The Research Article on Evaluation of Anti-Parkinson Activity of Alfalfa (*Medicago Sativa*) Through the Preclinical Studies In Swiss Albino Mice

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ABSTRACT
The anti-Parkinson's activity of Medicago sativa (Medicago Sativa) ethanolic extract is examined in this research. Some of the phytochemicals that have been identified thus far include glycosides, fixed oils, tannins, alkaloids, saponins, and flavonoids. The effects of medicinal ginseng on dopamine and catalase enzyme levels suggest that it may be useful in alleviating PD-related behavioral symptoms. One reason for its medicinal benefits is the presence of flavonoid components in the seed extract, namely baicalein and baicalin, which are recognized for their antioxidant capabilities. After administering Medicago sativa, the study plans to monitor changes in motor function, biochemical markers, and antioxidant status. Results like this should shed light on the pathogenesis of PD and point the way to potential novel treatments. To further understand how Medicago sativa exerts its anti-Parkinson effects, more research is required. Clinical translation of treatments based on Medicago sativa for the management of Parkinson's disease is supplemented by the results of this research project, which seek for increase pharmacological knowledge.

KEYWORDS: Medicago sativa, animal study, Parkinson, Extract, anti-oxidant, phytochemical screening

INTRODUCTION [01-20]
As far as Aristotle was concerned, the brain was just a "cooling structure," with the liver and heart being considered essential. A "encephalocentric" view supplanted the "cardiocentric" one because Alcmaeon of Croton recognized the brain as the origin of human intelligence. Although early anatomists likely used vivisection, Galenic ideas based on animal observations dominated the field for almost 1500 years.
As time goes on, PD worsens, a neuronal disorder that impacts movement. Nerve cells (neurons) in certain regions of the brain may become weak, damaged, or die off, leading to problems with movement of body, shaking, rigidity of the limbs or trunk, or imperfect body balance. Walking, speaking, and doing other basic tasks may become more challenging as these symptoms worsen. The fact that these symptoms may be seen in other diseases means that not everyone with them really has PD. In most cases, motor symptoms may be greatly reduced with medication or surgery, and efforts to discover a cure for PD are ongoing.
The precise development of PD is unknown; however, it is belief to be inherited and associated with cer-

tain genetic alterations in a large percentage of cases. In other words, the disease often does not run in families; most instances are unconnected. Scientists currently think that PD is usually brought on by a combination of hereditary susceptibility and some unknown environmental factor. Development of Parkinson's disease is elicited by the irreversible damaged or destruction of neurons, which are brain cells. Although the illness affects several brain areas, the bulk of symptoms are caused by neuronal loss in the specific part of brain called substantia nigra, a region at the brain's base. Dopamine is often released by dopaminergic neurons in this area.

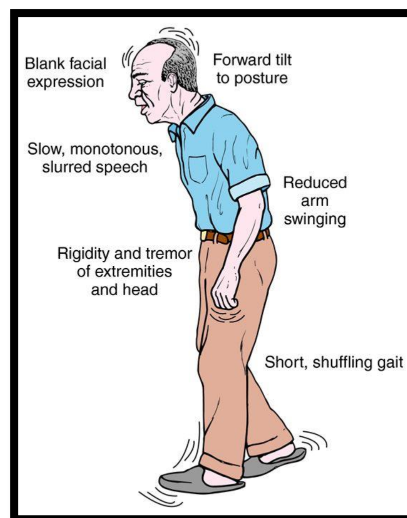

Fig 01- The condition of Parkinson disease

One such protein is alpha-synuclein. People with PD may see the occurrence of Lewy bodies, which are deposits of the protein called alpha-synuclein, in the injured brain cells. To this day, researchers still don't know what triggers Lewy bodies or how they exacerbate the disease. Some research suggests that defective protein removal in PD cells can cause harmful protein buildup and, ultimately, cell death. Protein clumps inside PD brain cells may contribute to neuronal death, according to new study.
A tree of generations. Several genes have been significantly linked to PD, including the alpha-synuclein gene, and many others have been shown to have a role. There is a small but real possibility that some environmental factors or genes might influence the same proteins and genes that are changed in inherited diseases.

MATERIAL & METHODS

The intention of this work is to ascertain the efficacy of treating animal models with haloperidol (5 mg/kg) to cause motor dysfunction or Parkinson-like symptoms, and then to evaluate the efficacy of treating these symptoms with bromocriptine (4 mg/kg) and L-DOPA. Sodium CMC (0.5%) is used as a medication suspension agent, and tissues are washed with ice-cold saline throughout the studies. Biochemical tests, especially those that assess oxidative stress, make use of buffer solutions such as ferric chloride and phosphate buffers. The actophotometer and rotarod equipment evaluate the motor activity and coordination of animals, while instruments such as the UV spectrophotometer examine metabolic changes. A CO_2 chamber is used for the painless death of animals, a deep freezer and lyophilizer are utilized for the storage and drying of samples, and a rotating vacuum evaporator is utilized for the removal of solvents.

COLLECTION AND AUTHENTIFICATION OF PLANT

World of Nature in Pune supplied the Medicago Sativa plants in October 2023, from which the plants were harvested. Alrsin Ayurvedic Pharmaceuticals in Andheri, Mumbai's Mr. Mahesh Atale, is a botanist, and he was given the sample voucher. After rinsing with tap water, the plant stems were left to dry in the shade at room temperature with the help of a fan. The stem was allowed to dry before being milled into a coarse powder and stored in a container.

EXTRACTION OF PLANT [21-32]

Medicago sativa (commonly known as Medicago Sativa) extraction using the Soxhlet extraction method involves repeatedly washing the plant material with a solvent to obtain its bioactive compounds. First, the dried and powdered Medicago sativa is placed in a Soxhlet apparatus, where it is packed in a thimble. A solvent, such as ethanol or methanol, is heated in a flask, and its vapors rise into the condenser, where they cool and drip onto the plant material. The solvent dissolves the plant's desired compounds and siphons back into the flask once the chamber fills. This cycle continues, allowing efficient extraction of compounds without using excessive solvent. After the extraction, the solvent is desiccation, resulting in the concentrated extract of Medicago sativa, which can be used for further analysis or applications.

PHYTOCHEMICAL SCREENING [33-40]

Preliminary chemical tests were carried out on the ethanolic extract of *Medicago sativa* for the determination of the presence of different phytoconstituents.

Table 01– The Phytochemical Screening Taste & its Description

Molecule	Taste	Description
Carbohydrates	Molisch test	2-3 ml of ethanolic extract was mixed with α-Naphthol in alcohol. To this added conc. H2SO4 from the side of the test tube. A violet-colored ring appear at the terminal of the two liquids indicates the existence of carbohydrates.
	Benedict's test	1 ml of ethanolic extract was mixed with 2 ml of Benedict's reagent. This mixture was heated in a hot water bath for at least 3 min or until a visible color change.
Proteins	Biuret test	2 ml of the ethanolic extract was mixed with 1 ml of sodium hydroxide. 2-3 drops of copper sulfate were added to the mixture. A change in color from blue to purple indicates the existence of proteins.
Steroids	Sulphur powder test	2 ml of ethanolic extract was taken in a test tube. Sulfur powder was sprinkled upon it. If the sulfur powder sinks to the bottom of the test tube, that indicates existence of steroids
Saponins	Froth test	The presence of saponins was determined by the Frothing test. The ethanol extract of *Medicago sativa* was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates the absence of saponins and stable froth for more than 1.5 cm indicated the presence of saponins.

Flavonoids	Lead acetate test	2 ml of ethanolic extract was treated with a few drops of lead acetate solution. The formation of a yellow-colored precipitate indicated the presence of flavonoids.
	Shinoda test	The ethanol extract of *Medicago sativa* was treated with a few drops of conc. HCL and magnesium turnings. The appearance of pink or red color within a few minutes indicated the presence of flavonoids.
Alkaloids	Hager's and Dragondroff's test	The ethanolic extract of *Medicago sativa* was dissolved in 2 N HCL. The mixture was filtered and the filtrate was divided into 2 equal portions. One portion was treated with an equal amount of Hager's reagent and the other portion was treated with an equal amount of Dragondroff's reagent. The brown and yellow precipitate indicated the presence of respective alkaloids.
Phenols and tannins-	Ferric Chloride test	0.5 gm of extract was boiled with 20 ml of water and filtered. To the filtrate, 0.1% FeCl3 solution was introduced. The deep black color indicated the presence of tannins.
	Dilute Potassium permanganate test	2ml of ethanolic extract of *Medicago sativa* was mixed with a few drops of dilute potassium permanganate solution. A positive test was concluded and decoloration of potassium permanganate was observed.
Fixed oil and fats test	Stain test	Ethanolic extract was kept on a filter paper and allowed to dry. A positive test was concluded as a translucent stain was formed on the paper when you held the paper up to the light.
Glycosides	Legal test	0.5 gm of the drug extract was mixed with 0.5 ml of pyridine. To this solution, a few drops of sodium nitroprusside was added. The formation of yellow precipitate indicates the presence of alkaloids

ANIMALS REQUIRED

The Albino Swiss Mice used in this research are males, 8-12 weeks old, and 20-30 grammes in weight. For the investigation, a grand total of 36 mice, six per group, are needed. To maintain reliability and accuracy in the experiment, the total number of animals needed is still 36. Miniature white mice from Switzerland. The sample size was 24 Swiss albino mice, with a weight span of 25-30 gm. The animals were housed in an air-conditioned, well-ventilated building along a consistent room temperature of 24°C, a 12:12 day: night period, with a relative humidity of 55-60%. The animals were placed in large polypropylene container like cages that were spread with rice husks and provided with bedding. Pellets and purified mineral water made up the animals' regular food. The research did not begin until the animals had been given a week to acclimatize. Evaluating the Anti-Parkinson Activity of Medicago sativa in Swiss Albino Mice is part of the thesis "Evaluation of Pharmacological Activity of selected plant part using Animal Model," and both the animals and the study protocol were accepted by CPCSEA under protocol number Proposal No. HRFT/IAEC/2023–2024/16.

GROUPING OF ANIMALS

In order to assess the effects of different test substances, this research used six groups of six Albino Swiss Mice. As a control, Group 1 receives 10 ml/kg of distilled water. Two milligrams per kilogram of haloperidol is administered intraperitoneally to Group 2. Two doses of haloperidol and bromocriptine, each at 2 mg/kg, are administered to Group 3. portions of 50 mg/kg, 150 mg/kg, and 300 mg/kg of AEE in conjunction with Haloperidol (probably a particular experimental drug) are administered to Groups 4, 5, and 6, respectively. There must be a total of 36 animals used in the investigation. This protocol makes good use of its resources by sharing animals from Groups 1, 2, and 3.

EVALUATION OF ANTIPARKINSON ACTIVITY [41-52]-

Rotarod Test -

The rotarod apparatus will be used to record motor coordination. Mice that can remain motionless on the moving rod for 120 seconds prior to treatment will be included in the trial. Before and after the animals are treated with the extract, researchers will measure how long it takes for them to descend off the revolving rod.

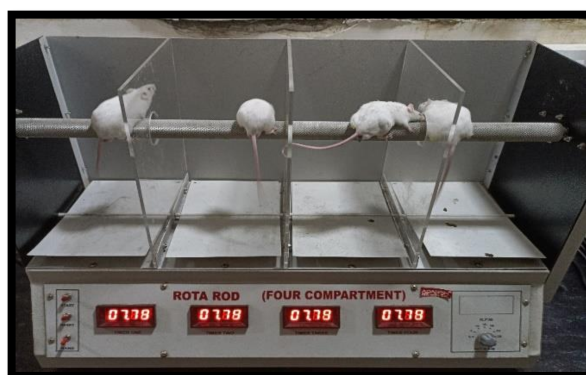

Fig 02- Determination of motor coordination pattern using rotarod.

Actophotometer Test-

It will be an actophotometer that measures the locomotor activity. One mouse block gets its own dedicated light beam thanks to the arrangement of six photocells across the bottom of the enormous cage, which measures 30 x 30 x 30 cm. When light rays hit the photocells, they activate; when an animal passes across the light beam, the light is interrupted, and the number of cut interruptions is recorded for 10 minutes.

Fig 03- Determination of locomotor activity using Actophotometer

Bar Test-

A bar test will be utilized record the level of catalepsy. In the bar test, the forepaw of the animal will be set on the horizontal bar located 3 cm above and parallel to the base. The time at which the animal set down its paw from the bar will be recorded. The maximum cut-off time for the bar test will be fixed at 300 sec.

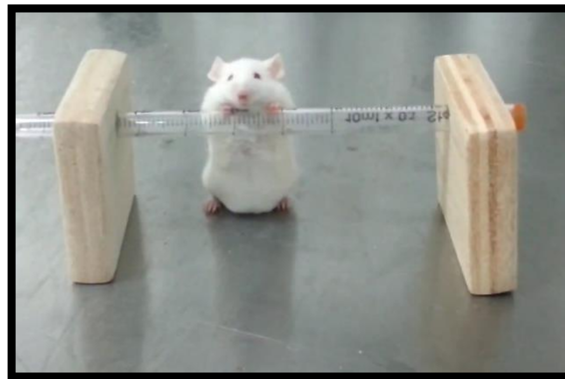

Fig 04- Determination of catalepsy using 3cm bar.

ESTIMATION OF BIOCHEMICAL PARAMETERS [53-65]-

Preparation of Brain sample-

A CO2 chamber was used to put an end to the animals' lives once the aforementioned factors had been evaluated. The brains were swiftly extracted and placed on a bed of icy salt water. We used a 0.1M Phosphate buffer (pH 8) to weigh and homogenise the tissues. Dopamine concentration and CAT test were both performed on the collected supernatant.

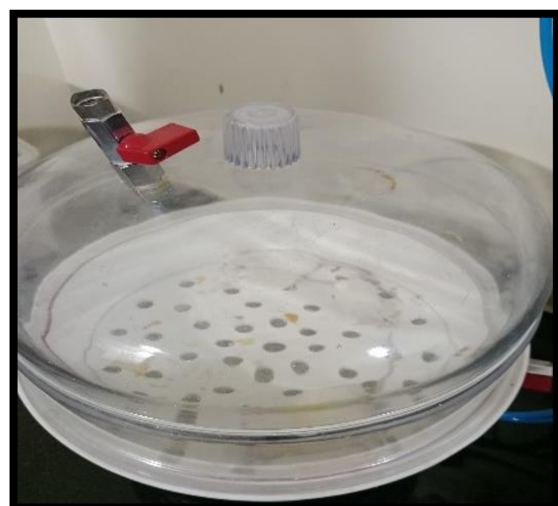

Fig 05 - Euthanization and removal of brain samples

Determination of dopamine by UV Spectrophotometer-
The dopamine level in mice brains was estimated by admixing homogenized supernatant liquid (1ml) with 1ml of ferric chloride (1.5 X 10-2 M) and 1ml of potassium ferricyanide (1.5 X 10-2 M) in 25ml distilled water. It was kept aside for 30min and the developed color was estimated using the UV-visible double beam spectrophotometer at 735nm (33).

Determination of Catalase-
The haloperidol-induced Parkinson's disease mice will be put to sleep in a CO2 chamber after their motor coordination, locomotor activity, and bar tests have been evaluated. Then, their brains will be removed. Without delay, they will be submerged in icy salt water. We will weigh the tissues and mix them well in a 0.1M phosphate buffer with a pH of 8. In order to assess Catalase activity, we will extract homogenates from mouse brains and place them in several test tubes. For the catalase test, we will utilize the supernatant.

DPPH Anti-oxidant Activity-
By using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals, the antioxidant activity of the sample compounds was assessed for their free radical scavenging activity (George et al., 1996). One milliliter of water were added to the test tube as the compound. The samples were incubated in the dark for 30 minutes after being mixed with 1 ml of 0.1% ethanolic DPPH. Afterwards, we tested the mixture's absorbance at 518 nm and looked for discoloration in the samples; a range of purple to yellow was judged strong positive, while a range of light pink was considered weak positive.
The percentage of DPPH radicals that the extract was able to suppress was determined using the following formula: The absorbance of the DPPH solution (Ac) and the extract (As) or vitamin C (Ac) are the two variables that determine the percentage of radical scavenging by DPPH. Half-life of DPPH radicals (IC50) was defined as the extract concentration at which half of the radicals were inhibited.

Results & Discussions
PHYSICOCHEMICAL CHARACTERISTICS-

Table 02- The Phytochemical analysis observation of plant

Phytoconstituents	Test	Observation
Carbohydrates	Molisch test	+ ve
	Benedict's test	+ ve
Proteins	Biuret test	+ ve
	Sulphur powder test	+ ve
Saponins	Froth test	+ ve
Flavonoids	Lead acetate test	+ ve
	Shinoda test	+ ve
Alkaloids	Hager's and Dragondroff's test	+ ve
Phenols and tannins	Ferric Chloride test	+ ve
	Dilute Potassium permanganate test	+ ve
Fixed oil and fats test	Stain test	+ ve
Glycosides	Legal test	- ve
Terpenoids	Salkowski test	+ ve

Through a battery of qualitative tests, the plant's phytochemical analysis identifies the existence of several bioactive chemicals. A good outcome in both the Molisch and Benedict's tests indicates the presence of carbohydrates. The Biuret and Sulphur Powder tests both demonstrate that proteins are present. A positive Froth test indicates the existence of saponins. The presence of flavonoids is supported with the good outcomes of the Lead acetate and Shinoda tests. Positive results from both the Hager's and Dragondroff's tests indicate the presence of alkaloids. Positive results from the Ferric Chloride and Dilute Potassium permanganate tests indicate the existence of tannins and phenols. It is the stain test that verifies the existence of fixed fats and oils. A negative Legal test indicates the absence of glycosides. A positive Salkowski test indicates the presence of terpenoids. This extensive investigation has shown that the plant is rich in phytoconstituents, some of which may have medicinal uses.

EVALUATION OF ANTIPARKINSON ACTIVITY-

Bar Test -

Table 03- Effect of bromocriptine and AEE on catalepsy in bar test (Catalepsy score in seconds)

Time interval in mins.	Vehicle control	Haloperidol control 1 mg/kg	Bromocriptine 5mg/kg	AEE 50mg/kg	AEE 150mg/kg	AEE 300mg/kg
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30	0 ± 0	6.32 ± 0.27	3.56 ± 0.24	6.18 ± 0.54	5.42 ± 0.32	5.16 ± 0.2
60	0 ± 0	8.42 ± 0.32	1.42 ± 0.45	8.3 ± 0.62	5.14 ± 0.21	2.36 ± 0.1
90	0 ± 0	13.47 ± 0.82	1.53 ± 0.64	12.56 ± 0.21	8.46 ± 0.46	2.57 ± 0.98
120	0 ± 0	20.11 ± 0.63	1.24 ± 0.47	21.42 ± 0.34	12.34 ± 0.24	1.53 ± 0.45
240	0 ± 0	20.66 ± 0.57	0.96 ± 0.23	20.54 ± 0.69	11.28 ± 0.58	1.03 ± 0.89

- Note: All values are expressed in mean ± SEM (n = 6). Significance: $^{**}p \leq 0.01$ when compared with *Medicago sativa* extract.

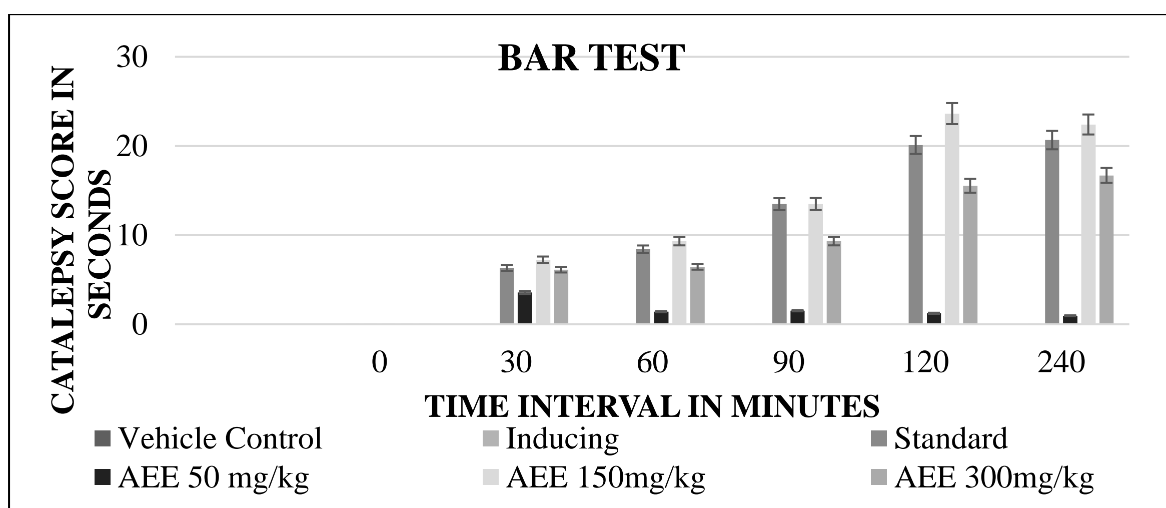

Fig 06- Effect of bromocriptine and AEE on catalepsy in bar test (Catalepsy score in seconds)

Using the bar test and measurements made at different intervals, the research evaluates the impact of Bromocriptine and different dosages of AEE on haloperidol-induced catalepsy in mice. At all time points,

the vehicle control group consistently does not exhibit catalepsy (0 ± 0 seconds). At 240 minutes, the catalepsy duration peaks at 20.66 ± 0.57 seconds in the haloperidol control group, which shows a gradual rise. A substantial reduction in catalepsy is seen with the use of bromocriptine (5 mg/kg), with a minimum score of 0.96 ± 0.23 seconds at 240 minutes ($p < 0.01$). The limited impact of AEE at 50 mg/kg is comparable to the control group treated with haloperidol. At 120 minutes, catalepsy scores peak at 21.42 ± 0.34 seconds. More effective decrease of catalepsy was seen at 300 mg/kg compared to 150 mg/kg, especially at 240 minutes (1.03 ± 0.89 seconds, $p < 0.01$). According to these findings, AEE is less efficacious at lower dosages but, like Bromocriptine, greatly mitigates haloperidol-induced catalepsy at larger levels

Rotarod Test-

The fall of time (in seconds) from the rotarod is significantly decreased in the haloperidol control group as compared to vehicle control group. It was improved in bromocriptine and AEE groups.

Table 04- Effect of bromocriptine and AEE on motor coordination using rotarod.

Treatment groups	**Fall of time (in seconds) Mean± SEM**
Vehicle control	91.90±1.105
Inducing Haloperidol 1 mg/kg	22.81±1.482
Standard Bromocriptine 5 mg/kg	73.72±1.348****
AEE 50 mg/kg	29.20±0.9712
AEE 150 mg/kg	37.26±1.36
AEE 300 mg/kg	67.29±1.110**

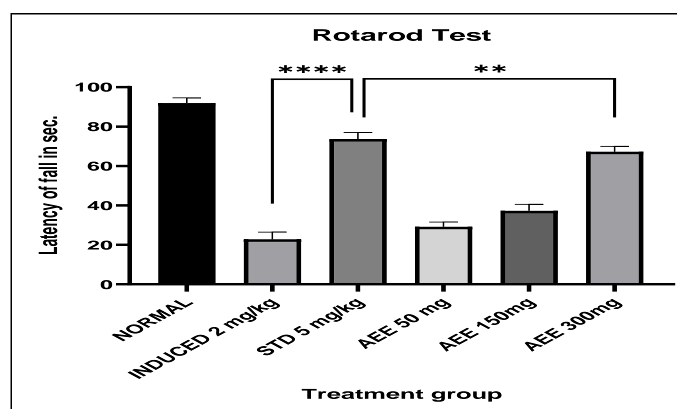

Fig 07- Effect of bromocriptine and AEE on motor coordination using Rotarod

Using the rotarod test, which measures the fall-off time in seconds, the research examines the effects of Bromocriptine and AEE on motor coordination in mice. A high fall-off time of 91.90±1.105 seconds is maintained by the vehicle control group, suggesting that their motor coordination is normal. The motor coordination is considerably diminished by haloperidol (1 mg/kg), and the effect wears off after 22.81±1.482 seconds. By raising the fall-off time to 73.72±1.348 seconds ($p<0.0001$), motor coordination is markedly improved with treatment with bromocriptine (5 mg/kg). The effectiveness of AEE at 50 mg/kg is not very high, and it takes 29.20±0.9712 seconds for the effects to wear off. With fall-off times of

37.26±1.363 seconds and 67.29±1.110 seconds, respectively, for AEE at 150 mg/kg and 300 mg/kg, the findings increase with increasing dosages, culminating in a substantial improvement ($p<0.01$) at the highest dose. These results indicate that AEE improves motor coordination dosage-dependently, with the maximum dose showing activity similar to Bromocriptine.

Actophotometer-

Random motor activity was significantly lessened in haloperidol control group as compared to vehicle control group. It was improved in bromocriptine and AEE groups.

Table 05 - Effect of bromocriptine and AEE on locomotor activity using Actophotometer.

Treatment groups	Ambulation counts / 10min mean ± SEM
Vehicle control	230.3±3.425
Inducing Haloperidol 1 mg/kg	25.33±2.568
Standard Bromocriptine 5mg/kg	177.8±3.423****
AEE 50 mg/kg	52.67±1.987
AEE 150 mg/kg	72.33±2.060
AEE 300 mg/kg	160±1.862**

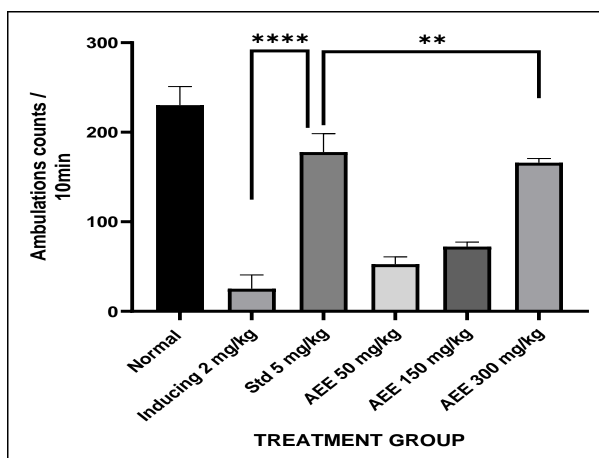

Fig 08- Effect of bromocriptine and AEE on locomotor activity using Actophotometer.

Using an actophotometer, the researchers tracked the number of times mice walked for 10 minutes to see how Bromocriptine and AEE affected their locomotor activity. With 230.3±3.425 ambulation counts, the vehicle control group demonstrates a high level of activity. A substantial decrease in locomotor activity to 25.33±2.568 counts is seen with haloperidol (1 mg/kg). The activity levels are considerably restored by 5 mg/kg of bromocriptine, leading to an increase in ambulation counts to 177.8±3.423 ($p<0.0001$). With 50 mg/kg of AEE, 52.67±1.987 counts are obtained, 72.33±2.060 counts at 150 mg/kg, and 160±1.862 counts at 300 mg/kg, which is a substantially enhanced level of activity ($p<0.01$). According to these findings, AEE enhances locomotor activity dose-dependently; the maximum dosage produced a significant recovery, although to a lesser extent than Bromocriptine.

ESTIMATION OF BIOCHEMICAL PARAMETERS-

Determination of Dopamine concentration by UV spectrophotometer:

The Haloperidol group shows a drop in the concentration of dopamine in relation to the vehicle control group. Bromocriptine and AEE groups show improvement in levels of dopamine indicating a potential treatment strategy for PD. AEE intermediate dose (150 mg/kg) showed the highest levels of increase in dopamine concentration when compared to AEE low (50 mg/kg) and high dose (300 mg/kg)

Table 06- Effect of bromocriptine and AEE on dopamine concentration using UV

Treatment Groups	Concentration of Dopamine (µg/ml)
Vehicle control	65±1.28
Inducing Haloperidol 1 mg/kg	18±1.63
Standard Bromocriptine 5mg/kg	75±.85****
AEE 50 mg/kg	22±1.92
AEE 150 mg/kg	35±0.72
AEE 300 mg/kg	54±0.38**

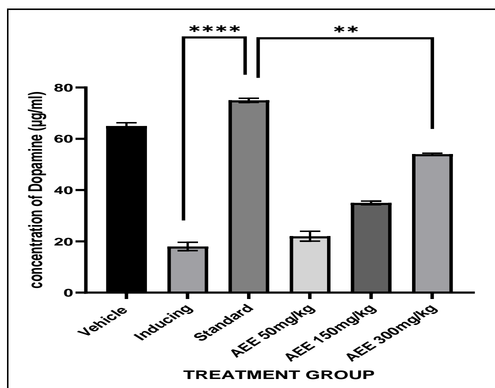

Fig 09- Effect of bromocriptine and AEE on dopamine concentration using UV.

In this work, the researchers used ultraviolet spectrophotometry to test how different dosages of AEE and bromocriptine affected the level of dopamine in mice. The dopamine levels at baseline was 65±1.28 µg/ml in the vehicle control group. The dopamine-blocking action of haloperidol is shown by the considerable reduction of dopamine levels to 18±1.63 µg/ml after injection of 1 mg/kg. Using bromocriptine at a dosage of 5 mg/kg considerably raises the concentration of dopamine to 75±0.85 µg/ml ($p<0.0001$), proving that it enhances the effects of dopamine. At 50 mg/kg, AEE leads to a 22±1.92 µg/ml dopamine concentration; at 150 mg/kg, it rises to 35±0.72 µg/ml; and at 300 mg/kg, it causes a considerable increase to 54±0.38 µg/ml dopamine levels ($p<0.01$). The findings indicate that the effects of AEE on the haloperidol-induced decrease in dopamine levels are dose-dependent, with a significant restorative effect seen at the highest dosage.

Determination of Catalase by UV spectrophotometer-

The level of catalase was decreased in the haloperidol group indicating the increase in ROS further leading to the progression of PD. On the other hand, catalase levels were improved in the bromocriptine and AEE groups. AEE intermediate dose (150 mg/kg) was found to significantly increase the levels of catalase when

compared to AEE low (50 mg/kg) and high (300 mg/kg) groups

Table 07 - Effect of AEE on the levels of Catalase (CAT) in the brain of haloperidol-

Treatment Groups	CAT (µmoles of H2O2 used/min/mg of protein)
Vehicle control	0.1243±0.001284
Inducing Haloperidol 1 mg/kg	0.0378±0.007561
Standard Bromocriptine 5mg/kg	0.1943±0.005948****
AEE 50 mg/kg	0.07752±0.001522
AEE 150 mg/kg	0.09183±0.003962
AEE 300 mg/kg	0.1106±0.0120447**

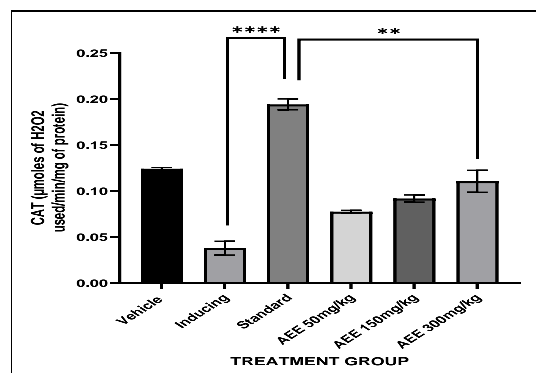

Fig 10- Effect of AEE on the levels of Catalase (CAT) in the brain of haloperidol

In this research, mice that were given haloperidol had their brain catalase (CAT) levels measured to see if AEE affected those levels. The baseline CAT activity for the vehicle control group was 0.1243±0.001284 µmoles of H2O2 used/min/mg of protein. Indicating oxidative stress, haloperidol (1 mg/kg) considerably lowers CAT activity to 0.0378±0.007561 µmoles. Bromocriptine, the usual treatment at 5 mg/kg, shows an antioxidant effect by dramatically increasing CAT activity to 0.1943±0.005948 µmoles ($p<0.0001$). The CAT activity is increased to 0.07752±0.001522 µmoles at 50 mg/kg, to 0.09183±0.003962 µmoles at 150 mg/kg, and to 0.1106±0.0120447 µmoles ($p<0.01$) at 300 mg/kg of AEE. These results indicate that AEE significantly protects against haloperidol-induced oxidative stress by increasing CAT activity in a dose-dependent manner.

DPPH Anti-oxidant Activity-

By using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals, the antioxidant activity of the sample compounds was assessed for their free radical scavenging activity (George et al., 1996). One milliliter of water were added to the test tube as the compound. The samples were incubated in the dark for 30 minutes after being mixed with 1 ml of 0.1% ethanolic DPPH. Afterwards, we tested the mixture's absorbance at 518 nm and looked for discoloration in the samples; a range of purple to yellow was judged strong positive, while a range of light pink was considered weak positive. The percentage of DPPH radicals that the extract was able to suppress was determined using the following formula: As a percentage, the DPPH radical scavenging efficiency is equal to [(Ac-As)/Ac] * 100. The absorption of the DPPH solution is denoted as Ac, whereas the absorption of the vitamin C extract is denoted as As. The table below shows that at concentrations of 10, 20, 50, and 100 µg/ml, the proportion of DPPH radicals inhibited by AME and vita-

min C varied with dosage.

Table 08- The % inhibition of AEE and Vit. C at different concentrations.

Concentration (µg/ml)	Vit C (% inhibition)	Medicago Sativa Extract (% inhibition)
10	38.54±1.021	24.70±1.38
20	49.73±0.938	30.01±0.021
50	66.12±1.320	39.28±1.960
100	79.25±0.801	52.12±1.081

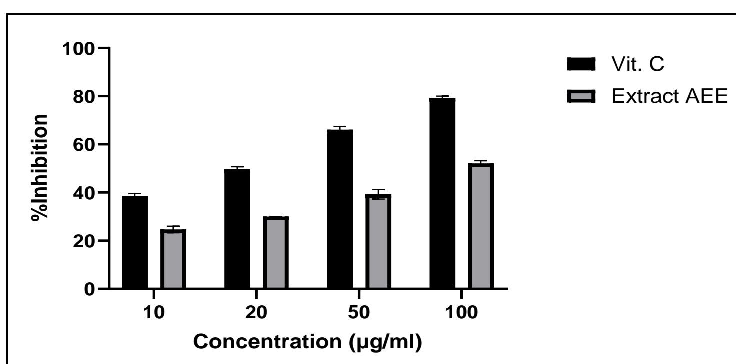

Fig 11- Inhibition percentage of DPPH radicals by AEE and vitamin C at different concentrations.

This research looks at how different amounts of vitamin C and Medicago sativa extract (AEE) hinder bacterial growth. Vit. C displays an inhibition of 38.54±1.021% at 10 µg/ml, while AEE displays an inhibition of 24.70±1.38%. With a concentration increase to 20 µg/ml, the inhibition of vitamin C reaches 49.73±0.938% and that of AEE reaches 30.01±0.021%. Vit. C inhibits AEE at a rate of 39.28±1.960% and 66.12±1.320% at 50 µg/ml. When the concentration is 100 µg/ml, the inhibitory effect of vitamin C is 79.25±0.801% and that of AEE is 52.12±1.081%. Vit. C consistently showed stronger percentage inhibition across all tested doses compared to AEE, but both substances exhibited dose-dependent inhibitory effects, according to the data.

CONCLUSION

Saponins, flavonoids, alkaloids, phenols, tannins, fixed oils, and glycosides were identified in the preliminary phytochemical investigation of the Medicago sativa ethanolic extract. Medicago sativa seems to have anti-Parkinson's action, according to the study. There is evidence that it alleviates behavioral symptoms while simultaneously raising dopamine and catalase enzyme levels. Baicalein and baicalin are two of the flavonoid components found in the seed extract that are responsible for its anti-oxidant capabilities. The potential action mechanism of Anti-Parkinson requires more in-depth research. Modifications in biochemical indicators, motor function, and antioxidant status are among the anticipated consequences of Medicago sativa therapy. Implications for understanding the pathogenesis of PD and discovering new treatment approaches are held by these results. To sum up, this thesis is an effort to further our knowledge of pharmacology and to provide the groundwork for the practical application of therapies based on Medicago sativa to the management of PD.

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