

EFFECT OF ETHANOLIC SEED EXTRACT OF *MUCUNA PRURIENS* (L.)DC. VAR.UTILIS ON HALOPERIDOL INDUCED TARDIVE DYSKINESIA IN RATS

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ABSTRACT

To identify the protective effect of ethanolic extracts of *Mucuna pruriens* (EEMP) seeds against haloperidol induced tardive dyskinesia. The effects of ethanolic extracts of *Mucuna pruriens* was studied using in-vivo parameters like vacuous chewing movements, tongue protrusions, transfer latency in elevated plus maze. Biochemical parameters (SOD, CAT, GSH, Dopamine, glutamate, lipid peroxidation and total protein content) of the ethanolic extracts of *Mucuna pruriens* were assessed at two dose levels (200 and 400mg/kg). Ethanolic extract of *Mucuna pruriens* at the dose level of 400mg/kg exerted significant protective effect against haloperidol induced tardive dyskinesia. Significant elevation of antioxidant enzymes levels was observed. The protective action of the extract could be due to its ability to increase the levels of antioxidant enzymes and suppress lipid peroxidation.

Keywords: *Mucuna pruriens*, Haloperidol, Tardive dyskinesia, Antioxidant enzyme, Dopamine, Glutamate.

1. INTRODUCTION

Mucuna pruriens Linn (MPL) is a popular Indian medicinal plant, which has long been used in Ayurvedic system of medicine for diseases including Parkinsonism. Roots, leaves and seeds of the plant are commonly used in the treatment of impotence, snake bite, diabetes, cancer and Parkinsonism. The endocarp of *Mucuna pruriens* is non-toxic and is 2-3 times more potent than levodopa in controlling hyperprolactinemia¹ motor symptoms of Parkinson's disease animal models.² *Mucuna pruriens* has also shown to exhibit neuroprotective effect by increase brain mitochondrial complex-I activity and significantly restoring dopamine and norepinephrine levels in Parkinsonism animal model.³ Phytochemical evaluation on the seeds revealed the presence of 5-indolic compounds, especially tryptamine and 5-hydroxytryptamine⁴, alkaloids like mucunine, mucunadine, purine and prurienine.⁵

Haloperidol (HAL) a widely used neuroleptic for the treatment of psychosis is limited by its tendency to produce a range of extrapyramidal movement disorders like tardive dyskinesia (TD), akathisia, dystonia and Parkinsonism.⁶ It has been postulated that the pathophysiology underlying TD may be oxidative stress.⁷ Since several published data's has shown the ability of *Mucuna pruriens* seeds to scavenge free radicals and enhance the levels of antioxidant enzymes. The present work was designed to study the protective effect ethanolic extracts of the seeds of *Mucuna pruriens* (EEMP) in tardive dyskinesia induced by chronic haloperidol treatment in rats.

2. MATERIALS AND METHODS

2.1. Plant material

The seeds of MPL were collected from palakkadu district, Kerala. June 2008. The plant was identified and authenticated by Dr. Sasikala Ethirajulu, Captain Sreenivasan research foundation, Chennai, Tamilnadu, India. The specimen voucher was deposited in the

Department of Pharmacology and toxicology, C.L. Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.

2.2. Preparation of the ethanolic extract of MPL.

Freshly collected seeds of *Mucuna pruriens* were dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (950 g) was passed through sieve number 40 and subjected to hot solvent extraction in a soxhlet apparatus using ethanol, at a temperature range of 40-80°C. Before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. A brownish black waxy residue was obtained. The percentage yield of ethanolic extract was 18.7% w/w.

2.3. Phytochemical screening

The freshly prepared seed extract of MPL was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorff's reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's, Fehling's and Benedict's reagent; proteins and amino acids with Millon's, Biuret, and xanthoprotein test. Saponins was tested using hemolysis method; Gum was tested using Molish's reagent and Ruthenium red; Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures.⁸

The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids +ve; Gums and mucilage + ve; Glycosides + ve; Saponins – ve; Terpenes + ve and Tannins + ve

Where + ve and – ve indicates the presence and absence of compounds.

2.4. Animals

Colony inbred strains of wistar rats of either sex weighing 150-250g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 p.m., $22 \pm 1^\circ\text{C}$ room temperature, in polypropylene cages. The animals were fed on standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. All the experiments were carried out between 9.00 and 12.00 hours. It is randomly distributed into four different groups with six animals in each group under identical conditions throughout the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals). (IAEC Reference number: IAEC/XIII/10/CLBMCP/2007-2008 dated 20-04-07)

2.5. Acute toxicity studies.

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD).⁹

2.6. Induction of orofacial dyskinesia

Haloperidol was purchased from Sigma (Aldrich, USA). Haloperidol (1.0mg/kg, s.c) was administered chronically to rats for a period of 21days to induce orofacial dyskinesia.¹⁰ All the behavioral assessments were carried out 24 h after the last dose of HAL.

2.7. In vivo parameters

2.7.1. Behavioral assessment of orofacial dyskinesia

On the test day the rats were placed individually in a small (30 x 20 x 30 cm) plexiglass observation chamber for the assessment of oral dyskinesia. The animals were allowed 10 min to get used to the observation chamber before behavioral assessments. The number of vacuous chewing movements and tongue protrusions were scored by the observer with the help of a hand-operated counter. In the present study vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion or vacuous chewing movements occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the chamber to permit observation of oral dyskinesia when the animal was facing away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min. In all the experiments the scorer was unaware of the treatment given to the animals.¹¹

2.7.2 Transfer latency on elevated plus maze

Cognitive behavior was assessed by using the elevated plus-maze learning task, which measures spatial long-term memory. Transfer latency (TL), the time in which the animal moves from the open arm to the enclosed arm was

utilized as an index of learning and memory process. The elevated plus-maze consisted of two open arms (50 x10 cm) and two closed arms (50 x10 x40 cm) with an open roof. The maze was elevated to a height of 50 cm from the floor. The animals were placed individually at the end of either of the open arms and the transfer latency was noted on the first day. If the animal did not enter an enclosed arm within 90 s, it was gently pushed in to the enclosed arms and the TL was assigned as 90 s. To become acquainted with the maze, the animals were allowed to explore the plus-maze for 20 s after reaching the closed arm and then returned to its home cage. Retention was examined 24 h after the first day trial. In the present study the transfer latency was quantified on days 2, 7, 14 and 22 on haloperidol treated animals.¹²

2.7.3. Locomotor activity

The locomotor activity was monitored using an actophotometer (IMCORP, India). Before subjecting the animal to cognitive task, they were individually placed in the actophotometer and the total activity count was registered for 10 min. The locomotor activity was expressed in terms of total photo beam interruption counts/10 min per animal. Increase in count was regarded as central nervous system stimulant activity and decrease in count was regarded as depressant activity.

2.8. Biochemical studies

2.8.1. Dissection and homogenization

Acute-reserpine treated animals on day 29th of treatment (or day 29 of the last reserpine injection) and chronic haloperidol treated animals on day 22nd after behavioral quantification were sacrificed by decapitation. The brains were removed, forebrain was dissected out and cerebellum was discarded. Brains were put on ice and the cortex, striatum and subcortical regions were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The postnuclear fractions for catalase assay were obtained by centrifugation of the homogenate at $1000 \times g$ for 20 min, at 4°C and for other enzyme assays, it was centrifuged at $12,000 \times g$ for 60 min at 4°C . The subcortical region of brain comprised all the remaining parts of the forebrain which included the hippocampus, thalamus, hypothalamus and other subthalamic structures. Whole brains (excluding cerebellum) of one set of animals from each group were separately stored at 0°C for dopamine estimation. On the day of experiment, forebrain was dissected into two parts: cortical and subcortical (including the striatum).

2.9. Estimation of antioxidant enzyme levels in rat brain.

2.9.1. Estimation of superoxide dismutase

To 1 ml of the sample, 0.25 ml of absolute ethanol and 0.15 ml of chloroform were added. After 15 min of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant obtained constituted the enzyme extract. The reaction mixture for auto-oxidation consisted of 2 ml of buffer, 0.5 ml of 2 mM pyrogallol and 1.5 ml of water. Initially the rate of auto-oxidation of pyrogallol was noted at an interval of 1 min for 3 min. The assay mixture for the enzyme contained 2ml of 0.1 M Tris

– HCl buffer, 0.5 ml of pyrogallol, aliquots of the enzyme preparation and water made up to 4 ml. The rate of inhibition of pyrogallol auto-oxidation after the addition of the enzyme was noted. The superoxide dismutase activity was measured by the inhibition of pyrogallol auto-oxidation at 420 nm for 10 min. One unit of superoxide dismutase is the amount of enzyme required to bring about 50% inhibition of auto-oxidation by pyrogallol. The enzyme activity was expressed in terms of units/min/mg protein.¹³

2.9.2. Estimation of Catalase

Homogenized the tissue with M/15 phosphate buffer at 1 to 4°C and centrifuged. Stirred the sediment with cold phosphate buffer and allowed to stand in the cold condition with occasional shaking. Repeat the extraction once or twice, supernatants are combined and used for assay. 3 ml of H₂O₂ phosphate buffer was taken in one cuvette, added 0.01 – 0.04 ml sample and read against a control cuvette containing enzyme solution without H₂O₂ phosphate buffer at 240 nm. Δt was noted for a decrease in the optical density from 0.450 to 0.400. This value was used for the calculations.¹⁴

2.9.3. Estimation of lipid peroxidation

0.2 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of sodium dodecyl sulphate and 1.5 ml of thiobarbituric acid were added. The volume of the mixture was made up to 4.0 ml with distilled water and then heated at 95°C in a water bath for 60 min. After incubation the tubes were cooled to room temperature and final volume was made to 5.0 ml in each tube. 5.0 ml n-butanol pyridine (15: 1) mixture was added and the contents were vortexed thoroughly for 2 minutes. After centrifugation at 3000 rpm for 10 min, the organic upper layer was taken and its optical density read at 532 nm against an appropriate blank without the sample.¹⁵

The levels of lipid peroxides were expressed as n moles of malondialdehyde (MDA)/min/mg protein in brain homogenate.

2.9.4. Estimation of reduced glutathione (GSH)

1ml of tissue homogenate was precipitated with 1 ml of 10% TCA. The precipitate was removed by centrifugation. To an aliquot of the supernatant was added 4 ml of phosphate solution and 0.5 ml of DTNB reagent. The color developed was read at 420 nm. The amount of glutathione in tissue is expressed as $\mu\text{g/mg}$ protein.¹⁶

2.9.5. Estimation of protein

The protein content of brain tissue was estimated using bovine serum albumin as standard.¹⁷

2.9.6. Estimation of brain glutamate level

Weighed quantity of brain portion was homogenized with 2 parts by weight of perchloric acid and centrifuge for 10 minutes at 3000 rpm. Adjust 3.0ml supernatant fluid to pH 9 with 1.0ml phosphate solution. Allow to stand 10 min. in an ice bath and then filter through a small, fluted filter paper. Allow to warm to room temperature, dilute 1:10 and take 1.0 ml for the assay. Absorbance was measured at

340nm. Similarly a blank reading at 340nm was measured.¹⁸ The level of glutamate was expressed as $\mu\text{mol/g}$ tissue.

2.9.7. Estimation of dopamine level in rat brain by spectrofluorimetry

Preparation of tissue extract

On the day of experiment rats were sacrificed, whole brain was dissected out and separated the subcortical region (including the striatum). Weighed a specific quantity of tissue and was homogenized in 3 ml HCl Butanol in a cool environment. The sample was then centrifuged for 10 min at 2000 rpm. 0.8 ml of supernatant phase was removed and added to an eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M HCl. After 10 min, shake the tube and centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay

To 0.02ml of the HCl phase, 0.005 ml 0.4 ml HCl and 0.01ml EDTA/ Sodium Acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1ml sodium thiosulphate in 5 M Sodium hydroxide. 10 M Acetic acid was added 1.5 min later. The solution was then heated to 100°C for 6 min.

When the samples again reach room temperature, excitation and emission spectra were read (330 to 375 nm) in a spectrofluorimeter. Compared the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank). Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005 ml bidistilled water and 0.1 ml HCl Butanol to 20 ng of dopamine standard.¹⁹

2.11. Statistical analysis

All values are expressed as mean \pm SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

3. RESULTS

3.1. Effect of chronic EEMP treatment on HAL induced Vacuous chewing movements

The effect of EEMP on the HAL-induced VCMs. Significant increase in the number of VCMs was observed on the 2nd, 7th, 14th and 22nd days of treatment with HAL, EEMP 200 and 400 mg/kg treated groups when compared with HAL treated group. EEMP at a dose of 400 mg/kg significantly (P<0.05) decreased the VCMs when compared to HAL treated group as shown in Fig 1.

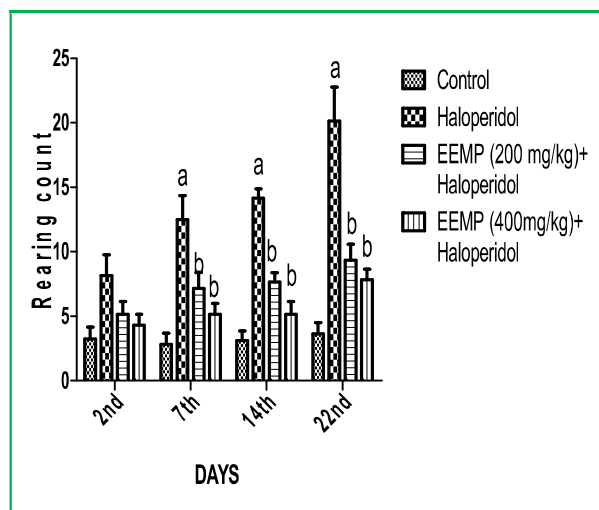


Figure 1: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) - induced vacuous chewing movements in rats. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.2. Effect of chronic EEMP treatment on HAL induced Tongue protrusions

The effect of EEMP on the HAL-induced TPs. Significant increase in the number of TPs was observed on the 2nd, 7th, 14th and 22nd days of treatment with HAL, EEMP 200 and 400 mg/kg treated groups when compared with HAL treated group. EEMP at a dose of 400 mg/kg significantly (P<0.05) decreased the TPs when compared to HAL treated group as shown in Fig 2.

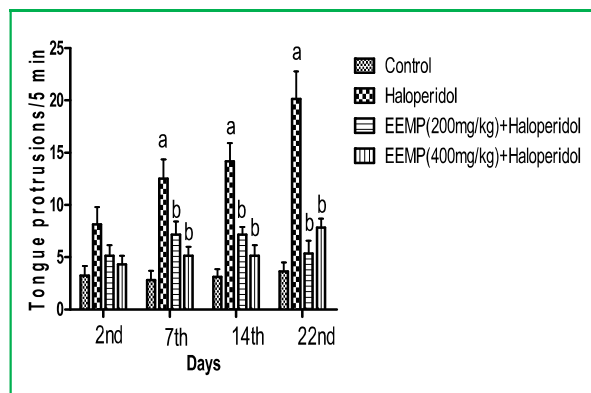


Figure 2: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL)-induced Tongue protrusions in rats. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.3. Effect of chronic EEMP treatment on elevated plus-maze performance of HAL treated rats

The transfer latency measured on days 2, 7, 14 and 22 the vehicle treated rats was drastically shorter than on the first day, indicating the ability of the rats to recall the learned aspect in a lesser period of time. However, the TL of HAL treated rats was found statistically significant (P<0.05) in comparison with vehicle treated animals on the respective day of observation, indicating the poor retention ability of

HAL treated rats. Chronic treatment with MEMP (200 and 400 mg/kg) significantly (P<0.05) and dose dependently shortened the TL latency of HAL treated rats as compared to HAL alone treated rats as shown in Fig 3.

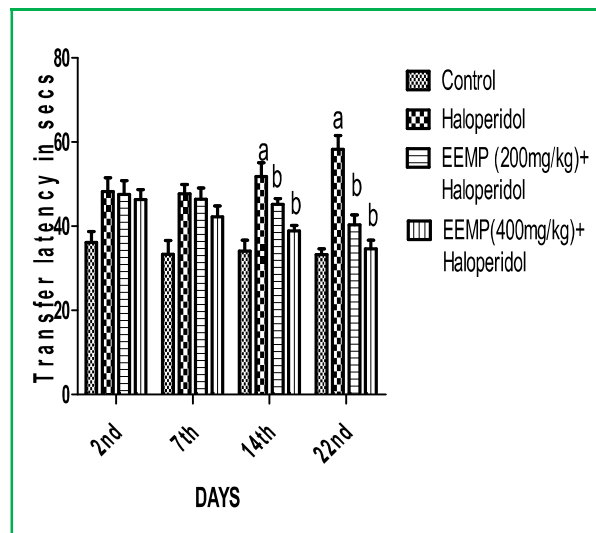


Figure 3: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL)-induced memory dysfunction in rats. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.4. Effect of chronic EEMP treatment on HAL induced alteration in locomotor activity

The effect of EEMP on the HAL-induced alteration in locomotor activity. HAL significantly decreased the locomotor activity (P<0.05) when compared to the control group. EEMP significantly reversed the HAL -induced decrease in locomotor activity as compared to the HAL group at doses of 200 and 400 mg/kg (P<0.05) as shown in Fig 4.

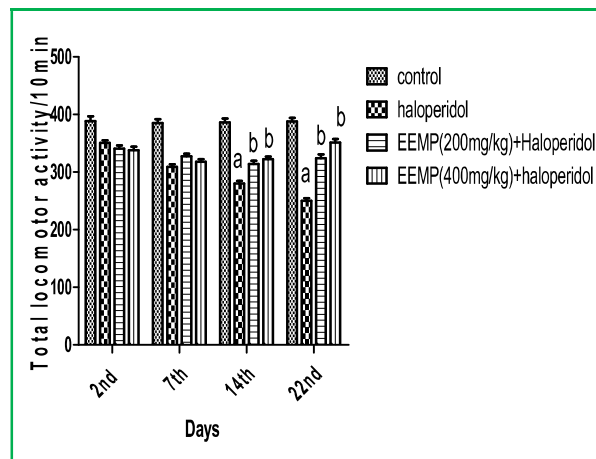


Figure 4: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL)-induced alteration in locomotor activity in rats. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.5. Effect of chronic EEMP on the brain antioxidant enzyme levels in chronic HAL treated rats

Chronic HAL treated rats showed decreased levels of antioxidant enzymes SOD and catalase in their brain homogenates. Chronic administration of EEMP (200 and 400 mg/kg) significantly reversed the HAL-induced decrease in brain SOD (Fig. 5) and catalase (Fig. 6) levels compared with only HAL treated rats.

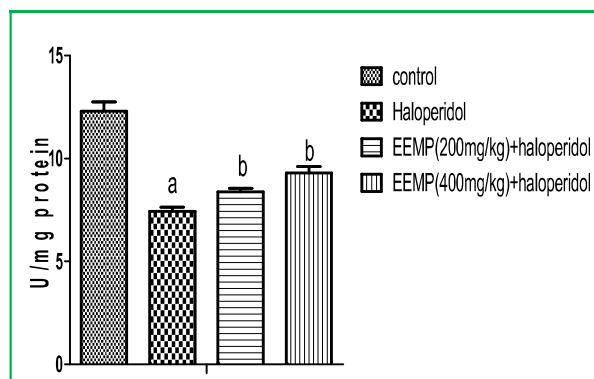


Figure 5: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated depletion in the level of brain antioxidant enzyme superoxide dismutase (SOD). Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

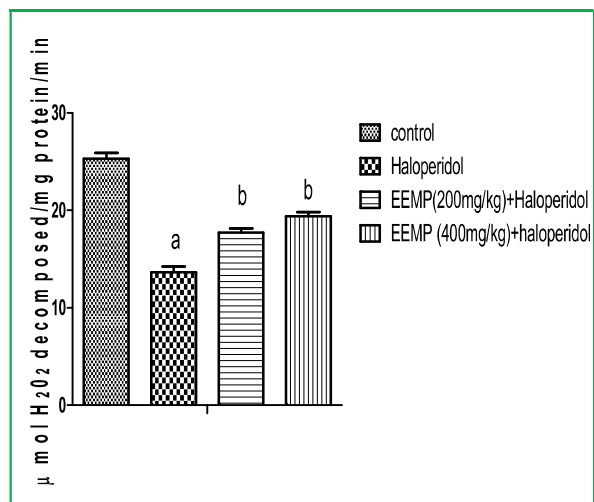


Figure 6: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated depletion in the level of brain antioxidant enzyme catalase (CAT). Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.6. Effect of chronic EEMP on the brain MDA level in chronic HAL treated rats

Chronic HAL treatment to rats on alternate days for 5 days induced lipid peroxidation as indicated by a significant (P<0.05) rise in brain MDA levels compared with the vehicle treated rats. Chronic administration of EEMP (200 and 400 mg/kg) to HAL treated animals significantly reversed the extent of lipid peroxidation compared with HAL only treated rats (Fig. 7).

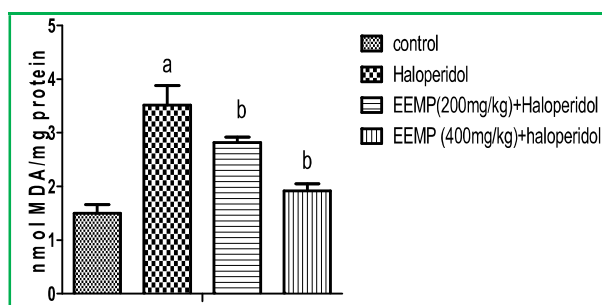


Figure 7: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated brain Malonyl dialdehyde (MDA) level. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.7. Effect of chronic EEMP on the brain glutathione (GSH) levels in chronic HAL treated rats

Statistical analysis of brain GSH levels showed a significant difference between the vehicle treated and HAL treated rats. However, chronic administration of EEMP (200 and 400 mg/kg) showed a significant increase in the level of GSH compared with HAL treated rats (Fig 8).

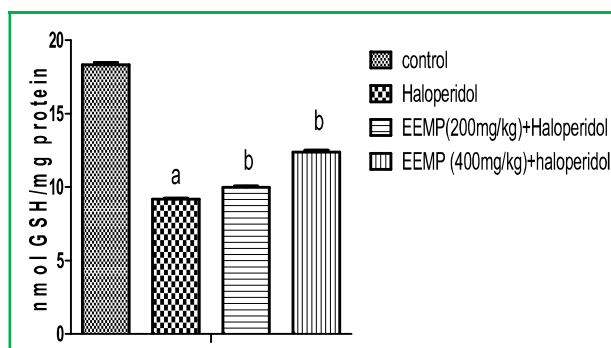


Figure 8: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated brain Reduced Glutathione (GSH) level. Values expressed as mean \pm SEM, n=6. ^aP < 0.05 compared with vehicle treated control group. ^bP < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.8. Effect of chronic EEMP on Dopamine levels in chronic HAL treated rats

As shown in Figure 9, the brain dopamine levels of HAL was significantly (P<0.05) reduced compared with the control group. However, groups administered with EEMP (200 and 400mg/kg) showed significant (P<0.05) increase in the level of dopamine in a dose dependent manner.

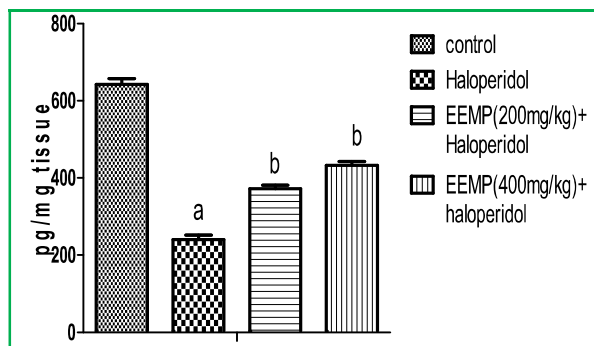


Figure 9: Effect of chronic administration of ethanolic extract of

Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain Dopamine level. Values expressed as mean \pm SEM, $n=6$. ^a $P < 0.05$ compared with vehicle treated control group. ^b $P < 0.05$ compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.9. Effect of chronic EEMP on L-Glutamate level in chronic HAL treated rats

Figure 10, shows the brain L-glutamate levels of HAL was significantly ($P < 0.05$) increased compared with the control group. However, groups administered with EEMP (200 and 400mg/kg) showed significant ($P < 0.05$) decrease in the level of L-Glutamate.

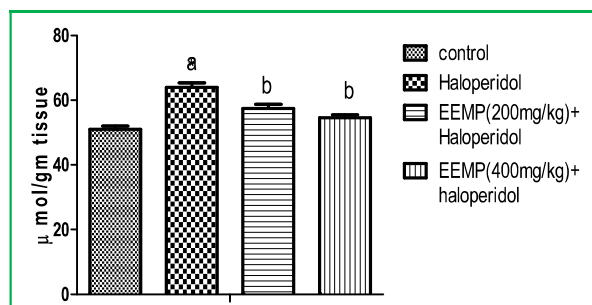


Figure 10: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated brain L-Glutamate level. Values expressed as mean \pm SEM, $n=6$. ^a $P < 0.05$ compared with vehicle treated control group. ^b $P < 0.05$ compared with HAL treated groups. (ANOVA followed by Dunnett's test).

3.10. Effect of chronic EEMP on the total protein levels in chronic HAL treated rats

HAL treated groups indicated a significant ($P < 0.05$) decrease in total protein content (fig.11) when compared with the vehicle treated group. Which was significantly increased by the EEMP treated groups when compared with HAL treated group.

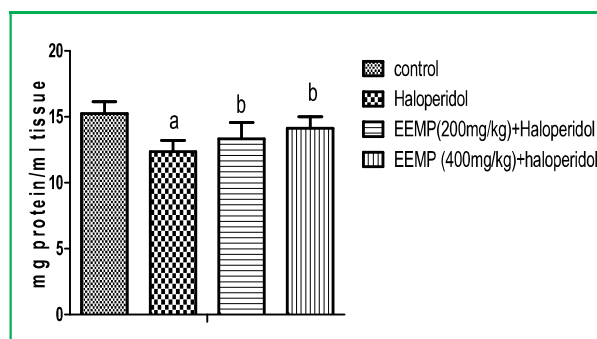


Figure 11: Effect of chronic administration of ethanolic extract of *Mucuna pruriens* (EEMP) on haloperidol (HAL) mediated brain Total protein level. Values expressed as mean \pm SEM, $n=6$. ^a $P < 0.05$ compared with vehicle treated control group. ^b $P < 0.05$ compared with HAL treated groups. (ANOVA followed by Dunnett's test).

4. DISCUSSION

The present study has revealed the protective effect of EEMP on haloperidol induced tardive dyskinesia. EEMP reversed the increased frequencies of VCM and TP induced by chronic haloperidol treatment. We also observed an increment in the levels of antioxidant

enzymes SOD and CAT in the treated groups. Lipid peroxidation was ameliorated in EEMP treated animals and significant improvement was also seen in transfer latency of rats on elevated plus maze and locomotor activity. Reports have indicated that excess production of free radicals (oxidative stress) are associated with neuroleptic use and might contribute to the onset of TD.²⁰ Oxidative stress is a shift towards oxidation in oxidation-reduction reaction, which leads to cellular damage and is indicated by oxidized products of lipids and proteins.²¹ This effect can be related to the reduction in specific antioxidant mechanisms, such as decreased GSH levels and low level of antioxidant defence system such as SOD and catalase.²² Sagara, using rat primary cortical neurons and mouse hippocampal cell line HT-22 showed that haloperidol caused a sequence of cellular alterations that lead to cell death and the production of reactive oxygen species.²³ Neuronal loss in the striatum of animals chronically treated with neuroleptics has been reported.^[24] Haloperidol induced TD and oxidative stress can be prevented by dietary supplements like antioxidants and essential fatty acids.²⁵

The commonly used animal model for TD is the rat model in which VCMs and TPs are induced by chronic neuroleptic treatment. Neuroleptics act by blocking dopamine receptor²⁶ and increase catecholamine turnover, which in turn leads to excessive free radical generation. Increased metabolism of catecholamines produce large amount of free radicals which are cytotoxic.²⁷ Haloperidol decreases the genetic expression of MnSOD, CuZnSOD and CAT and thus decreases the enzymatic activity of SOD, GSH, and CAT.²⁸ Chronic haloperidol treatment increases lipid peroxidation and also nucleic acid peroxidation.²⁹ SOD dismutates superoxide radicals to form hydrogen peroxide, which is decomposed to water and oxygen by glutathione peroxidase and catalase, thereby preventing formation of hydroxyl radicals.³⁰ Our findings of decreased enzymatic and nonenzymatic antioxidants and increased lipid peroxidation in haloperidol treated group support the involvement of oxidative stress in TD. EEMP dose dependently decreased the elevated lipid peroxidation levels and increased the levels of SOD and CAT.

Glutathione primarily acts by reducing inactive disulfide linkages of enzymes to active sulfhydryl group, while the sulfhydryl group of glutathione becomes oxidized. Hence glutathione plays an important role in protecting membrane peroxidation and also reduces hydrogen peroxide by the action of glutathione peroxidase.³¹ We observed increased levels of GSH which may further contribute to the antioxidant effect of *Mucuna pruriens*. Alcoholic extract of *Mucuna pruriens* has shown to possess antilipid peroxidation effect which is due to removal of superoxide and hydroxyl radicals. Hence the decreased level in lipid peroxidation in the treated group may be due to the elevated levels of antioxidant enzymes SOD and CAT. TD developed by chronic administration of haloperidol is associated with an increase in glutamate followed by calcium influx.³² The estimated glutamate levels in our study revealed a dose dependent reduction in glutamate level in EEMP treated animals. Increased density of dopamine receptors³³ and decreased levels of

dopamine³⁴ is observed in chronic haloperidol treatment. The decreased levels of dopamine may contribute to the production of excess free radicals. EEMP has shown an increase in dopamine levels which may suppress the development of supersensitivity due to increased levels of dopamine receptors.

Beyond the development of orofacial dyskinesia, haloperidol has been associated with learning deficits in animals.³⁵ MP has shown a 15% and 35% increase in learning and memory retrieval.³⁶ In the present study, co administration of EEMP with haloperidol, showed a significant improvement in memory, which might be due to the increased levels of neurotransmitters. All the first generation antipsychotics have shown to increase serum prolactin levels.³⁷ *Mucuna pruriens* has shown to inhibit the hyperprolactinemic effect of chlorpromazine in human. Thus the ability of *Mucuna pruriens* to inhibit increased levels of prolactin may have a protective action against TD. The ability to restore dopamine, norepinephrine and serotonin in substantia nigra, increased complex I activity and the phytoconstituents like flavonoids, saponins and terpenes present in the seeds of *Mucuna pruriens* may contribute to its neuroprotective effect.

Although further studies are needed to explain how *Mucuna pruriens* prevents the development of vacuous chewing movements and tongue protrusion in rats induced by chronic haloperidol administration, the correlation between TD and EEMP observed in the present study provide further evidence of the possibility that the antioxidant potential of EEMP and the ability of MPL to restore the neurotransmitter levels may have a protective role in the development of haloperidol induced dyskinesia.

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