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Evaluation of Antidyskinetic Effects of Ethanolic Extract of Mucuna Pruriens Seeds on Haloperidol-Induced Motor Dysfunction in Rats

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Abstract: *To determine if ethanolic extracts of Mucuna pruriens (EEMP) seeds may prevent tardive dyskinesia brought on by haloperidol. Using in-vivo metrics such as vacuous chewing motions, tongue protrusions, and transfer delay in raised plus maze, the effects of Mucuna pruriens ethanolic extracts were investigated. At two dosage levels (200 and 400 mg/kg), the biochemical parameters (SOD, CAT, GSH, Dopamine, glutamate, lipid peroxidation, and total protein content) of the mucuna pruriens ethanolic extracts were evaluated. At a dosage level of 400 mg/kg, Mucuna pruriens ethanolic extract had a notable protective effect against tardive dyskinesia caused by haloperidol. Antioxidant enzyme levels were found to be significantly elevated. The extract's capacity to inhibit lipid peroxidation and raise antioxidant enzyme levels may be the cause of its protective effects.*

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

I. INTRODUCTION

Popular Indian medicinal plant Mucuna pruriens Linn (MPL) has been utilized in Ayurvedic treatment for Parkinsonism. Impotence, snake bite, diabetes, cancer, and Parkinsonism are treated using plant roots, leaves, and seeds. The non-toxic endocarp of Mucuna pruriens controls hyperprolactinemia1 motor symptoms of Parkinson's disease animal models 2-3 times better than leavodopa. 2 In Parkinsonism animal models, Mucuna pruriens increases brain mitochondrial complex-I activity and restores dopamine and norepinephrine levels. 3 Seed phytochemistry showed 5-indolic substances including tryptamine and 5-hydroxytryptamine4, alkaloids such mucunine, mucunadine, prurine, and prurienine. 5

Haloperidol (HAL), a neuroleptic used to treat psychosis, may cause extrapyramidal movement disorders as tardive dyskinesia (TD), akathisia, dystonia, and Parkinsonism. 6 TD may be caused by oxidative stress. 7 Several studies have indicated that Mucuna pruriens seeds scavenge free radicals and boost antioxidant enzymes. Ethanolic extracts of Mucuna pruriens seeds (EEMP) were tested for their preventive efficacy against tardive dyskinesia caused by prolonged haloperidol administration in rats.

II. MATERIALS AND METHODS

Plant material

The MPL seeds were gathered in Kerala's Palakkadu district. June of 2008. Dr. Sasikala Ethirajulu of the Captain Sreenivasan Research Foundation in Chennai, Tamilnadu, India, discovered and verified the plant. The specimen voucher was placed at the C.L. Baid Metha College of Pharmacy's pharmacology and toxicology department in Chennai, Tamilnadu, India.

Preparation of the ethanolic extract of MPL.

Mucuna pruriens seeds that had just been harvested were ground into a coarse powder after being shade-dried. A weighed amount of the powder (950 g) was run through sieve number 40 and then heated to between 40 and 80°C

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using ethanol in a soxhlet device for solvent extraction. The marc was thoroughly dried and weighed both before to and after each extraction. In a rotating vacuum evaporator, the filtrate was evaporated to dryness at 40°C with lowered pressure. The result was a waxy, brownish-black residue. 18.7% w/w was the ethanolic extract yield as a percentage.

Phytochemical screening

The presence of chemical components in the newly made MPL seed extract was assessed qualitatively. The following chemicals and reagents were used to undertake phytochemical screening of the extract: Using Mayer's, Hager's, and Dragendorffs reagents for alkaloids; sodium acetate, ferric chloride, and amyl alcohol for flavonoids; lead acetate and gelatin for phenolic compounds and tannins; Molish, Fehling, and Benedict reagents for carbohydrates; and Millon, Biuret, and xanthoprotein tests for proteins and amino acids. Hemolysis was used to test saponins, Molish's reagent and Ruthenium red were used to test gum, 10% sodium hydroxide was used to test coumarin, and concentrated sulfuric acid was used to test quinones. By using conventional processes, they were recognized by distinctive color changes. 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.

Animals

For the pharmacological investigations, colony inbred strains of wistar rats weighing 150–250g of either sex were used. The animals were housed in polypropylene cages with regular circumstances (day/night rhythm) from 8 a.m. to 8 p.m. at room temperature of 22 °C. The regular pelleted meal (Hindustan Lever Pvt Ltd., Bangalore) and unlimited water were fed to the animals. Before the studies, the animals were kept in polypropylene cages for a week to become acclimated to the lab environment. Every experiment was conducted between 9:00 and 12:00. Throughout the trials, it is divided into four groups at random, each consisting of six animals, all of whom are kept in the same circumstances. The Institutional Animal Ethical Committee (IAEC) of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) gave its approval to the experimental procedure. (**IAEC Reference number: IAEC/XIII/10/CLBMCP/2007-2008 dated 20-04-07)**

Acute toxicity studies.

In accordance with OECD rules 423, an acute toxicity research was conducted on the extracts to determine a safe dosage using the acute oral toxic class approach. 9.

Induction of orofacial dyskinesia

We bought haloperidol from Sigma (Aldrich, USA). Rats were chronically given haloperidol (1.0 mg/kg, s.c.) for 21 days in order to cause orofacial dyskinesia. 10. Every behavioral evaluation was conducted 24 hours after the previous HAL dosage.

In vivo parameters

Behavioral assessment of orofacial dyskinesia

To evaluate oral dyskinesia, the rats were separately housed in a tiny (30 x 20 x 30 cm) plexiglass observation room on test day. Prior to behavioral evaluations, the animals were given ten minutes to acclimate to the observation room. The observer used a hand-operated counter to assess the number of vacuous chewing motions and tongue protrusions. In the current research, single mouth openings in the vertical plane that are not focused on tangible objects are referred to as vacuous chewing actions. Vacuous chewing motions or tongue protrusion that had place during a grooming session were not considered. Every time the rat started grooming, the count was halted, and it resumed when the grooming ceased. When the animal was looking away from the observer, mirrors were positioned behind the chamber's back wall and under the floor to allow for the observation of oral dyskinesia. For five minutes, the behavioral indicators of oral dyskinesia were regularly assessed. The scorer had no idea how the animals were being treated in any of the tests. 11.

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Transfer latency on elevated plus maze

The elevated plus-maze learning test, which gauges spatial long-term memory, was used to evaluate cognitive behavior. An indicator of learning and memory processes is transfer latency (TL), which is the amount of time it takes an animal to shift from an open arm to an enclosed arm. Two 50 x 10 cm open arms and two 50 x 10 x 40 cm closed arms with an open canopy made up the raised plus-maze. The labyrinth was raised fifty centimeters from the ground. On the first day, the transfer latency was recorded after each animal was positioned separately at the end of either open arm. The animal was gently pushed into an enclosed arm and the TL was set to 90 seconds if it did not enter within that time. After arriving at the closed arm, the animals were given 20 seconds to explore the plus-maze to get a feel for it before being sent back to their home cage. Twenty-four hours following the first day of the study, retention was assessed. In the current investigation, the rats treated with haloperidol had their transfer latency measured on days 2, 7, 14, and 22. Twelve

Locomotor activity

An actophotometer (IMCORP, India) was used to track the locomotor activity. The animals were individually put in the actophotometer and their overall activity count was recorded for ten minutes prior to being given a cognitive test. Total light beam interruption counts/10 minutes per animal were used to quantify locomotor activity. A rise in count was thought to be a stimulant activity of the central nervous system, whereas a fall in count was thought to be a depressive activity.

Biochemical studies

Dissection and homogenization

Animals treated with acute resperpine on day 29 of therapy (or the 29th day after the last resperpine injection) and chronic haloperidol on day 22 following behavioral quantification were decapitated. The brains were taken out, the cerebellum was thrown away, and the forebrain was dissected. The cortex, striatum, and subcortical areas of the brains were separated and weighed while they were placed on ice. A tissue homogenate containing 10% (w/v) was made in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 1000 × g for 20 minutes at 4°C to get the postnuclear fractions for the catalase test, and at 12,000 × g for 60 minutes at 4°C to acquire the postnuclear fractions for the other enzyme assays. All of the remaining forebrain components, including the hippocampus, thalamus, hypothalamus, and other subthalamic structures, were included in the subcortical portion of the brain. For dopamine measurement, one pair of animals from each group had their whole brains (not including the cerebellum) kept separately at 0°C. The cortical and subcortical (including striatal) regions of the forebrain were separated on the day of the experiment.

Estimation of antioxidant enzyme levels in rat brain.

Estimation of superoxide dismutase

0.25 ml of 100% ethanol and 0.15 ml of chloroform were added to 1 ml of the sample. The suspension was centrifuged after being shaken in a mechanical shaker for 15 minutes, and the resulting supernatant was the enzyme extract. 1.5 ml of water, 0.5 ml of 2 mM pyrogallol, and 2 ml of buffer made up the auto-oxidation reaction mixture. For three minutes, the rate of pyrogallol auto-oxidation was first recorded at one-minute intervals. Two milliliters of 0.1 M Tris-HCl buffer, half a milliliter of pyrogallol, aliquots of the enzyme preparation, and four milliliters of water comprised the enzyme test combination. Following the addition of the enzyme, the rate at which pyrogallol auto-oxidation was inhibited was recorded. The suppression of pyrogallol auto-oxidation at 420 nm for 10 minutes was used to gauge the superoxide dismutase activity. To bring about a 50% suppression of auto-oxidation by pyrogallol, one unit of superoxide dismutase is needed. Units/min/mg protein were used to represent the enzyme activity. 13.

Estimation of Catalase

Centrifuged the tissue after homogenizing it with M/15 phosphate buffer at 1–4°C. Cold phosphate buffer was used to stir the sediment, and it was then left to stand in the cold environment with periodic shaking. After one or two extractions, the supernatants are collected and employed in the experiment. At 240 nm, a control cuvette containing an enzyme solution devoid of H2O2 phosphate buffer was read against a cuvette containing 3 ml of

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H2O2 phosphate buffer and 0.01–0.04 ml of sample. A drop in optical density from 0.450 to 0.400 was observed. The computations were performed using this value. 14

Estimation of lipid peroxidation

1.5 ml of thiobarbituric acid, 0.2 ml of sodium dodecyl sulphate, 0.2 ml of tissue homogenate, and 1.5 ml of 20% acetic acid were added. After adding distilled water to bring the mixture's volume up to 4.0 ml, it was heated for 60 minutes at 95 °C in a water bath. Following incubation, the tubes were allowed to cool to room temperature, and each tube's final volume was adjusted to 5.0 ml. After adding 5.0 ml of the n-butanol pyridine (15:1) combination, the liquid was vigorously vortexed for two minutes. Following 10 minutes of centrifugation at 3000 rpm, the organic top layer was removed, and its optical density at 532 nm was measured against a suitable blank that did not include the sample. 15.

The brain homogenate's lipid peroxide levels were reported as n moles of malondialdehyde (MDA)/min/mg protein.

Estimation of reduced glutathione (GSH)

One milliliter of 10% TCA was used to precipitate one milliliter of tissue homogenate. Centrifugation was used to remove the precipitate. Four milliliters of phosphate solution and half a milliliter of DTNB reagent were added to an aliquot of the supernatant. At 420 nm, the color produced was measured. Glutathione content in tissue is measured in µg/mg protein. 16

Estimation of protein

Bovine serum albumin was used as a reference to assess the protein content of brain tissue. 17

Estimation of brain glutamate level

The brain portion was weighed, homogenized with two parts perchloric acid by weight, and centrifuged for ten minutes at 3000 rpm. Use 1.0 ml of phosphate solution to bring the 3.0 ml supernatant fluid's pH down to 9. After 10 minutes of standing in an ice bath, strain through a little piece of fluted filter paper. Take 1.0 ml for the test, dilute 1:10, and let warm to room temperature. At 340 nm, absorbance was measured. At 340 nm, a blank reading was also obtained.

18 The expression for the glutamate level was µmol/g tissue.

Estimation of dopamine level in rat brain by spectrofluorimetry

Preparation of tissue extract

Rats' whole brains were dissected and the subcortical region—including the striatum—was isolated on the day of the experiment. A predetermined amount of tissue was weighed and then homogenized in three milliliters of HCl Butanol in a cold setting. After that, the sample was centrifuged at 2000 rpm for 10 minutes. After being extracted, 0.8 ml of the supernatant phase was put into an Eppendorf reagent tube with 0.25 ml of 0.1 M HCl and 2 ml of heptane. To separate the two phases, shake the tube and centrifuge it under the same conditions after ten minutes. The aqueous phase was used for the dopamine test, whereas the upper organic phase was disposed of.

Dopamine assay

0.01 ml of EDTA/Sodium Acetate buffer (pH 6.9) and 0.005 ml of 0.4 ml HCl were added to 0.02 ml of the HCl phase. 0.01 ml of iodine solution was then added for oxidation. After two minutes, 0.1 ml of sodium thiosulphate was added to 5 M sodium hydroxide to terminate the process. After 1.5 minutes, 10 M acetic acid was added. After that, the solution was heated for six minutes to 100°C.

Excitation and emission spectra (330–375 nm) were recorded in a spectrofluorimeter after the materials had returned to ambient temperature. compared an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank) with the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank). The oxidation step reagents were added in reverse order (sodium thiosulphate before iodine) to provide tissue blanks for the test. 20 ng of dopamine standard was mixed with 0.005 ml bidistilled water and 0.1 ml HCl Butanol to create internal reagent standards. 19.

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Statistical analysis

The mean ± SEM is used to represent all values. Non-parametric ANOVA and Dunnett's multiple comparison tests were used to examine the data, while Graph Pad PRISM software was used to assess the remaining data. P-values less than 0.05 were regarded as substantially different.

III. RESULTS

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.

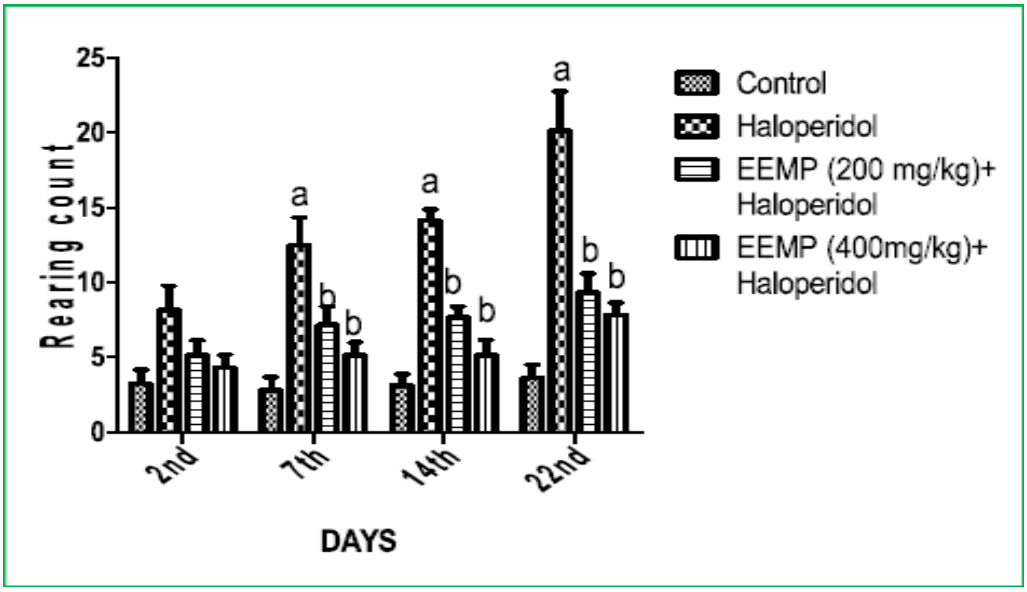

Figure 1: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) - induced vacuous chewing movements in rats. Values expressed as mean ± SEM, n=6. [a]P <0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA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

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

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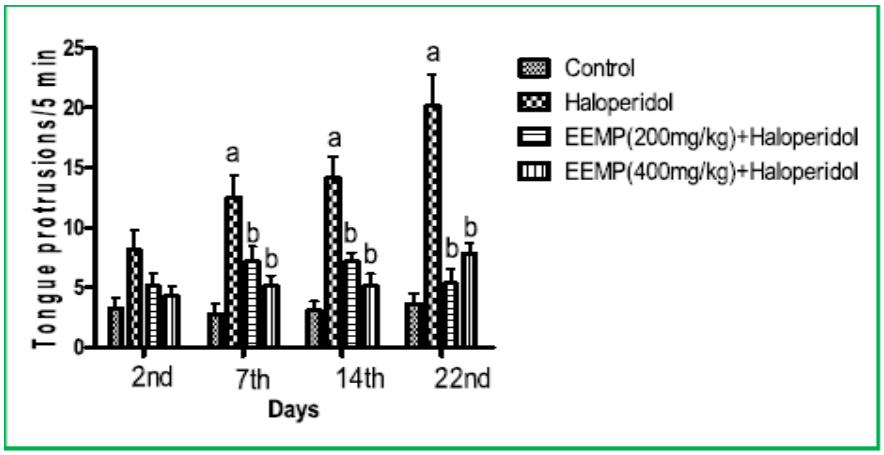

Figure 2: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL)-induced Tongue protrusions in rats. Values expressed as mean ± SEM, n=6. aP < 0.05 compared with vehicle treated control group. bP < 0.05 compared with HAL treated groups. (ANOVA followed by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

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

The vehicle-treated rats' transfer latency on days 2, 7, 14, and 22 was much lower than on the first day, suggesting that they could remember the learnt material faster. The poor retention capacity of HAL-treated rats was shown by the fact that, on the day of observation, the TL of HAL-treated rats was statistically significant (P<0.05) when compared to vehicle-treated animals. As seen in Figure 3, the TL latency of rats treated with HAL was considerably (P<0.05) and dose-dependently decreased by chronic treatment with MEMP (200 and 400 mg/kg) in comparison to rats treated with HAL alone

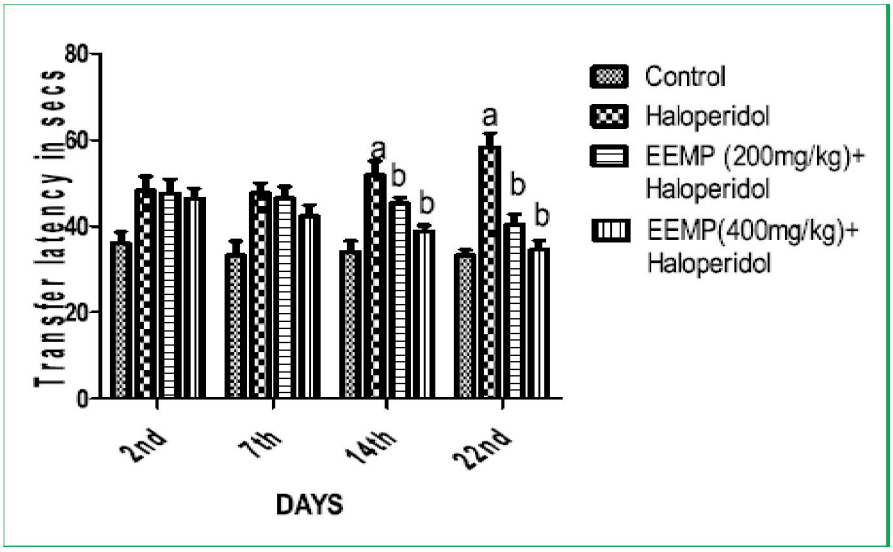

Figure 3: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL)-induced memory dysfunction in rats. Values expressed as mean ± SEM, n=6. aP < 0.05 compared with vehicle treated control group. bP <
0.05 compared with HAL treated groups. (ANOVA followed by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

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

The impact of EEMP on the change in locomotor activity brought on by HAL. The locomotor activity was considerably reduced ($P<0.05$) by HAL in comparison to the control group. Figure 4 shows that at dosages of 200 and 400 mg/kg, EEMP effectively corrected the HAL-induced reduction in locomotor activity when compared to the HAL group ($P<0.05$).

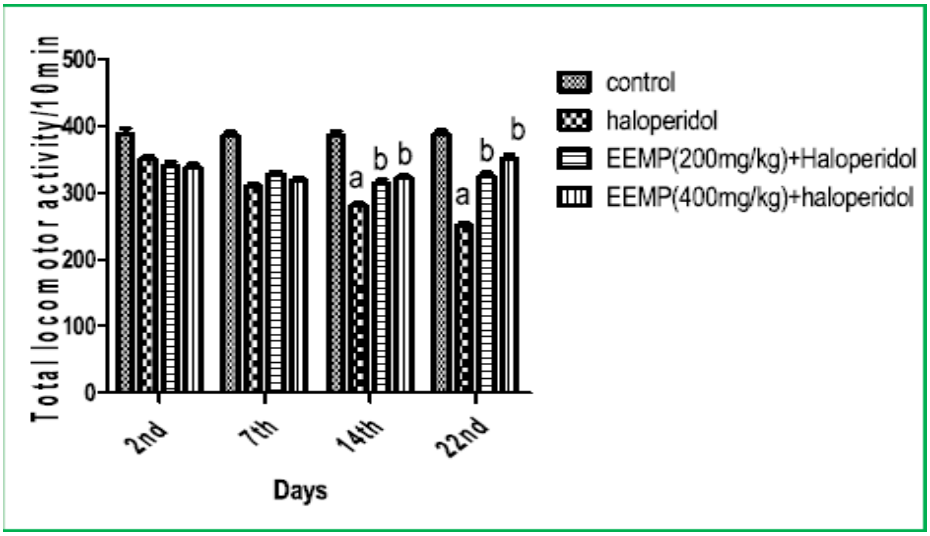

Figure 4: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL)-induced alteration in locomotor activity in rats. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups.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

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

Rats given chronic HAL had lower brain homogenates of the antioxidant enzymes SOD and catalase. When compared to rats that were solely given HAL, the chronic treatment of EEMP (200 and 400 mg/kg) dramatically reversed the decline in brain SOD (Fig. 5) and catalase (Fig. 6) levels caused by HAL.

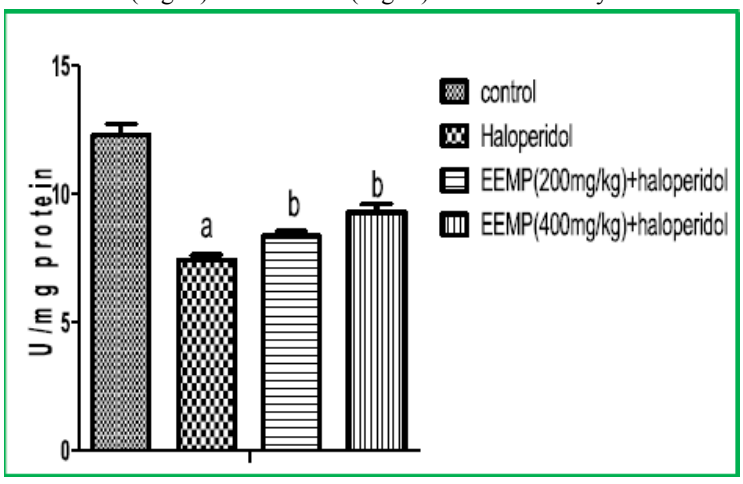

Figure 5: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated depletion in the level of brain antioxidant enzyme superoxide dismutase (SOD). Values expressed as mean ± SEM, n=6. [a]P <0.05 compared with vehicle treated control group. [b]P < 0.05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

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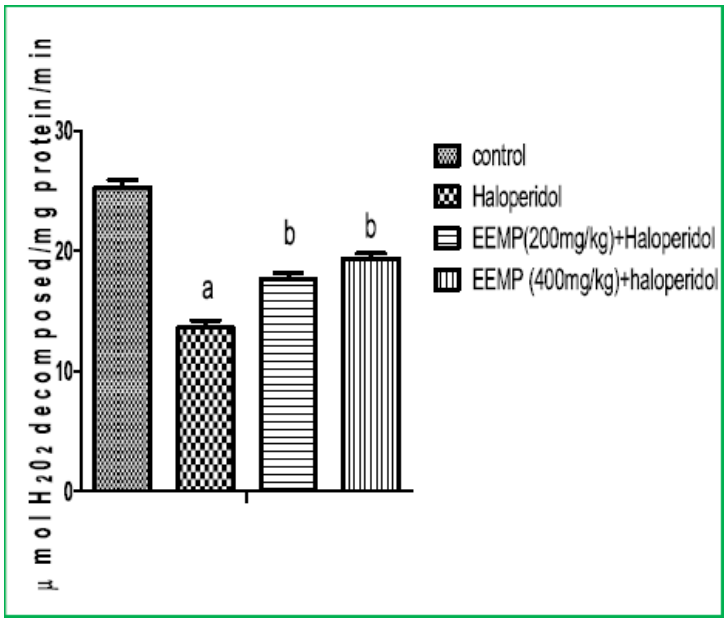

Figure 6: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated depletion in the level of brain antioxidant enzyme catalase (CAT). Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared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

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

When compared to rats treated with a vehicle, animals given chronic HAL on alternate days for five days showed a substantial (P<0.05) increase in brain MDA levels, indicating lipid peroxidation. When EEMP (200 and 400 mg/kg) was administered over an extended period of time to rats treated with HAL, the degree of lipid peroxidation was considerably reversed (Fig. 7).

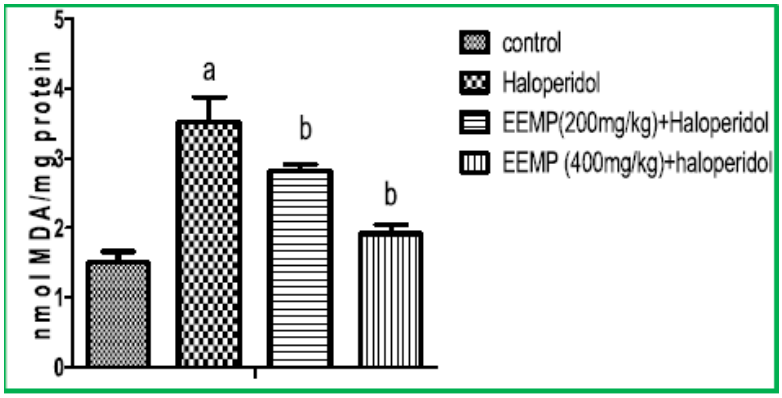

Figure 7: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain Malonyl dialdehyde (MDA) level. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA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

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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).

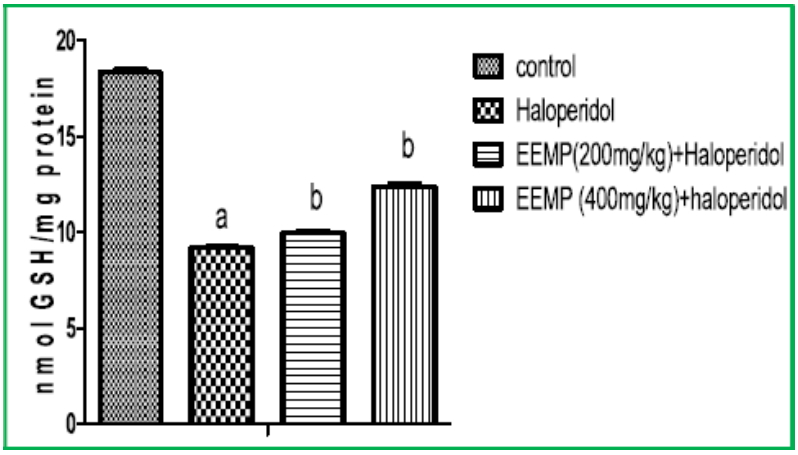

Figure 8: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain Reduced Glutathione (GSH) level. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA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

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

Figure 9 illustrates that HAL's brain dopamine levels were considerably (P<0.05) lower than those of the control group. However, dopamine levels in the groups given EEMP (200 and 400 mg/kg) increased significantly (P<0.05) in a dose-dependent manner.

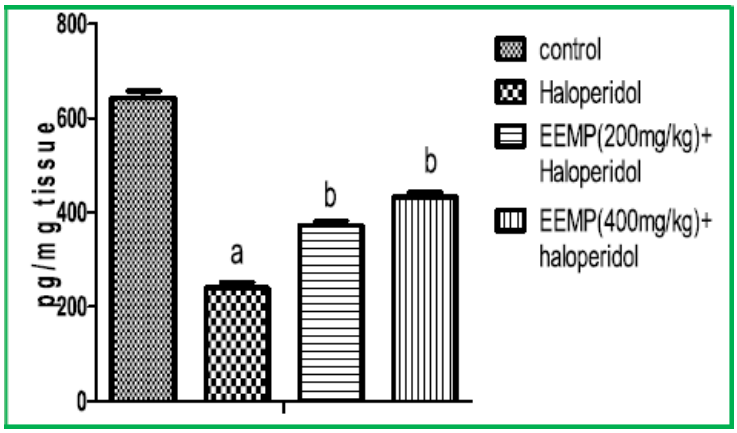

Figure 9: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain Dopamine level. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA followed by Dunnett's test).

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

Figure 10 demonstrates that, in comparison to the control group, the brain L-glutamate levels of HAL were considerably (P<0.05) higher. However, there was a substantial (P<0.05) drop in L-glutamate levels in the groups who received EEMP (200 and 400 mg/kg).

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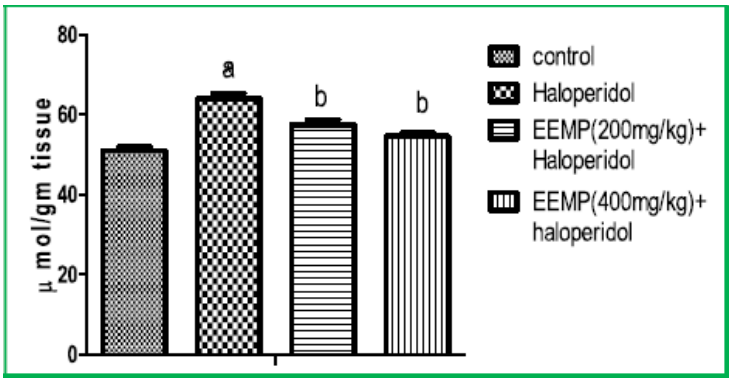

Figure 10: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain L-Glutamate level. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA followed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

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

When compared to the vehicle-treated group, the HAL-treated groups showed a substantial (P<0.05) reduction in total protein content (fig. 11). which, in contrast to the HAL-treated group, was considerably raised by the EEMP-treated groups.

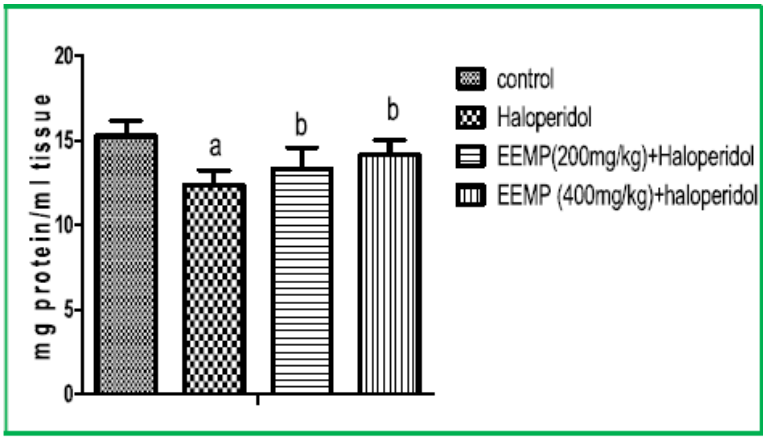

Figure 11: Effect of chronic administration of ethanolic extract of Mucuna pruriens (EEMP) on haloperidol (HAL) mediated brain Total protein level. Values expressed as mean ± SEM, n=6. [a]P < 0.05 compared with vehicle treated control group. [b]P < 0.05 compared with HAL treated groups. (ANOVA followed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

IV. DISCUSSION

This research found that EEMP protects against haloperidol-induced tardive dyskinesia. Chronic haloperidol therapy raised VCM and TP frequencies, whereas EEMP restored them. The treated groups also had higher SOD and CAT levels. EEMP reduced lipid peroxidation and improved rats' transfer latency on raised plus maze and locomotor activity. Research suggests that neuroleptic usage may cause TD by increasing free radical generation (oxidative stress). 20 Oxidated lipids and proteins suggest oxidative stress, which damages cells. 21 This impact may be caused by low GSH levels and SOD and catalase levels. 22 In rat primary cortical neurons and mouse hippocampus cell line HT-22, Sagara found that haloperidol promoted cell death and reactive oxygen species production. 23 Chronically treated mice with neuroleptics lose striatal neurons. [24] Food supplements including antioxidants and essential fatty acids help reduce haloperidol-induced TD and oxidative stress. 25

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Chronic neuroleptic therapy induces VCMs and TPs in rats, the most frequent TD animal model. Neuroleptics inhibit dopamine receptor26 and enhance catecholamine turnover, causing increased free radical production. High catecholamine metabolism produces harmful free radicals. 27 Haloperidol reduces MnSOD, CuZnSOD, and CAT genetic expression and enzymatic output of SOD, GSH, and CAT. 28 Lipid and nucleic acid peroxidation increase with chronic haloperidol use. 29 SOD dismutases superoxide radicals to hydrogen peroxide, which glutathione peroxidase and catalase degrade to water and oxygen, avoiding hydroxyl radicals. 30 Haloperidol treatment lowered enzymatic and nonenzymatic antioxidants and enhanced lipid peroxidation, supporting oxidative stress in TD. EEMP dose-dependently reduced lipid peroxidation and enhanced SOD and CAT.

Glutathione oxidizes its sulfhydryl group and reduces enzyme disulfide connections to active sulfhydryl groups. Thus, glutathione peroxidase lowers hydrogen peroxide and protects membranes. 31 Mucuna pruriens' antioxidant impact may be enhanced by higher GSH levels. Due to superoxide and hydroxyl radical elimination, Mucuna pruriens alcohol extract reduces lipid peroxidation. Thus, greater SOD and CAT levels may explain the treated group's lower lipid peroxidation. Chronic haloperidol treatment increases glutamate and calcium influx. 32 Our investigation found EEMP-treated mice had dose-dependent glutamate reductions. Dopamine receptor density33 and levels34 increase in chronic haloperidol therapy. Dopamine deficiency may increase free radicals. EEMP increases dopamine levels, which may reduce supersensitivity owing to dopamine receptors.

Other than orofacial dyskinesia, haloperidol may cause learning problems in animals. 35 MP improves learning and memory between 15% and 35%. 36 In this research, EEMP and haloperidol co-administration improved memory, perhaps owing to higher neurotransmitter levels. All first-generation antipsychotics raise serum prolaction. 37 Mucuna pruriens inhibits chlorpromazine's hyperprolactinemic action in humans. Thus, Mucuna pruriens' prolactin-inhibiting effect may protect against TD. Mucuna pruriens seeds may protect the brain by restoring dopamine, norepinephrine, and serotonin in the substantia nigra, increasing complex I activity, and containing flavonoids, saponins, and terpenes.

Although more research is needed to determine how Mucuna pruriens prevents vacuous chewing movements and tongue protrusion in rats induced by chronic haloperidol administration, the correlation between TD and EEMP in this study suggests that EEMP's antioxidant potential and MPL's ability to restore neurotransmitter levels may protect against it.

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