

# Antiparkinsonian and Antioxidant Effects of Hydroalcoholic Extract of *Camellia sinensis*, *Asparagus racemosus*, *Mucuna pruriens* and their Combination

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## ABSTRACT

**Objectives:** Because of environmental risk factors Parkinson's disease rate doubled in last decades. In upcoming decades rate of Parkinson's disease is expected to be 12 million people in aging population. Present treatments are having huge side effects and requires more combinations. Hence objective of this study is to provide herbal combination for Parkinson's disease with reduced side and adverse effects. **Methods:** The antiparkinsonian activity of HECS, HEAR, HEMP and Mixture was evaluated by using haloperidol induced catalepsy, reserpine induced hypolocomotion, tacrine induced vacuous chewing movements and orofacial brusts. Antioxidant activity was assessed by using DPPH radical and H<sub>2</sub>O<sub>2</sub> scavenging assay. The results were analyzed by repeated measure ANOVA followed by Dunnett's test. **Results:** Significant reduction ( $P < 0.05$ ) in haloperidol-induced catalepsy was observed in the all groups at the doses of 30 and 100 mg/kg when given orally. Mixture 30 mg/kg showed extremely significant ( $P < 0.001$ ) reduction in duration of catalepsy. Pretreatment with Mixture at 100mg/kg was significantly ( $P < 0.05$ ) reduces Reserpine induced hypolocomotion which is more significant as compared with other treatments. Similarly, HECS 30 mg/kg and mixture was more effective ( $P < 0.05$ ) than remaining extracts in reducing tacrine induced Vacuous

chewing movements. In tacrine induced orofacial brust Mixture and HEAR 30 mg/kg shows extremely significant ( $P < 0.001$ ) reduction of orofacial brusts. Similarly Mixture and HECS 100 mg/kg is more effective than other treatment in tacrine induced tongue protrusion. In DPPH scavenging assay, all the extracts exhibited free radical scavenging activity. In DPPH assay the IC<sub>50</sub> value of ascorbic acid, HECS, HEAR, HEMP and Mixture (1:1:1) was 14.99, 18.44, 26.51, 23.19 and 20.47 µg/ml respectively. **Conclusion:** 1:1:1 mixture show extremely significant antioxidant and anti-parkinsonian activity as compare with individual hydroalcoholic extract. Thus the studied combination possess potent antiparkinsonian effect.

**Key words:** Antioxidant, Antiparkinsons, *Camellia sinensi*, *Asparagus racemosus*, *Mucuna prurines*, Haloperidol, Reserpine, Tacrine.

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## INTRODUCTION

Parkinson's Disease (PD) is a complex multi-framework, neurodegenerative sickness. In spite of the fact that dominantly saw as motor ailment, it likewise has incapacitating non-motor highlights, which are as often as possible missed and not treated. The degeneration of dopaminergic neurons is viewed as the underlying driver of the trademark traditional engine side effects and non-motor indications. Significant treatment objectives are to increase striatal dopamine levels with antecedent replacement and additionally decrease its breakdown. As the ailment advances, a consistent increment in the portion of levodopa is inescapable. Be that as it may, higher dosages cause engine intricacies of dyskinesia and dystonia and bargain clinical treatment. Based on the studies, it is known that mitochondrial dysfunction, Neuronal death in substantial nigra and changed oxidative stress are the two crucial cellular stress parameters playing important role in PD pathogenesis. In parkinsons disease tremors, bradykinesia, stiffness in limbs and torso and postural instability are the four main symptoms. The focal point of the executives is the alleviation of the clinically prevailing motor side effects of PD. In any case, presently, there is an expanding acknowledgment of the non-motor highlights of PD, which additionally need consideration. These highlights are rest aggravations, constipation, psychological decay, melancholy, dread, tension, bladder

issues, weight changes, weakness and loss of vitality, autonomic brokenness/hypotension and sexual issues. These can be predominant and incapacitating in a sizeable number of patients, influencing a mind-blowing nature. PD additionally has pre-malady manifestations like blockage and loss of smell, decreased outward appearances, soft tone, rest changes, wooziness which go before months to decades before clinical determination of PD. The current restorative technique against PD essentially depends on reestablishing the ideal degree of dopamine (DA) and its related flagging pathways, for which Levodopa or L-DOPA (L-3, 4-dihydroxyphenylalanine), a forerunner of dopamine is regulated to the PD patients. L-DOPA gives introductory advantage by hindering the malady movement; be that as it may, long haul benefits are impossible. Additionally, it is likewise directed in blend with carbidopa, a fringe decarboxylase inhibitor. This aides in reducing the reaction of L-DOPA which principally incorporate gastrointestinal and cardiovascular issues. Another procedure for PD treatment is the utilization of monoamine oxidase B (MAO-B) inhibitors. The movement of MAO-B compound is expanded by virtue of DA metabolism which hoists oxidative stress and mitochondrial dysfunctions. Until this point, mitochondrial brokenness and adjusted oxidative stress are viewed as the conceivable system prompting neuronal cell death. Therefore, therapeutic approaches

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that optimize ROS and enhance mitochondrial function are being thoroughly considered in the current scenario. There is a generous extension to benefit of the modalities of conventional information in the administration for both the non-motor and pre-disease highlights of PD. Exploration during the most recent multi decade has distinguished a few plants that show restorative property against neurodegenerative issues like PD and AD.<sup>1,2</sup> Therefore, the present study aims to investigate antioxidant and neuroprotective activity of *Mucuna pruriens*, *Camellia sinensis* and *Asparagus racemosus* in combination to get rid from side effects of L-DOPA and also development of novel therapeutic approach against PD to address each cardinal sign of PD by using these herb with different potential.

*Mucuna pruriens* L. (Family: *Fabaceae*) is a tropical legume plant usually known as velvet beans. *Mucuna pruriens* is generally exploited for the treatment of Parkinsonism and dissimilar sorts of sexual debilities. It also acts as an aphrodisiac, anti-snake venom, antidiabetic, antioxidant and have a strong potential to improve the fertility of sperm. It has immense medicinal properties specifically due to high L-DOPA (L-3,4-dihydroxyphenylalanine) content and its role in parkinsons disease. Besides L-DOPA it also contains glycoside, gallic acid, glutathione, Levodopa. It improves locomotor and behaviour function, alleviate oxidative stress, metal chelation, mitochondrial and synaptic function.<sup>3,4</sup>

*Camellia sinensis* is popularly known as Green Tea belonging to Theaceae family. Green tea and its bioactive constituents are most popular for their cell reinforcement properties, prompting clinical examinations in ailments related with responsive oxygen species, for example, malignancy or cardiovascular and neurodegenerative maladies. The most important phytoconstituents of *Camellia sinensis* are polyphenolic compounds known as catechins including Epigallocatechin Gallate (EGCG), Catechin (C), Epicatechin (EC), Gallocatechin (GC), Gallocatechin Gallate (GCG), Epigallocatechin (EGC) and Epicatechin Gallate (ECG). Flavonols add values to the oxidative damage prevention capacities of tea leaves. The aglycones of the fundamental flavonols in tea leaves are quercetin, kaempferol and myricetin. Polyphenols, for example, epigallocatechin gallate, because of their mitigating and antioxidant impacts, are known to introduce neuroprotective properties, what could clarify their advantages in neurodegenerative illnesses.<sup>5-7</sup>

*Asparagus racemosus*, otherwise called *shatavari* (family Asparagaceae), is a significant therapeutic plant endemic to tropical India and Thailand. It is notable medication in Ayurvedic rasayana that forestall maturing, increment life span and improve mental capacity. Also, it is suggested in Ayurvedic messages for the avoidance and treatment of gastric ulcers, dyspepsia and as a galactagogue. The significant dynamic mixes in the root are steroidal saponins, for example, asparacoside, shatavarin IV, V and XI and different constituents, for example, racemosol and asparagamine. It indicated high free radical searching just as neurotropic modulatory properties in infections related with neuron cell misfortune. The anti-PD compounds present in *A. racemosus* include racemosol and rhamnose.<sup>8,9</sup>

The neuroprotective effects of *Mucuna pruriens*, *Camelia sinensis* and *Asperagus racemosus* extract in PD animal model have been reported by many researchers. However, the neuroprotective effect of combination is not reported hence present study aims at studying antiparkinsonian and antioxidant activity of these herbs in order to minimized side effects of available therapies and addressed motor and non-motor symptoms.

## MATERIALS AND METHODS

### Experimental Animals

Experiments were performed utilizing male Wistar rodents (Rats) weighing 180-200g. Creatures were kept up at 22°C ± 2°C on a

standard pellet diet and tap water *ad libitum*. Institutional Animal Ethics Committee for Animal Experiments of Sanjivani College of Pharmaceutical Education and Research, Kopargaon approved the study under the protocol SCPER/CPCSEA/IAEC/2019-20/01 and all experiments were conducted in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Behavioral tests were performed during the light cycle between 10.00 a.m. and 4.00 p.m.

### Drugs and Chemicals

Tacrine and Reserpine were purchased from Sigma, Aldrich, Mumbai. All other chemicals used were of analytical grade and purchased from standard manufacturer.

### Plant material and extraction

Dry powder of *Mucuna pruriens* seeds, *Asperagus racemosus* roots and *Camelia sinensis* leaves was purchased from local market and were authenticated from Department of Pharmacognosy, Sanjivani College of Pharmaceutical Education and Research, Kopargaon.

Hydroalcoholic extracts were prepared using Soxhlet's extractor. The extracts were filtered and dried. Extracts were subjected to phytochemical screening.<sup>10</sup> The extracts were administered in doses of 30 and 100 mg/kg (p.o.). Control group was given only vehicle in equivalent volume of plant extract.

### Experimental design

Animals were randomly divided into following groups of 5 animals each.

Group I - Control

Group II - Haloperidol (0.5 mg/kg) or Reserpine (1 mg/kg) or Tacrine (5 mg/kg),

Group III - HEMP (30 mg/kg),

Group IV - HEMP (100 mg/kg), Group V - HECS (30 mg/kg),

Group VI - HECS (100 mg/kg),

Group VII - HEAR (30 mg/kg),

Group VIII - HEAR (100 mg/kg),

Group IX - 1:1:1 Mixture (30 mg/kg),

Group X - 1:1:1 Mixture (100 mg/kg).

These groups were used for treatment of Parkinson's symptoms.

### Assessment of anti-parkinsonian activity

#### *Haloperidol induced catalepsy*

Male Wistar rats (weighing 180–200 g) were divided into ten groups of five each. Rats were pretreated with vehicle, HECS (30 and 100 mg/kg, p.o.), HEAR (30 and 100 mg/kg, p.o.), HEMP (30 and 100 mg/kg, p.o.), 1:1:1 HECS:HEAR:HEMP (30 and 100 mg/kg, p.o.) 30 min before haloperidol (0.5 mg/kg, intra-peritoneally). Post administration of haloperidol duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 and 180 min utilizing bar test. Both the forepaws of the animals were placed on a wooden bar raised above the ground. The cutoff time (time for which animal was placed on elevated bar) was 300 sec.<sup>11,12</sup>

#### *Tacrine induced jaw movements*

The observation chamber consisted of a clear Plexiglas box measuring 28×28×28 cm<sup>3</sup>, which had a wire mesh floor. The box was elevated 42 cm from the surface of the table, allowing behavioral observation from all angles. Rats were divided into groups and treated with vehicle, HECS (30 and 100 mg/kg, p.o.), HEAR (30 and 100 mg/kg, p.o.), HEMP (30 and 100 mg/kg, p.o.) and 1:1:1 HECS:HEAR:HEMP (30 and 100 mg/kg, p.o.). After 20 min, tacrine (5 mg/kg i.p.) was administered and the

number of chewing movements, orofacial bursts and tongue protrusions were measured every ten min for 60 min.<sup>13,14</sup>

### Reserpine-induced hypolocomotion

Reserpine was injected intraperitoneally at a dose of 1 mg/kg in a suspension with Tween-80, 1 h after the mice were treated with vehicle, HECS (30 and 100 mg/kg, p.o.), HEAR (30 and 100 mg/kg, p.o.), HEMP (30 and 100 mg/kg, p.o.) and 1:1:1 HECS:HEAR:HEMP (30 and 100 mg/kg, p.o.). The effect of the study compounds on reserpine-induced hypolocomotion was assessed using Actophotometer (Dolphine, India). The locomotor activity of the animals was measured for 2 min, at 2 h, 3h and 4h after reserpine had been administered.<sup>15</sup>

### Antioxidant activity

#### DPPH scavenging assay

The free radical scavenging activity of the HEMP, HECS and HEAR was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of HEMP, HECS and HEAR solution in water at various concentrations (2-1000 µg/ml). The mixture was incubated for 45 min at room temperature and the absorbance was measured at 517 nm against the corresponding blank solution. Ascorbic acid was used as reference standard. Percentage inhibition of DPPH free radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = [(Ac - At)/Ac] \times 100$$

Where *Ac*: absorbance of the control and *At*: absorbance of the extract or reference standard. The antioxidant activity was expressed as IC<sub>50</sub>. The IC<sub>50</sub> value was defined as the concentration in µg/ml of the extract that inhibits the formation of DPPH radicals by 50%.<sup>16-18</sup>

### Statistical analysis

Results were expressed as mean ± SEM. Significant differences between groups were determined by analysis of variance test followed by Dunnett's test.

## RESULTS

### Haloperidol Induced catalepsy

In haloperidol induced cataleptic animal model mixture 30 mg/kg shows extremely significant reduction in cataleptic phase as shown in Figure 1. Followed by mixture 30 mg/kg, 100 mg/kg also shows extremely significant reduction in catalepsy. Almost all the hydroalcoholic extracts shows significant reduction in catalepsy induced by haloperidol but when combined in 1:1:1 proportion mixture shows extremely significant reduction with reduced dose of individual extract of *Camellia sinensis*, *Asparagus racemosus* and *Mucuna pruriens* as revealed in Figure 1.

### Reserpine Induced Hypolocomotion

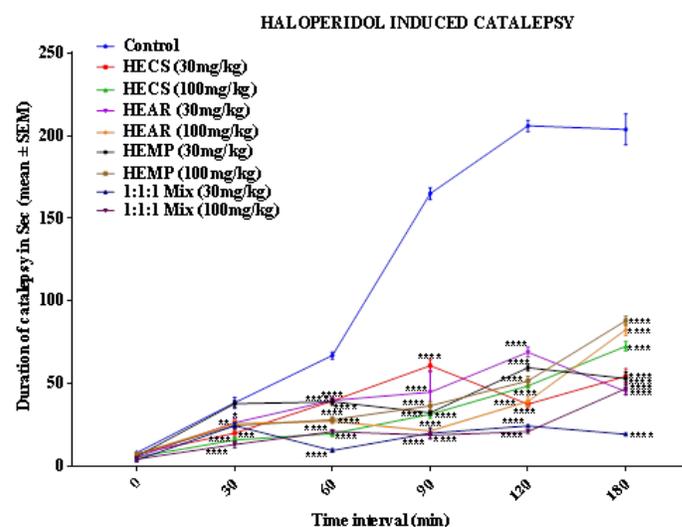
Hydro alcoholic extract of *Camellia sinensis*, *Asparagus racemosus* and *Mucuna pruriens* are studied for locomotor activity and hypolocomotion was induced with reserpine. In reserpine induced hypolocomotion hydro alcoholic extract shows significant increase in locomotor activity but as compare with low dose of 30 mg/kg, 100 mg/kg of HECS, HEAR and mixture shows more significant increase in locomotor activity. Mixture of 100 mg/kg shows extremely significant increase in locomotion and mixture 30 mg/kg shows very significant effect at first and third hour as exposed in Figure 2.

### Tacrine Induced Vacuous Chewing Movement (VCM)

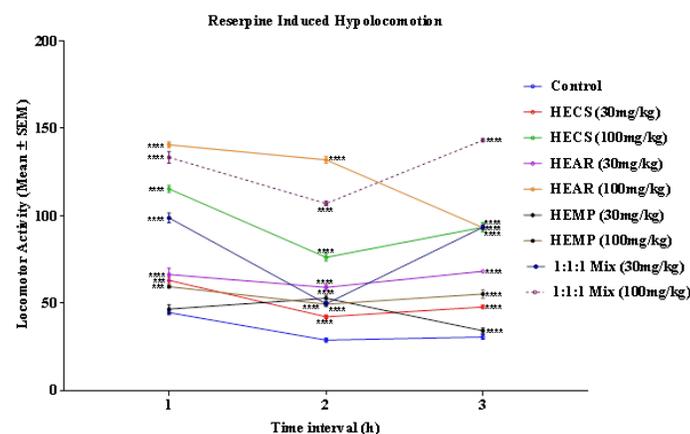
In tacrine induced vacuous chewing movements hydroalcoholic extract of *Camellia sinensis* at dose of 100 mg/kg shows extremely significant reduction in vacuous chewing movements followed by 30 mg/kg. Mixture of 30 mg/kg and 100 mg/kg subsequently shows very significant reduction in tacrine induced vacuous chewing movements followed by significant reduction observed in HEAR and HEMP at 30 and 100 mg/kg dose as presented in Figure 3.

### Tacrine Induced Orofacial Brust (OB)

In tacrine induced orofacial brust mixture at 30 mg/kg shows extremely significant reduction as compare to 100 mg/kg. Apart from that HECS 100 mg/kg and HEAR 30 mg/kg individually shows extremely significant reduction in orofacial brust. HECS 30 mg/kg, HEAR 100 mg/kg, HEMP 30 mg/kg and HEMP 100 mg/kg shows significant reduction in tacrine induced orofacial brust individually as shown in Figure 4.



**Figure 1:** Effect of HECS, HEAR, HEMP and its combination on Haloperidol induced catalepsy. All the values are expressed as mean ± SEM; n=5, \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, \*\*\*\**p*<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).



**Figure 2:** Effect of HECS, HEAR, HEMP and its combination on Reserpine induced Hypolocomotion. All the values are expressed as mean ± SEM; n=5, \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, \*\*\*\**p*<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).

### Tacrine Induced Tongue Protrusion (TP)

In tacrine induced tongue protrusion Mixture 30 mg/kg and 100 mg/kg shows extremely significant reduction in tongue protrusion along with individual treatment of HECS 30 mg/kg, HECS 100 mg/kg, HEAR 30 and 100 mg/kg, HEMP 30 and 100 mg/kg shows significantly reduction in tongue protrusion as displayed in Figure 5.

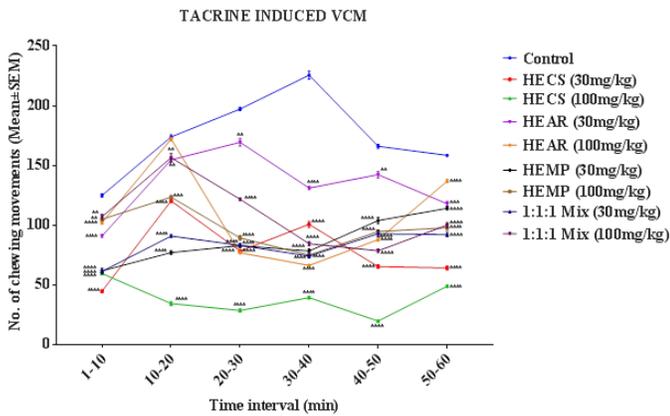
### DPPH scavenging assay

HECS shown strong DPPH scavenging activity followed by 1:1:1 mixture. The scavenging effect of HECS, HEAR, HEMP and mixture was comparable to ascorbic acid. The IC<sub>50</sub> value of Ascorbic acid, HECS, HEAR, HEMP and mixture was 14.99, 18.44, 26.51, 23.19 and 20.47 µg/ml respectively. As compared with ascorbic acid HECS and mixture shows strong antioxidant effect as revealed in Figure 6.

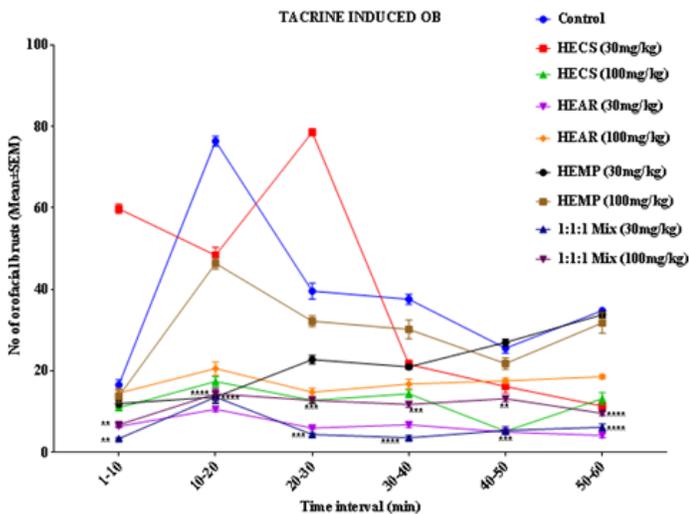
## DISCUSSION

Blocked dopamine D2 receptor shows extrapyramidal side effect as catalepsy,<sup>19</sup> haloperidol blocks D2 receptor and causes development of

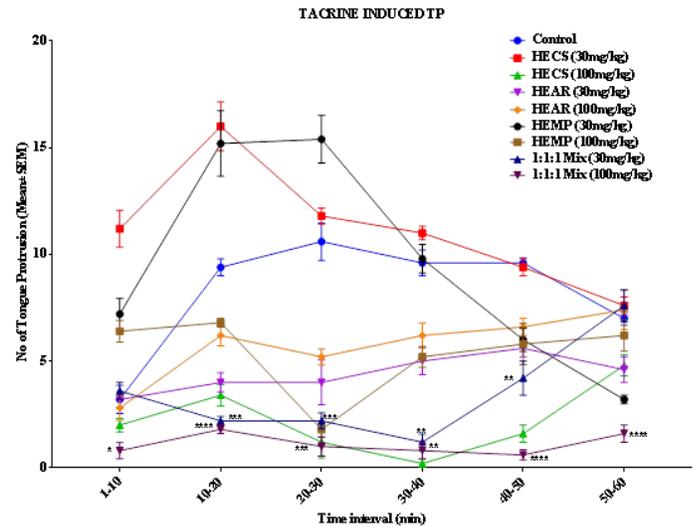
catalepsy in mice. Nishchal *et al.* (2014) states that antipsychotic drugs such as haloperidol used to treat schizophrenia causes loss of correction in externally imposed posture and development of behavioral state called catalepsy.<sup>20</sup> *Camellia sinensis* has epigallocatechine gallate like polyphenols which are an active ingredient shows curation in protective properties of brain against specific diseases and improvement in brain functioning like mental health and boosted mood.<sup>21</sup> Various active polyphenols present in *Camellia sinensis* plays important role in reduction in catalepsy. Nabavi *et al.* (2017) proved that catechins present in *Camellia sinensis* reduces depression by means of inhibiting monoamine oxidase (MAO).<sup>22</sup> Elsaid *et al.* (2015) stated that accumulation of reactive oxygen species produces oxidative stress in brain causes neurotoxicity and antioxidant property of *Asparagus officinalis* have potentials to protect neuronal damage caused by reactive oxygen species.<sup>23</sup> As



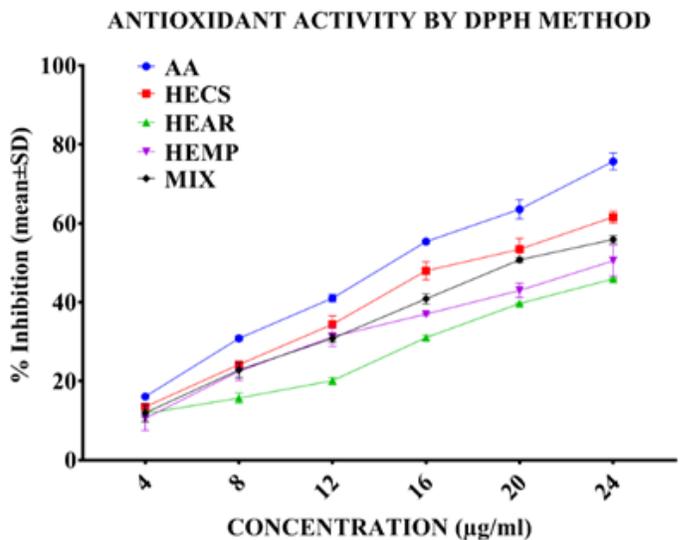
**Figure 3:** Effect of HECS, HEAR, HEMP and its combination on Tacrine induced VCM. All the values are expressed as mean ± SEM; n=5, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).



**Figure 4:** Effect of HECS, HEAR, HEMP and its combination on Tacrine induced OB. All the values are expressed as mean ± SEM; n=5, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).



**Figure 5:** Effect of HECS, HEAR, HEMP and its combination on Tacrine induced TP. All the values are expressed as mean ± SEM; n=5, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 significant compared to control (repeated measures ANOVA followed by Dunnett's test).



**Figure 6:** Percent inhibition shown by Ascorbic acid, HECS, HEAR, HEMP and 1:1:1 Mixture in DPPH scavenging assay. All the values are expressed in mean ± SD.

per the study of Periera *et al.* (2020) reduction in striatal dopamine levels and short-term memory impairments are seen after Reserpine administration in rats which mimics the pre-motor phase of Parkinson's disease.<sup>24</sup> Dopamine depletion causes hypersensitivity of D2 receptors in corticostriatal terminals hence dopamine metabolism results in reduced dopamine release in synaptic cleft shows early and late impairment in behavior and social recognition tasks (Periera *et al.* 2020).<sup>24</sup> Dopamine and 5 hydroxy tryptamine are responsible for regulation of mood in brain, however our study reveals that improved in locomotion activity with *Camellia sinensis* was might be due to its effect on increased levels of 5 hydroxy tryptamine in brain region.<sup>25</sup> In present study we observe that the extract of *Camellia sinensis* significantly shows improvements in haloperidol induced catalepsy, reserpine induced hypolocomotion and tacrine induced vacuous chewing movements and are due to the direct or indirect effect of chemical constituents present in extract. Estrogen plays vital role in various parts of brain via estrogen receptors. One of the major function associated with estrogen via estrogen receptor in hippocampus and cerebral cortex region is modulating the function of brain-derived neurotrophic factor levels and improving learning and memory impairments.<sup>8</sup> Lalert *et al.* (2013) have revealed in previous study that root extract of *Asparagus racemosus* reverses learning and memory impairments in ovariectomized rats.<sup>26</sup> The effect of the root extract was due to direct effect on the estrogen receptor without enhancing the estrogen levels.<sup>8</sup> Various chemical components present in *Asparagus racemosus* root extract such as steroidal saponins (like asparacosides, shatavarin iv, v and xi), racemosal and asparagine are might be shows significant neuroprotection. Present study result reveals that the root extract of *Asparagus racemosus* shows significant improvements in catalepsy, hypolocomotion and vacuous chewing movements are also due to the presence of different chemical constituents. Reserpine by showing effect on vesicular monoamine transporter 2 proteins reduces storage for biogenic amines and causes monoamine oxidase to actively deplete biogenic amines such as serotonin, dopamine and norepinephrines (Hedgecok *et al.* 2019).<sup>27</sup> Lebowitz and Khoshbouei (2020) stated that dopamine signaling simplified as neurobiological substrate for salient stimuli and as per the stimuli goal oriented movement.<sup>28</sup> As dopamine plays an important role in movement, reserpine induced depletion of dopamine causes movement related disorganization such as hypolocomotion. *Mucuna pruriens* contains main and active chemical constituent as L-dopa which has a dose dependant toxic effect with other chemical constituents present in *Mucuna pruriens* such as hallucinogenic tryptamines and anti-nutritional factors phenols and tannins.<sup>29,30</sup> Extract of *Mucuna pruriens* seed due to presence of chemical constituent such as L-dopa possess anti-parkinsonian effect at various animal models used for development of parkinsonian symptoms. Tacrine causes increase in acetylcholine levels at cholinergic synapses by blocking enzyme called acetylcholinesterase which is responsible for hydrolysis of acetylcholine. Cousins *et al.* (1997) reported that enhanced concentration of acetylcholine by anticholinesterase tacrine produces parkinsonian side effects such as bradikinesia, rigidity, tremor and in rats it shows purposeless chewing movement.<sup>31</sup> Hence for study we are using tacrine induced vacuous chewing movement animal model for motor effects. DPPH assay is reliable parameter for measurement of antioxidant activity. All the extracts shown antioxidant potentials due to various chemical constituents present in extract as compared with ascorbic acid but HECS and 1:1:1 mixture shows extremely significant antioxidant potentials as compare with other treatment groups.

## CONCLUSION

Present study reveals that hydroalcoholic extracts of *Camellia sinensis*, *Asparagus racemosus* and *Mucuna pruriens* possess significant effect on

haloperidol induced catalepsy, reserpine induced hypolocomotion and tacrine induced vacuous chewing movements individually but when combination was used for the same study shows extremely significant effects in dose related manner because of number of additional chemical constituents and their lower concentrations present in the mixture.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## ABBREVIATIONS

**AA:** Ascorbic acid; **Ac:** Absorbance of control; **At:** Absorbance of extract; **DPPH:** 2,2 -diphenyl - 1 - picrylhydrazyl; **HEAR:** Hydroalcoholic extract of *Asparagus racemosus*; **HECS:** Hydroalcoholic extract of *Camellia sinensis*; **HEMP:** Hydroalcoholic extract of *Mucuna pruriens*; **MIX:** Mixture; **OB:** Orofacial brush; **VCM:** Vacuous chewing movement.

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