IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF ANTI-PARKINSON ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANTS OF AMMANNIA BACCIFERA LINN

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Abstract
Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by the degeneration of dopaminergic neurons in the substantia nigra, leading to characteristic motor deficits. The present study investigates the potential anti-Parkinson activity of the ethanolic extract of whole plants of *Ammannia baccifera Linn*, a traditionally used medicinal herb with antioxidant and neuroprotective properties. The extract was subjected to phytochemical screening, followed by evaluation in experimental animal models of Parkinson's disease induced by 6-hydroxydopamine (6-OHDA) and haloperidol. Behavioral assessments, including the rotarod test, catalepsy test, and open field test, were employed to evaluate motor function. Biochemical markers of oxidative stress and dopaminergic activity were also analyzed. Results demonstrated significant improvement in motor coordination and reduction in cataleptic scores in extract-treated groups compared to controls. The extract also attenuated oxidative damage and supported dopaminergic function, suggesting a neuroprotective mechanism. This study supports the therapeutic potential of *Ammannia baccifera* in managing Parkinson's disease and warrants further investigation into its active constituents and mechanisms of action.

Keyword*:* Parkinson's disease, *Ammannia baccifera*, rotarod test, catalepsy test, neuroprotective

INTRODUCTION

Neurodegenerative diseases (NDD) are a group of illness with diverse clinical importance and etiologies. NDD include motor neuron disease such as Cerebellar disorders, Alzheimer's disease (AD), Lewy body dementia (LBD), Multiple sclerosis (MS), Huntington's disease (HD), Parkinson's disease (PD) and Schizophrenia. Among these Alzheimer's disease and Parkinson's disease are the most common neuro degenerative diseases.

Parkinson Disease

Parkinson's disease is a brain disorder that leads to shaking, stiffness, and difficulty with walking, balance, and coordination. PD is a chronic progressive neurodegenerative disorder characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and wide spread presence of alpha synuclein (aSyn), an intracellular protein. Dopamine deficiency in the basal ganglia leads to classical Parkinson an motor symptoms such as brady-kinesia, tremor, rigidity and later postural instability. Parkinson's symptoms usually begin gradually and get worse over time. As the disease progresses, people may have difficulty walking and talking. They may also have mental and behavioral changes, sleep problems, depression, memory difficulties, and fatigue. Both men and women can have Parkinson's disease. However, the disease affects about 50 percent more men than women. Parkinson's disease occurs when nerve cells, or neurons, in an area of the brain that controls movement become impaired and/or die. Normally, these neurons produce an important brain chemical known as dopamine. When the neurons die or become impaired, they produce less dopamine, which causes the movement problems of Parkinson's. Scientists still do not know what causes cells that produce dopamine to die. People with Parkinson's also lose the nerve endings that produce norepinephrine, the main chemical messenger of the sympathetic nervous system, which controls many automatic functions of the body, such as heart rate and blood pressure. The loss of nor epinephrine might help explain some of the non-movement features of Parkinson's, such as fatigue, irregular blood pressure, decreased movement of food through the digestive tract, and sudden drop in blood pressure when a person stands up from a sitting or lying-down position. Many brain cells of people with Parkinson's contain Lewy bodies, unusual

clumps of the protein alpha-synuclein. Although some cases of Parkinson's appear to be hereditary, and a few can be traced to specific genetic mutations, in most cases the disease occurs randomly and does not seem to run in families. Many researchers now believe that Parkinson's disease results from a combination of genetic factors and environmental factors such as exposure to toxins. Parkinson disease was described in 1817 by a Physician named James Parkinson, published an essay on the Shaking Palsy. Later, the term Parkinson Disease was coined by William Sander in 1865 and he termed PD as is one of the chronic diseases associated with aging. The pathophysiology of Parkinson's disease is death of dopaminergic neurons as a result of changes in biological activity in the brain with respect to Parkinson's disease (PD). There are two proposed major mechanisms for neuronal death in Parkinson's Disease which includes protein aggregation in Lewy bodies and changes in cell metabolismor mitochondrial function, neuro-inflammation.

NEED OF THE STUDY

Various synthetic medicines are prescribed for Parkinson disease but they exert side effects. Still there is a challenge to the medical system for Management of Alzheimer and Parkinson disease without any side effects. Consequently, the search for natural drugs from medicinal plants is being increased because of its fewer side effects, willingly availability and low cost. Thus, the scientific validation of medicinal plants traditionally used in the treatment and management of Alzheimer's and Parkinson's disease is demanded. Ammannia baccifera L. belonging to the family Lythraceae is distributed commonly through out India, often as a weed in rice fields. In Ayurveda, the leaves are considered to have laxadive, stomachic, hepatopathy and aphrodisiac properties and are useful for treating strangury. In Unani they are used as an appetizer and for the treatment of rheumatic pains and fevers. In Siddha the whole plant is used in the treatment of glandular swellings, anticancer (Rajoriya et al., 2025) leukorrhea, snake-bite poisoning abscesses, ulcers and polyuria. Leaves contain lawsone as the major chemical constituent. The herb is reported to possess anti-typhoid, anti-tubercular and anti-tumor properties (Parrotta, 2001; Khare, 2007). The different extracts of the plant are reported to have antimicrobial activity against human and plant pathogenic fungi (Ray et al., 2004). Four flavonol and three flavone glycosides quercetin 3--d-glucoside, rutin, luteolin 7-d-glucoside, isorhamnetin 3-rutinoside, apigenin 7--d-glucoside vitexin and kaempferol 3-rhamnoglucoside were isolated and characterized from Ammannia coccinea (Graham et al., 1980). Free rhamnetin and its 3-rhamnosylglucoside were found to be present in Ammannia multiflora (Balraj and Nagarajan, 1981).

In the present study, we investigated the anti-parkinson activity of the methanol extract of Ammannia baccifera using several experimental models of rats for the purpose of validating its ethnomedical use. Natural active principles, endowed with anti-inflammatory activity, frequently exhibit antioxidant power; moreover many plant extracts interacting with several biosynthetic pathways of inflammation mediators in general possess antioxidant property (Speroni et al., 2006). The plant A. baccifera L. is reported to contain vitamin C, Tannins, Flavonoids, Betulinic acid, Lupeol, Lawsone, Ellagic acid, Quercetin, Hentriacontane, Dotriacontanol and ß-sitosterol glucoside, Triacontan-1, 30 diol and some other phyto-constituents which are responsible for the medicinal and pharmacological activities. On the basis of literature and documentation of existing uses of Ammannia baccifera Linn, an effort has been made to establish the scientific validity to investigate anti-parkinson activity.

RESEARCH METHODOLOGY

Selection of the Plant Material

The isolation of secondary plant metabolites begins with the selection of the plant, the most critical aspect of the project. In order to locate a plant, previously in folklore practices, one should turn to the discipline of ethanobotany. Ethanobotany literally means "people's botany" and is defined as the study of plants important to primitive people. Ethanobotany can include present day people and involves inter disciplinary study surrounding a core of botany with chemistry, pharmacology and anthropology among others.As the medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. The library search will yield detail of folk medicinal plants utilized in particular area. This will ensure the availability of plants for collection purposes. So, for the selection of a medicinal plant with an active anti-parkinson and anti-alzheimer activity, includes discussion with a tribal medical practitioner for the traditional and tribal uses of the plants which have been used for neurological diseases. The complied list of plants must therefore be subjected to a literature survey to confirm that the plant has not been previously investigated for anti-alzheimer and anti-parkinson activity. In addition to the verification that the plant constituents have yet to be identified, note must be taken of which plant parts were utilized and how they were culturally prepared. Based on the ethnopharmacological survey and literature review, the plant Ammannia baccifera Linn was selected for the present work.

Collection and Authentication of the Plant Material

The fresh plants of Ammannia Baccifera Linn were collected from the paddy fields of Mettukadai village, Erode district, Tamilnadu, India, during the month of September 2024. The plant was taxonomically identified and authenticated by the Botanist Dr. A. Balasubramanian, ABS Botanical Garden, Salem.

Extraction of Plant Material

The fresh plants were air-dried under shade and then coarsely powdered using a mechanical grinder. The powder was then passed through sieve no.40 and stored in an airtight container for the extraction. About 500gms of powder has been used for the process of extraction. The cleaned and powdered material of whole plants of Ammannia baccifera Linn were used for extraction purpose. About 500gms of powdered material was evenly packed in a Soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as Petroleum ether, Ethanol and Aqueous successively. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 Hrs. The aqueous extraction was carried out by cold-maceration process.

Methods of Extraction

• Hot Percolation process **• Cold Maceration process**

Solvents used

• Petroleum ether **• Ethanol** **• Distilled water**

Preparation of Extracts

Petroleum ether extract of whole plants of Ammannia baccifera Linn:

The shade dried coarsely powdered whole plants of Ammannia baccifera Linn. (500gm) were extracted with petroleum ether (60-80˚C), for 72 hrs. After completion of extraction, the defatted extracts were filtered while hot through Whatmann filter paper (No.10) to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10. Then the concentrated extract was transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Dark greenish brown coloured extract was obtained. The concentrated extract was then kept in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies (Rajoriya et al., 2025; Rajoriya et al., 2024).

Ethanol extract of whole plants of Ammannia baccifera Linn:

The main marc left after Pet. ether extraction was dried and then extracted with ethanol 95% v/v (75-78˚C), for 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark greenish coloured extract was obtained. The extract was then stored in a desiccator to remove the excessive moisture. The dried extract was then packed in an air tight glass container for further studies.

Aqueous extract of whole plants of Ammannia baccifera Linn:

The marc left after ethanol extraction was again dried and then macerated with distilled water in a 2 litre round bottom flask for 72 hrs and 10 ml of chloroform was added to avoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown coloured extract was obtained and it was stored in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies. The percentage yields of the above extracts were expressed in Table 1.

Identification of Phytochemical active constituents preliminary phytochemical studies

The extracts obtained (Petroleum ether, Ethanol and Aqueous) were subjected to the following preliminary phytochemical studies.

Test for Alkaloids

1. Dragendorff's test: To 2 mg of the extracts, 5ml of distilled water was added; 2M Hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

2. Hager's test: To 2 mg of the extracts were taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids.

3. Wagner's test: 2 mg of extract were acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicates the presence of alkaloids.

4. Mayer's test: To a few drops of the Mayer's reagent, 2mg of extracts were added. Formation of white or pale-yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates

1. Anthrone test: 2 mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue colour indicates the presence of carbohydrates.

2. Benedict's test: 2 mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 5ml of Benedict's solution was added and boiled for 5minutes.Formation of brick red coloured precipitate indicates the presence of carbohydrates.

3. Fehling's test: 2 mg of extracts were `shaken with 10ml of water, filtered and the filtrate was concentrated. To this 1ml mixture of equal parts of Fehling's solution A and B were added and few minutes. Formation of red or brick red coloured precipitate indicates the presence of reducing sugar.

4. Molisch's test: 2 mg of extracts were shaken with 10ml of water, filtered and the filtrate as concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of α – naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red – violet ring appears, indicating the presence of carbohydrates which disappears on the addition of excess of alkali.

Test for Flavonoids

Shinoda's test: 2mg of extracts were dissolved in 5ml of ethanol and to this, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish, or brown colour indicates the presence of flavonoids. With Conc. Sulphuric acid test: Yellow orange colour (anthocyanins), yellow to orange colour (flavones) and orange to crimson (flavonones).

Test for Glycosides

Molisch's test: 2 mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To these 2-3 drops of Molisch's reagent was added, mixed and 2ml of conc. Sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides.

Test for proteins and Free amino acids

Small quantity of the extracts was dissolved in few ml of water and treated with following reagents.

Million's reagent: Appearance of red colour shows the presence of protein and free amino acid.
Ninhydrin test: Appearance of purple colour shows the presence of proteins and free amino acids.
Biurets test: Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of pink or purple colour shows the presence of proteins and free amino acid.

Test for Gums and Mucilage
Precipitation with 95% alcohol: Small quantities of the extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for Saponins
Foam test: In a test tube containing about 5ml of extracts, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicates the presence of saponins.

Test for Sterols
Liebermann - Burchard's test: 2 mg of dry extracts were dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along sides of the test tube. Formation of green colour indicates the presence of steroids.
Salkowski Reaction: 2 mg of dry extracts were shaken with chloroform, to the Formation of red colour indicated the presence of steroids.

Test for Fixed Oils:
Spot test: Small quantities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop a phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soap partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Phenolic compounds and Tannins
Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.
Dilute ferric chloride solution (5%)-violet color.
1% solution of Gelatin containing 10% sodium chloride- White precipitate.
10% lead acetate solution- White precipitate

Pharmacological Screening
Animals
Healthy adult Wistar rats, weighing 180–220 g, were used and acclimatized to laboratory conditions for one week. All animals were housed in well-ventilated polypropylene cages (12 hrs light and 12 hrs dark schedule) at 25℃ and 55–65% RH. The rats were provided with a standard diet. Rats were freely allowed to commercial pelleted rats chow and water ad libitum. In accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animal Ethics Committee (IAEC) has approved the experimental study to carry out. The CPCSEA approval number is Vedic/CCSEA/IAEC/11, Dated 11.04.2025.

Anti-Parkinson's ACTIVITY
Haloperidol-Induced Catalepsy in Rats
All the animals were divided into 5 groups (n = 6)
1. Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group
2. Group II: Administered haloperidol (1 mg/kg, i.p.) daily for a period of 7 days, served as the negative control group.
3. Group III: Received Syndopa (10 mg/kg body weight) as standard drug
4. Group IV: Administered extract at the doses of 200 mg/kg body weight intra-peritonial
5. Group V: Administered extract at the doses of 400 mg/kg body weight intra-peritonial

Haloperidol was given 30 minutes prior to standard and test drug administration. Bodyweight changes and behavioral assessments were carried out before the start of the treatment. Various parameters like Catalepsy (Bar test), Locomotor activity (acto-photometer test), and Muscle Rigidity (Rota-rod test) were measured in all animals.

Chlorpromazine-Induced catalepsy in Rats
All the animals were divided into 5 groups (n = 6)
Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group
Group II: Administered chlorpromazine (3 mg/kg, i.p.) daily for a period of 21 days, served as the negative control group.
Group III: Received Syndopa (10 mg/kg body weight) as standard drug
Group IV: Administered extract at the doses of 200 mg/kg body weight intra-peritonially
Group V: Administered extract at the doses of 400 mg/kg body weight intra-peritonially
Chlorpromazine was given 30 minutes prior to standard and test drug administration. Bodyweight changes and behavioural assessments were carried out before the start of the treatment. Various parameters like Catalepsy (Bar test), Locomotor activity (Actophotometer test), and Muscle Rigidity (Rota-rod test) were measured in all animals.

BEHAVIORAL ASSESSMENT

Catalepsy bar test

Catalepsy is a state of activity characterized by muscle rigidity associate with failure to correct an externally induced oblique posture for a protracted amount of time. The standard bar test is used for the assessment of catalepsy. Antipsychotic agents usually increase hypersomnia, thereby providing a measure of the extrapyramidal side-effects observed in humans exposed to chronic antipsychotics. Catalepsy induced by the typical neuroleptic agents in rodents can be used as a model for extrapyramidal effects in PD. Catalepsy is most typically measured by the standard bar technique consists of inserting an animal, after administration of a neuroleptic such as haloperidol/CPZ in a position with its front legs resting on a bar suspended on top of the ground. The intensity of catalepsy is measured by the length of time the subject maintains this externally induced abnormal posture.

Catalepsy was measured by a grading technique given below.

Step I-0 Rat moved normally when placed on the table.

Step II-0.5 Rat moved only when touched/pushed.

Step III-0.5 Front paws of the rats were placed alternately on a 3 cm high

block. If the rat failed to correct the posture within 15 sec, a score

of 0.5 for each paw was added to the score of step 1.

Step IV-1.0 The front paws of the rat were alternately placed on a 9 cm high block.

If the rat failed to correct the posture within 15 sec, a score of 1 for each paw was added to the scores of step I and II. Thus, 3.5 is a highest score for an animal.

Rotarod activity test

Rotarod apparatus has a horizontal grooved rod rotating at a fixed speed. The rats are made to balance on this rod. Dependent upon their motor co-ordination, Central nervous activity, and grip strength the animal either stays on the rotating rod for a specific time and after that falls down on the platform of each compartment. The floor of each compartment has sensors that deactivate the timers and the exact fall off time for each rat is displayed on the respective display. A cut-off time of 180 seconds was maintained throughout the experiment. The average results were recorded as the fall of time. In free riding, the mouse holds the rotating rod and rotates with it. Hence, free ridings are considered as a sensitive parameter related to grip strength and muscle coordination (Rajoriya et al., 2024).

Locomotor activity test

The spontaneous locomotor activity was monitored using a digital actophotometer equipped with infrared-sensitive photocells. The apparatus was placed in a darkened, light and sound attenuated, and ventilated testing room. Each interruption of a beam generated an electrical impulse that was denoted on a digital counter. Each animal was observed over a period of 1 min following haloperidol and chlorpromazine administration and values were expressed as counts per min.

RESULT AND DISCUSSION

Plant derived natural products such as flavonoids, phenolic compounds, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant activity. There has been growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intuse research into their potential benefits to human health. One of their main properties in this regard is their antioxidant activity, which enable them to attenuate the development of neurodegenerative diseases. Antioxidant plays an important role in inhibiting and scavenging free radicals, thus providing protection to degenerative diseases. Realizing the fact, this research was carried out to evaluate the anti-parkinson activities of extracts of *Ammannia baccifera* Linn. Based on ethano pharmacological literature, the plant was collected from the paddy fields of Mettukadai village, Erode district, Tamilnadu, India, during the month of September 2019. The collected plant was identified and authenticated by a Botanist.

Determination of Extractive values of Whole plant of *Ammannia Baccifera* Linn.

The shade dried coarsely powdered whole plants of *Ammannia baccifera* Linn. were extracted by using different solvents of increasing polarity by continuous hot percolation process using Soxhlet apparatus and aqueous extracts by cold maceration method. Extractive values were presented in Table 1.

Table 1: Extractive Values of Whole Plants of *Ammannia Baccifera* Linn

Plant name	Parts used	Method of extraction	Yield in percentage		
			Petroleum Ether	Ethanol	Aqueous
Ammannia baccifera Linn.	Whole plant	Continuous Hot Percolation and Cold Maceration	5.6	9.3	15.6

Phyto-chemical Evaluation

The phyto-constituents present in the various extracts were identified by performing chemical tests and the results were showed in Table 2.

Petroleum Ether	Chlorophyll, Starch, Fat, Fixed oil.
Ethanol	Carbohydrates, Glycosides, Tannins, Saponins, Flavonoids and Phenolic compounds.
Aqueous Extract	Carbohydrates, Glycosides, Flavonoids and Phenolic compounds.

From the above stated extracts, ethanolic extract showed the presence of more phyto-constituents. Hence, ethanolic extract (EEAB) was selected for the pharmacological evaluation.

Table 6.2: Preliminary Phyto-chemical studies of extracts of whole plant of *Ammannia Baccifera* Linn.

S. No	Constituents	Tests	Petroleum Ether	Ethanol Extract	Aqueous Extract
1.	Alkaloids	Mayer's test	-	-	-
		Dragendorff test	-	-	-
		Hager's test	-	-	-
		Wager's test	-	-	-
2.	Sterols	Libermann's Burchard test	-	+	+
		Salkowski's test	-	+	+
3.	Carbohydrates	Molisch reagent	-	+	+
		Fehling reagent	-	+	+
		Benedict reagent	-	+	+
		Anthrone test	-	+	+
4.	Fixed Oils and Fats	Spot test	+	-	-
5.	Phenolic	$FeCl_3$	-	+	+
		Gelatin test	-	+	+
		Lead acetate test	-	+	+
6.	Protein	Biuret test	-	-	-
		Ninhydrin test	-	-	-
		Xanthoprotein test	-	-	-
		Millon's reagent	-	-	-
7.	Saponins	Foam test	-	+	-
8.	Tannins	Gelatin test	-	+	-
9.	Gum and Mucilage	$FeCl_3$	-	+	-
		Precipitation with 95% alcohol	+	-	-
10.	Flavonoids	Shinoda's test	-	+	+
		Conc. H_2SO_4	-	+	+
11.	Glycosides	Molisch's test	-	+	+

Pharmacological evaluation (Anti-Parkinson Activity)

Haloperidol Induced Model

Effect of EEAB on Haloperidol-induced Catalepsy in Rats

All the animals were evaluated using a catalepsy bar test for the assessment of catalepsy for a week. The control animals (group-I) shown a catalepsy time of about 1.5-2.5 seconds during their entire observation period. All the groups shown a significant change in the catalepsy time on day 0. On day 7 Group-II animals (haloperidol alone) were found to be more retaining on the bar for a longer duration as compared to group-I. Group-III (200mg/kg) and Group-IV (400mg/kg) (pre-treated with different doses of extract) showed a significant reduction in the catalepsy time as compared to Group-II. Group-V animals (Syndopa) significantly reduced the catalepsy time as compared to Group-II on day 7. The values were indicating that EEAB treated groups (group-III and group-IV) significantly reduces the catalepsy time on day 7. The results were shown below in Table 3.

Table 3: Effect of Ethanolic extract of *Ammannia Baccifera* on Haloperidol Induced Catalepsy in rats

S. No.	Treatment	Time (seconds)	
		0th day	7th day
1	Group I (Propylene glycol - 5ml/kg)	1.91±0.21	2.34±0.32
2	Group II (Haloperidol – 1mg/kg)	3.43±0.26	19.20±0.60
3	Group III (HP + EEAB – 200mg/kg)	2.80±0.31	9.61±0.48
4	Group IV (HP +EEAB – 400mg/kg)	2.42±0.19	7.92±0.76
5	Group V (HP + Syndopa – 10 mg/kg)	2.27±0.31	6.52±0.56

All the values were expressed as mean± SEM and n=6 in each group; All the data were analyzed by one-way ANOVA method; P values <0.05 are considered to be significant.

Effect of EEAB on Haloperidol-induced Hypolocomotion in Rats

All the animals were evaluated for locomotor activity using Actophotometer. The locomotor activity score of group-I was found to be 70-73 counts/min throughout the week. For group-II, the activity score was reduced to 49.51 ± 0.61 on day 7. It showed a decrease in the locomotor activity on group-II (haloperidol) as compared to group-I (vehicle)., Animals pre-treated with EEAB (group-III and group-IV) showed a significant increase in the locomotor activity when compared to group-II. Group-V animals showed an increase in the locomotor activity as compared to group-II. Group-IV animals showed a much significant increase in the activity score similar to that of group-V animals. The results were shown below in Table 4.

Table 4: Effect of ethanolic extract of *Ammannia Baccifera* on Haloperidol Induced Hypolocomotion in rats

S. No.	Treatment	Locomotor activity (counts/min)	
		0th day	7th day
1.	**Group I (Propylene glycol 5 ml/kg)**	**70.42±0.61**	**71.80±0.23**
2.	**Group II (Haloperidol – 1 mg/kg)**	**65.10±0.46**	**49.51±0.61**
3.	**Group III (HP + EEAB – 200 mg/kg)**	**66.61±0.40**	**63.84±0.39**
4.	**Group IV (HP +EEAB – 400 mg/kg)**	**67.13±1.04**	**64.24±0.73**
5.	**Group V (HP + Syndopa – 10 mg/kg)**	**69.21±1.10**	**65.72±1.02**

All the values were expressed as mean± SEM and n=6 in each group. All the data were analyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Effect of EEAB on Haloperidol-induced Muscular Rigidity in Rats

Muscular rigidity was evaluated by using a Rotarod apparatus. The mean fall-off time was considered to be an indicator of muscular rigidity. The mean fall-off time of group-I was found to be 95-100 seconds during the entire weekly observation. All the groups shown a non-significant difference in muscular rigidity on day 0 and then showed a significant difference in muscular rigidity on day 7 except. Group - I. Group – II showed a significant reduction in the mean fall-off time when compared to group-I. Group-III and Group-IV significantly shown the reduction in mean fall-off time compared to group-II. Group-V showed a significant increase in the mean fall-off time as compared to group-II. The results were shown below in Table 5. The results coincide with the previous reported article.

Table 5: Effect of Ethanolic extract of *Ammannia Baccifera* on Haloperidol Induced Muscular Rigidity in Rats

S. No.	Treatment	Fall off time (Counts/min)	
		0th day	7th day
1.	Group I (Propylene glycol 5 ml/kg)	96.31±0.37	95.83±0.81
2.	Group II (Haloperidol – 1 mg/kg)	92.22±1.64	76.54±0.96
3.	Group III (HP + EEAB – 200 mg/kg)	93.42±0.23	88.31±0.72
4.	Group IV (HP +EEAB – 400 mg/kg)	95.14±0.76	90.71±0.88
5.	Group V (HP + Syndopa – 10 mg/kg)	94.20±1.21	95.13±0.91

All the values were expressed as mean± SEM and n=6 in each group. All the data were analyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

CHLORPROMAZINE INDUCED MODEL

Effect of EEAB on Chlorpromazine-induced Catalepsy in Rats

Animals were evaluated by using bar test for the assessment of catalepsy for weekly observation for a period of 21 days. All the animals were evaluated on day 0, day 7, day 14, day 21 after treatment. Group-I animals showed catalepsy score between 2.0-2.5 seconds during their entire observation period. Group-II animals showed a significant increase in catalepsy time when compared to group-I on day 7. On day 14, group-II animals still showed a significant increase in the catalepsy time compared to group-I and the time increases on day 21. Group III and Group IV slightly reduce the catalepsy time after a week compared to group-II. On day 14, the reduction increases and on day 21, group-V has shown a much more significant reduction in the catalepsy time. Group-V animals showed a significant reduction in the catalepsy time as compared to group-II on day 7 and the reduction in catalepsy time increases after each week. On day 21, group-V showed a much significant reduction in catalepsy time compared to group-II. The results were shown in Table 6.

Table 6:Effect of Ethanolic Extract of *Ammannia Baccifera* on Chlorpromazine Induced Catalepsy in Rats

S. No.	Treatment	Time (Second)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol - 5ml/kg)	2.12±0.52	2.31±0.36	2.40±0.48	2.43±0.62
2.	Group II(Chlorpromazine – 3 mg/kg)	4.31±0.40	9.81±0.31	16.42±0.47	20.37±0.82
3.	Group III (CPZ + EEAB –200mg/kg)	2.90±0.51	5.24±0.64	7.94±0.88	9.63±0.49
4.	Group IV (CPZ +EEAB –400mg/kg)	2.73±0.46	4.92±0.80	7.14±0.94	7.82±0.76
5.	Group V (CPZ + Syndopa –10 mg/kg)	2.31±0.52	4.40±0.63	6.76±0.74	7.05±0.96

All the values were expressed as mean± SEM and n=6 in each group. All the data were analyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Effect of EEAB on Chlorpromazine-induced Hypolocomotion in Rats

All the animals were evaluated for locomotor activity using Actophotometer. All the animals were evaluated on day 0, day 7, day 14, day 21 after treatment. The locomotor activity score of group-I was found to be 65-75 counts/min for the entire observation period. Group-II animals showed a significant reduction in the locomotor activity score when compared to group-I on day 7 and the reduction in the locomotor activity score increases on day 14, and 21. Group III and Group IV showed gradual increase in the locomotor activity score on day 7, day 14, and day 21 as compared to group-II. Group-V showed an increase in the locomotor activity score on day 7 when compared to group-II and the values were significant. Group-V animals showed much significant increase in the locomotor activity score on day 14 and day 21. The results were shown below in Table 7.

Table 7: Effect of Ethanolic Extract Of *Ammannia Baccifera* On Chlorpromazine Induced Hypolocomotion in Rats

S. No.	Treatment	Treatment Locomotor activity (counts/minute)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol - 5ml/kg)	69.23±1.08	71.41±1.14	73.62±0.89	74.34±1.02
2.	Group II (Chlorpromazine -3mg/kg)	64.25±1.34	58.02±1.05	47.43±0.81	36.81±1.34
3.	Group III (CPZ + EEAB –200mg/kg)	66.31±0.75	64.31±0.66	62.48±0.44	61.06±1.15
4.	Group IV (CPZ +EEAB –400mg/kg)	66.82±1.16	65.21±1.34	64.71±0.87	64.13±1.40
5.	Group V (CPZ + Syndopa –10 mg/kg)	67.10±1.64	67.52±1.38	68.24±0.94	68.82±0.82

All the values were expressed as mean± SEM and n=6 in each group. All the data were analyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

Effect of EEAB on Chlorpromazine-induced Muscular Rigidity in Rats

Muscular rigidity was evaluated using the Rota-rod apparatus. The mean fall-off time was considered to be an indicator of muscular rigidity. All the groups showed a reduction in muscular rigidity after each week except group-I. The mean fall-off time of group-I was found to be 88-92 seconds during the entire observation period. Group-II showed a significant reduction in the mean fall-off time on day 7 when compared to Group-I and the reduction level increases on day 14, and day 21. Group-III and Group-IV slightly showed a significant increase in the fall-off time when compared to group-II on day 7. The fall-off time increases after each week on day 14, and on day 21. The results were shown below in table.no.12. The results coincide with the previous reported article.

Table 6.8: Effect of Ethanolic extract of *Ammannia Baccifera* on Chlorpromazine Induced Muscle Rigidity in rats

S. No.	Treatment	Treatment Fall off time (seconds)			
		0th day	7th day	14th day	21st day
1.	Group I (Propylene glycol - 5ml/kg)	88.20±1.14	88.62±1.20	89.30±0.94	89.71±1.26
2.	Group II (Chlorpromazine-3mg/kg)	83.00±0.78	69.52±1.40	61.04±1.53	42.82±1.28
3.	Group III (CPZ + EEAB –200mg/kg)	84.22±1.41	83.61±1.37	84.71±1.63	85.43±1.25
4.	Group IV (CPZ +EEAB –400mg/kg)	85.11±0.86	84.30±1.39	83.42±1.08	85.62±0.93
5.	Group V (CPZ + Syndopa –10 mg/kg)	86.61±0.85	85.70±1.46	84.38±1.03	88.37±1.33

All the values were expressed as mean± SEM and n=6 in each group. All the data were analyzed by one-way ANOVA method. P values <0.05 are considered to be significant.

DISCUSSION

Anti-Parkinson's activity

Catalepsy is a behaviour or nervous condition of animals characterized by muscular rigidity and fixity of posture for a prolonged period known as akinesia [66,67]. Catalepsy is a well-known motor symptom of Parkinson's disease. Group-III and Group-IV were found to reduce the catalepsy time in animals similar to that of standard drug. Moreover, Locomotor activity is considered to be an indicative of movement which is impaired or affected in PD which is known as bradykinesia. It is considered to be a cardinal motor symptom of PD. Hence, the locomotor index can be an indicator of Parkinsonism. Group-III and Group-IV were found to increase the locomotor index comparatively than group-II. Muscular rigidity is also known as muscle stiffness characterized by the inability of the muscles to relax. It is also regarded as the main motor symptom of Parkinson's disease. Fall-off time from the rod indicates the level of rigidity in animals. Thus, muscular rigidity also can be an index of Parkinsonism. Group-III and Group-IV were found to show prevention of reduction in fall-off time. This indicates that ethanol extract of *Ammannia baccifera* Linn. has a neuroprotective effect and hence may have a role in anti-parkinsonism activity.

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