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Research Article

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Pharmacological Evaluation of Antioxidant Activity of Methanolic Extract of *Mucuna Pruriens* Seeds.

C. Madhavi Latha*, SK. Salma Sulthana

Department of Pharmacology, Jagans College of Pharmacy, Jangalakandriga, Muthukur, Nellore -524346, A.P, India

ABSTRACT

Antioxidant activity of methanolic extract of *Mucuna pruriens* L. (Fabaceae) seeds were evaluated in male Wister albino rats. The methanolic extract of *Mucuna pruriens* L. 2000mg/kg (MEMP) was administered orally to different groups of rats for two times at an interval of 12 hours & continued for 30 days. MEMP suppressed the accumulation of lipid peroxidation in the plasma as well as maintained the activities of antioxidant enzymes such as superoxide dismutase (SOD) & catalase. The present investigation confirmed the antioxidant effect of the methanolic extract of *Mucuna pruriens*.

Keywords: Antioxidant enzymes, *Mucuna pruriens* L.

ARTICLE INFO

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*Corresponding Author

C. Madhavi Latha
Department of Pharmacology,
Jagans College of Pharmacy,
Muthukur, Nellore -524346, A.P, India
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1. Introduction

Antioxidant are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are harmful byproducts generated during

normal cell aerobic respiration. It is widely accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause

pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, parkinson's diseases, mongolism, ageing process & perhaps dementias. Generally, food antioxidants act reducing agents, reversing oxidation by donating electrons & hydrogen ions. There is a worldwide trend towards the use of natural antioxidants to enhance health and food preservation is of current interest.

Mucuna pruriens L. a member of the family Fabaceae. It is one of the popular and important medicinal plants indigenous to tropical countries like India is a constituent of more than 200 indigenous drug formulations.¹⁰⁰⁻¹⁰³ *Mucuna* finds traditional use in a number of diseases. Roots are used in the treatment of nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, and fever and as febrifuge and tonic. Leaves are aphrodisiac, tonic, and are useful in ulcers, inflammation, helminthiasis, cephalalgia and general debility. Seeds are used in snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhoea, sterility, gout, delirium, dysmenorrhoea, diabetes, and cancer¹⁰⁴⁻¹⁰⁶. It has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease.

2. Materials and Methods

Plant Collection

The seeds of *Mucuna pruriens* (Linn.) were collected from Thirunelveli, South India in the month of May 2009 and authenticated by Dr. Sasikala Ethirajulu, Research officer, pharmacognosy, Central Research institute for Siddha, Chennai-106.

Preparation of Plant Extract

The seeds of *Mucuna pruriens* were dried under shade and pulverized using a standard pulveriser. A weighed quantity of Powered seeds was subjected to continuous hot extraction in soxhlet apparatus with methanol at 60-80 °C. The filtered extract was evaporated under reduced pressure using rotatory vacuum evaporator until all solvent was removed to give a dark colored molten extract of 30.4% w/w. The methanolic extract *Mucuna pruriens* (Linn.) was suspended in 1% sodium carboxy methyl cellulose and used for the study.

Experimental Animals

Adult Wister rats of either sex weighing 180-250 Gms were used in pharmacological and toxicological studies. The inbred animals were procured from the animal house of C.L.Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97. The animals were maintained in a well ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1°C with humidity at 55±5%. Animals were fed with pellet chow, except during experimentation and water *ad libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

The Acute Oral Toxicity Studies:

The acute oral toxicity study was done according to the OECD guidelines 423 (Acute toxic class method). A single administration of starting dose of 2000 mg/kg b.w /p.o of the MEMP was administered to 3 rats and observed for 3

days. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed.

Experimental Design

Thirty male Wister rats weighing 180 to 250 gm were randomly divided into five groups of six each and kept in polypropylene cages for 5 days prior dosing for acclimatization to the laboratory conditions. The drugs were administered in constant volume of 0.2ml /100gm body weight .The control group animals received the vehicle in the same volume p.o.

Group 1:

Administered vehicle 1% sodium carboxy methyl cellulose. Fed with basal rodent chow and served as normal control or positive control.

Group 2:

Administered vehicle 1% sodium carboxy methyl cellulose. Fed with high fat diet and served as HFD negative control group.

Group 3:

Administered lower dose of MEMP (200mg/kg) which was suspended in 1% sodium carboxy methyl cellulose, p.o., and fed with high fat diet served as lower dose group (LD).

Group 4:

Administered higher dose of MEMP (400mg/kg) which was suspended in 1% sodium carboxy methyl cellulose, p.o., and fed with high fat diet served as higher dose group (HD). On day 30, animals were sacrificed by cervical dislocation. Blood was collected from jugular vein. Then the biochemical parameters are carried out.

Biochemical estimation

The biochemical parameters like lipid peroxidation, superoxide dismutase, & catalase activities were estimated on 30th day.

Statistical Analysis

The results are expressed as standard error mean ± SEM. Comparison between the groups were performed by one-way analysis of variance (ANOVA) followed by Dunnet's 't' test.

3. Results and Discussion

Oxidative stress is associated with peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance (TBARS). The concentration of LPO products may reflect the degree of oxidative stress. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense against ROS by lowering the steady state level oxygen. SOD scavenges the superoxide ions produced as cellular byproducts. SOD is a major defense for aerobic cells combating the toxic effect of superoxide radicals. The SOD activity is determined by the ability of the enzyme to inhibit auto oxidation of epinephrine. CAT is a heme protein, localized in the microperoxisomes. It reduces hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisome. The enzyme catalyses the decomposition of H₂O₂ to water and oxygen and thus protects the cell from oxidative damage by H₂O₂.

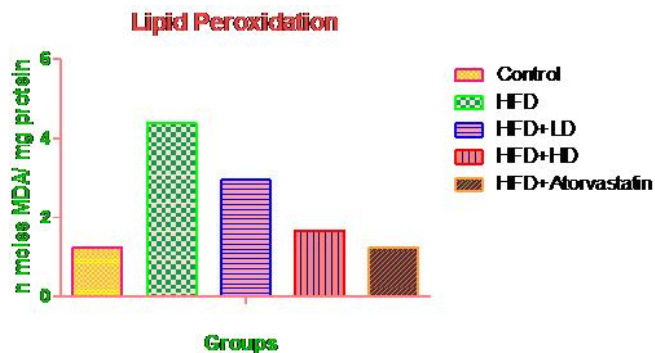


Figure 1: Effect of MEMP on Hepatic Lipid Peroxidation

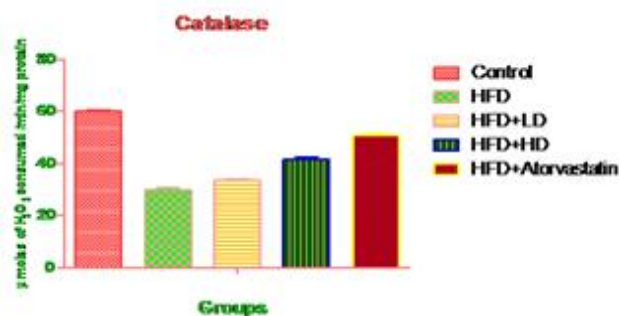


Figure 3: Effect of MEMP on Hepatic Catalase Level

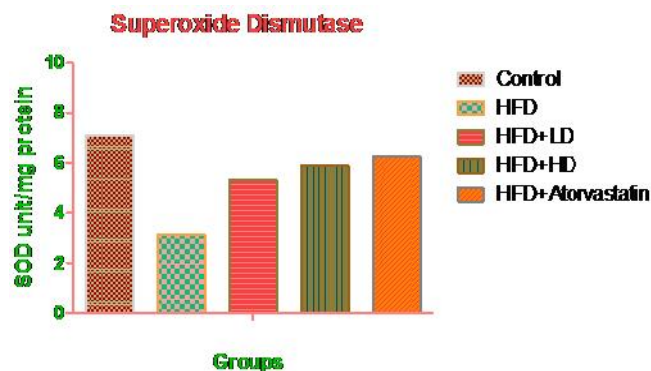


Figure 2: Effect of MEMP on Hepatic Superoxide Dismutase Level

4. Conclusion

The drug demonstrated for antioxidant properties in *in vivo* antioxidant models. Probable mechanism may be due to the reduced cholesterol absorption, deranged endogenous lipid synthesis, and enhanced fecal cholesterol excretion. MEMP also showed *In-vivo* antioxidant properties by facilitating the increased formation of free radical scavenging enzymes like SOD and catalase and reduced the extent of lipid peroxidation. It can be concluded that all the effects contribute for reducing the CVD due to atherosclerosis. *In-vitro* target validation and the innate mechanisms responsible for anti oxidant mechanisms must be studied in detail further.

Table 1: Effect of MEMP on Hepatic Lipid Peroxidation

Groups	Normal Control I	HFD Control II	HFD+LD III	HFD+HD IV	HFD + Atorvastatin V
Lipid Peroxidation (n moles of MDA/ mg Protein)	1.27 ± 0.97	4.39 ± 0.56 ^{a**}	2.96 ± 0.76 ^{b**}	1.67 ± 0.46 ^{b**}	1.26 ± 0.78 ^{b***}

- Comparisons were between: a- Group I vs. II, b- Group II vs. III, IV, and V.
- Values are expressed as mean ± SEM of 6 animals.
- Symbols represent statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

Table 2: Effect of MEMP on Hepatic Superoxide Dismutase Level

Groups	Normal Control I	HFD Control II	HFD+LD III	HFD+HD IV	HFD + Atorvastatin V
Superoxide dismutase (unit/mg protein)	7.12 ± 0.64	3.13 ± 0.42 ^{a***}	5.35 ± 0.33 ^{b*}	5.89 ± 0.69 ^{b**}	6.27 ± 0.58 ^{b**}

- Comparisons were between: a- Group I vs. II, b- Group II vs. III, IV, and V.
- Values are expressed as mean ± SEM of 6 animals.
- Symbols represent statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

Table 3: Effect of MEMP on Hepatic Catalase Level

Groups	Normal Control I	HFD Control II	HFD+LD III	HFD+HD IV	HFD + Atorvastatin V
CAT (μ moles of H ₂ O ₂ consumed /min/mg protein)	60.12 ± 0.53	29.75 ± 0.68 ^{a***}	33.45 ± 0.54 ^{b**}	41.57 ± 0.98 ^{b***}	50.68 ± 0.47 ^{b***}

- Comparisons were between: a- Group I vs. II, b- Group II vs. III, IV, and V.
- Values are expressed as mean ± SEM of 6 animals.
- Symbols represent statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.
- Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

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