et al. 1995). Majumdar et.al, 1979, has reported that the phenol constituents of the seed and flower oil of *Mesua* have powerful antiasthmatic effect. So far, no study has evaluated its hepatoprotective effects and induction of antioxidants in stringent infectious conditions. In this study an attempt has been made for the evaluation of antioxidative and hepatoprotective properties of *Mesua* in artificially challenged Balb/c mice.

2. Material and Method

2.1. Plant material and extraction

The flowers of *M. ferrea* were collected in April, 2008 from Kathmandu, Nepal and were authenticated at Department of Botany, University of Delhi, India. Collected flowers were shade dried for one week and powdered in a mixer blender. The powdered flowers (550 g) were extracted for its bioactive compounds at ambient temperature (22±2 °C) successively with 95% ethyl alcohol (1000 ml, 5 times) in Soxhelt apparatus. During extraction, solvents were changed after every 24 h. The alcohol from the pooled extracts was removed by distillation under reduced pressure at 40-45 °C leaving behind a powdered material (1.3g) which was used in saline for dose preparation.

2.2. Chemicals

All chemicals used in the study were of analytical grade and were obtained from Sigma-Aldrich, USA and Himedia India ltd., Mumbai.

2.3. Animals and experimental design

50 female pure strain Balb/c mice (16±2 g) were procured from local animal supplier in Delhi and were kept in metal cages by following the animal keeping guidelines issued by the University of Delhi. A normal 12L:12D light cycle was maintained. Temperature was maintained at 23±2°C throughout the experiment. Mice were acclimatized for one week and fed with normal mouse ration and tap water adlibitum. Mice were segregated in 5 groups (n=10). Group I was served as control normal (CT-NR), group II was control infected (CT-IN) and was not treated with any extract, groups III (T-I), IV (T-II) and V (T-III) were treated with 50,100 and 200 mg of extract/kg BW of mice. Infection was artificially installed by intraperitoneal administration of S. aureus (0.5ml in saline, $2x10^5$ cfu) in mice groups CT-IN, T-I, T-II and T-III at the onset of experiment. After 1h of the infection, methanolic extract of *M. ferrea* was introduced intraperitoneally in designated doses up to 7th day of infection. Blood and liver samples were taken before (0d) and after one week of experiment (7d). Blood was taken from each mice by orbital sinus venipuncture. Mice (n=6) were sacrificed for liver samples. All samples were frozen immediately in -20°C for further analysis.

2.4. Determination of serum biochemical and hematological parameters

Liver samples were homogenized in ice-cold buffer (100mM Tris-HCl, 0.1mM EDTA and 0.1% Triton X-100 (V/V) at a ratio of 1:9 (W/V). Homogenates were centrifuged at 30,000x g for 30min. Supernatant was collected and frozen at –20°C until analyzed.

2.5. Analytical methods

AST, AAT, CPK and ALKP were measured by standard kits following manufacturer's instructions (Bayer diagnostics, Gujarat, India). Creatinine and urea were measured by following instructions supplied with standard diagnostic kits (Aspen laboratories, Delhi, India) using Star-21-biochemistry analyzer, SEAC, India).

All enzymatic antioxidant assays were carried out using a Fluorometer (Labtech, Australia). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by the method of McCord and Fridovich, 1969, based on the inhibition of INT (iodo-p-nitro tetrazolium violet) reduction by O_2 generated by xanthine-xanthine oxidase. Catalase (CAT; EC 1.11.1.6) activity was determined by using the method of Lartillot et al. (1988). Briefly CAT activity was determined spectrophoto metrically at 240 nm using a specific absorption coefficient at 0.0392 cm^2 μmol^{-1} H_2O_2 and CAT activity was calculated as µmol H_2O_2 decomposed/mg protein/min. Glutathione peroxidase (GP_X; EC 1.11.1.9) activity was measured by the use of consecutive glutathione reductase reaction and oxidation of NADPH, with t-butyl hydroperoxides as substrate as described by Mannervik, 1985. A unit of GPx activity was defined as the amount of GPx needed to reduce initial glutathione concentration which was calculated according to Flohè and Gunzler (1984) and expressed as unit per mg protein. Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Calberg and Mannervik (1975), with little modifications by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted

of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH and 0.15 mM GSSG.

2.6 Statistical Analysis

Experiment was done in triplicates and data was statistically evaluated using student's T- test in SPSS version-11. The values were considered significant when ($p<0.05$).

3. Results

Liver is the main site of all the antioxidant actions and combats any infectious state in animals. It is the principal organ which converts all the medicines, drugs or any other chemical into their readily eliminated forms which can be extracted from the vertebrate body. In present study, *in vivo* liver antioxidant parameters in response to an artificially installed infection were evaluated in *Mesua* treated and control groups. Data is recorded on 0 and 7^{th} day of infection and summarized in Table.1. Liver superoxide dismutase (SOD) level was significantly enhanced ($p<0.05$) in CT-IN group in comparison to treated groups. Whereas, SOD activity was significantly restored ($p<0.05$) in T-II fed group (Table 1.) than other two treated groups. In CT-NR group SOD activity was found stable throughout study period. Liver Catalase (CAT) activity was significantly reduced ($p<0.05$) in all treated groups when compared to CT-IN group. CAT activity was stable in CT-NR group. However, CAT activity in T-II was significantly restored ($p<0.05$) among all the treated groups. This might be due to potential scavenging of free oxy radicals by *Messua* Extract. Similarly, In CT-IN group, GPx activity was significantly declined ($p<0.05$) when compared to treated groups. Among treated groups, no significant ($p<0.05$) change was observed in GPx activity except in T-II fed group, where GPx activity was retained ($p<0.05$) significantly. No ($p>0.05$) significant difference was observed in GPx activity of CT-NR group.

Liver Glutathione (GSH) activity of CT-IN was significantly increased ($p<0.05$) than treated groups. T-II treated group showed significant ($p<0.05$) restoration of GSH activity among treated groups, whereas, No significant difference ($p>0.05$) was observed in GSH activity of T-I and T-III fed group. CT-NR, GSH activity was observed stable with no significant ($p>0.05$) difference. Glutathione reductase (GR) activity in CT-IN was significantly higher ($p<0.05$) compared to Mesua treated groups. T-II group showed significantly lowest ($p<0.05$) GR activity. Whereas, T-I and T- III fed group showed significantly higher ($p<0.05$) GR activity in comparison to T-II fed group. AST is a measure of liver health in vertebrates. AST activity was changed significantly ($p<0.05$) in CT-IN when compared to treated groups. AST activity was restored ($p<0.05$) significantly in T- II fed group among all treated groups. Significantly lowest ($p<0.05$) AST activity was observed in T-I in treated groups (Table-II). ALT activity in CT-IN group was significantly increased ($p<0.05$) in comparison to treated groups. Among treated groups, T- III and T-I group showed significantly lower ($p<0.05$) ALT activity where as in T- II, ALT activity was significantly restored ($p<0.05$). No significant ($p>0.05$) difference was observed in Creatinine activity in treated and control groups (Table-II). Among treated groups, T-II group showed lowest % change in Creatinine activity. Similarly, no significant ($p>0.05$) difference was observed in CPK activity of all treated and control groups. However, least change was observed in CPK activity of T-II fed group (Table-II). Urea content was significantly ($p<0.05$) higher in CT-IN group, where as in *Messua* administered groups, urea level was significantly ($p<0.05$) controlled with least urea level in T-II administered mice group (Table-II).

4. Discussion

SOD and CAT considered as primary antioxidant enzymes as they are involved in the direct elimination of ROS (reactive oxygen species). SOD catalyzes the dismutation of superoxide radicals and CAT catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals. Both are imperative defense enzyme against oxygen radicals in any stressful conditions (McCord and Fridovich, 1969). The inhibition of SOD and CAT activities as a result of infection in CT-IN group and restoration of SOD in different doses *Mesua* administered groups is clearly depicting the antioxidant nature of *Mesua* leaves. There was a significant restoration in SOD and CAT levels in 100mg/kg of *Messua* methanolic extract administered group among all the treated groups also suggested that 100mg/kg of dose of *Messua* leaves in suitable of infections of *S.aureus*. Winter et al., 1999 reported similar results when he observed decrease in CAT and SOD activity in mice which might be due to loss of Mn-SOD activity in cells due to ROS (reactive oxygen species) or due to the loss of mitochondria, leading to a decrease in total SOD and CAT activity in the liver.

Glutathione, a potent inhibitor of neoplasticity process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process. The lowering in levels of GSH and GR in CT-IN group and its

restoration in *Messua* treated group indicates the potential of *Messua* methanolic extract as an inhibitor of intracellular oxidative stress. GR, the most important biomolecules protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GR. GPX, a selenium-containing enzyme present in significant concentrations, detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione. In present study, *Mesua* extract restored the hepatic free radical scavenging enzymes, GR and GSH in infection bearing stressed mice to normal levels. The *Messua* treated animals at the doses of 50, 100, and 200 mg/kg inhibited the body weight, and also brought back the hematological parameters to more or less normal levels. In stressed CT-IN group, elevation in AST and ALT activities and their restoration to normal levels in liver established *Mesua* as a potent antioxidant plant. This restoration of AST and ALT levels in *Mesua* treated groups could attribute to the control of increased gluconeogenesis and ketogenesis observed in infectious conditions due to the high activities of transaminases.

Table. I. Liver enzymatic antioxidants in *Mesua* treated and control mice groups (mean ± S.E.) [a b].

Parameters	Day	CT-NR	CT-UN	T-1	T-II	T-III
Urea	0	61.21±4.154	63.61±2.574	62.67±5.348	62.58±1.597	64.24±1.254
(mg dl^{-1})	7	60.25±5.2.36[a]	104.58±3.874[b]	94.34±5.102	76.91±4.198[b]	77.67±4.521[b]
Creatinine	0	48.74±6.41[a]	48.35±3.547[b]	57.21±1.124[b]	42.19±2.215[b]	51.39±2.014[b]
(mg dl^{-1})	7	47.51±5.454[a]	71.65±2.369[b]	48.12±2.178	40.58±1.487[b]	47.61±1.547
ALT	0	61.55±4.48[a]	59.18±2.184	56.57±1.458[b]	56.43±4.657[b]	64.87±2.047
(IU L^{-1})	7	71.55±7.013[a]	84.18±1.048[b]	87.35±1.427[b]	78.61±3.624[b]	87.28±1.325[b]
AST	0	98.54±5.968[a]	108.31±4.658[b]	121.19±8.213[b]	108.49±5.210[b]	108.87±6.351[b]
(I U L^{-1})	7	104.54±6.124[a]	133.25±5.058[b]	88.18±7.42[b]	79.49±3.421[b]	91.68±6.321[b]
CPK	0	55.48±4.125[a]	55.39±0.598[b]	62.68±4.120[b]	52.18±1.274[b]	57.49±3.104
(IU L^{-1})	7	57.64±7.120[a]	78.47±3.425[b]	60.48±5.126[b]	59.87±3.412[b]	66.42±5.785[b]
ALP	0	129.48±4.369[a]	118.68±4.785[b]	152.39±9.421[b]	159.75±5.103[b]	171.48±6.412[b]
(IU L^{-1})	7	124.86±8.107[a]	149.08±7.054[b]	126.68±1.954[b]	122.58±9.452[b]	123.89±2.574[b]

[a b] the difference is significant (p>0.05) in a row between treated and control groups

Table.II: Biochemical parameters of mice plasma in *Mesua* treated and control mice groups (mean ± S.E.) [a b].

Parameters	Day	CT-NR	CT-UN	T-1	T-II	T-III
SOD	0	44.25±3.524	47.88±4.189	40.74±2.547	44.87±3.74	44.88±5.741
(U/mg protein)	7	47.416±5.574[a]	72.647±6.784	64.714±5.125[b]	61.412±6.41[b]	66.74±4.521[b]
CAT	0	47.343±3.124	44.69±2.456	46.542±3.458	43.217±5.889	49.852±2.962[b]
(U/mg protein)	7	48.647±1.47[a]	68.127±2.154[b]	61.254±3.197[b]	52.631±1.057[b]	61.143±1.001[b]
GR	0	7.658±.458	7.894±.471	7.674±.398	7.364±.287	6.387±.412
(U/mg protein)	7	8.874±.178[a]	5.687±.410[b]	7.314±.128[b]	7.541±.137	6.287±.743
GPx	0	3.912±.112	4.178±.421	3.125±.416	3.144±.586	3.051±.157
(U/mg protein)	7	3.488±.285[a]	6.195±.523[b]	3.423±.120[b]	2.452±.187[b]	3.172±.356[b]
GSH	0	2.258±.258	2.187±.236[b]	2.47±.278	2.87±.189	2.321±.088
(U/mg protein)	7	3.896±.285[a]	4.257±.125	2.512±.421[b]	2.047±.458	3.47±.174[b]

[a b] significant change (p>0.05) in antioxidant level antibiotic treated and control group

5. Conclusion

We conclude that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, etc, present in *Messua* might be responsible for its potent antioxidant activity in bacterial infections. Optimum dose of methanolic extract of *Messua ferrea* was found to be 100 mg/kg of body weight/day.

6. Acknowledgement

Author is highly acknowledged to Council of Scientific and Industrial Research (CSIR) for providing financial assistance to author and department of science and technology (DST) India for providing financial support for the scientific work.

References

1. Kirtikar, K.R.; Basu, B.D. Indian Medicinal Plants, Vol. 1, International Book Distributors: Dehradun, India; 1995; pp 274-277.
2. Majumder R, Dastidar S. G., Basu S. P., Majumder A., and Singh S. K., Antibacterial potentiality of Mesua ferrea Linn. flowers,Phytotherapy research, 2004, 18, 10, 824-6.
3. Bergmeyer, H.U. In: H.U. Bergmeyer, Editor (2nd ed.), Methods of Enzymatic Analysis vol. 1, Academic press, New York, 1974, p. 438-442.
4. Calberg, I., and Mannervik, B., Purification and characterization of the flavo enzyme glutathione reductase from mice liver, J. Biol. Chem., 1975, 250, 5475–5480.
5. L. Flohè and W.A. Gunzler, Assay of glutathione peroxidase. Methods Enzymol., 1984, 105, 114–121
6. McCord, J.M. and Fridovich, I., Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein), J. Biol. Chem., 1969, 44, 1969, 6049–6055.
7. Kaplan A., Sazabo, L.L., Clinical chemistry: interpretation and techniques, Lee and Fibiger, Philapedelphia, 1983, 452-489.
8. Lartillot, S., Kedziora, P. and Athias, A., Purification and characterization of a new fungal catalase, Prep. Biochem., 1988, 18, 241–246
9. Mannervik, B., Glutathione peroxidase. Methods Enzymol. 1985,113, 490–495.
10. Shankaranarayanan, D., Gopalakrishnan, C. and Kameswaran, I., Pharmacological profile of mangostin and its derivatives., Arch. int. Pharmacodyn., 1979 b,. 239, 257-269.
11. Winter, C. A. and Porter, C. C., Effect of altemiceion in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters, J. Anter. Pharm. Ass., 1999, 46, 515-520.

*****

*****