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ANTI-CATALEPTIC ACTIVITY OF ETHANOL EXTRACT OF *AGERATUM CONYZOIDES* L.

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ABSTRACT

In the present study we evaluated the anticataleptic efficacy of ethanol extract of *Ageratum conyzoides* L. in haloperidol induced catalepsy in rats. Scientific evaluation of this claim using experimental model Anticataleptic activity using block method, Locomotor activity in actophotometer and Exploratory behavior in hole board apparatus. From the observations of above studies it could be envisaged, that the protective effect of ethanol extract of *Ageratum conyzoides* L. against symptoms of Parkinson's disease (catalepsy) may be due to regulation in neurotransmitters such as dopamine, serotonin, glutamate which are playing an important role in protection of catalepsy and antioxidant properties. Further studies have to be conducted on the various extracts and isolated principles of the plant for their effects on other CNS disorders.

Keywords: *Ageratum conyzoides* L., Catalepsy, Parkinsonism, CNS disorders.

INTRODUCTION

Central Nervous System associated diseases are appearing as a major threat in the future because of increasing mental stress, work load and strain which are essential in developing world. Unknowingly this throws in to a state where there is more chance of CNS disorders. Atmospheric pollutants, toxins also causing neurodegenerative diseases like Parkinson's disease and Alzheimer's disease. Herbal drugs are having diversified uses are always an alternative option to the synthetic drugs which are well known for their adverse effects. Since the existing antiparkinson's drugs encounter many side effects and need for prolonged treatment including questionable efficacy in the treatment, may cause Parkinson's related movement problems like hallucinations and orthostatic hypotension. These reasons force the area of research to find new and improved treatments which will encounter the adverse effects and drawbacks of the existing treatments. Long term treatment with haloperidol, a classical neuroleptic drug widely used for the treatment of schizophrenia and affective disorders can lead to Parkinson's like symptoms. It blocks dopamine receptors, and concomitant increase in turnover of this amine may contribute haloperidol toxicity due to generation of free radicals and increased lipid peroxidation. Dementia has been associated with drug-induced parkinsonism and has been suggested to support the role of underlying brain damage.

A wide variety of pathological conditions including cancer, rheumatoid arthritis, Alzheimer's disease, diabetes mellitus, ischemia, atherosclerosis and Parkinson's disease appear to have etiological relation to the reactive oxygen species (ROS) induced and free radical mediated oxidation of biomolecules, which take place in conditions with inadequate antioxidant defence stress. Prevention of the initial cellular damage caused by these species has been the subject of intense investigation and resulted in the discovery of several naturally occuring or synthetic substances, which have been accredited as potent antioxidants [1].

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Ageratum conyzoides L. (Compositae) is found in many parts of Kenya . The plant is traditionally used for its antispasmodic, haemostatic, antiasthmatic and insecticide activities and for the treatment of wounds and *Staphylococcus aureus* infections [2-5]. The plant extract is a cardiodepressant on isolated rabbit heart, a neuromuscular blocker, hypotensive and calcium channel blocker, and has antispasmodic effects on isolated rabbit ileum [6]. This present study carried out to assess the validity of the folkloric uses of this plant in brain disorders and establish the possible mechanisms of pharmacological action. Scientific evaluation of this claim using experimental model Anticataleptic activity using block method, Locomotor activity in actophotometer and Exploratory behavior in hole board apparatus. This folklore claim was supported in our study by various behavioral studies.

METERIALS AND METHODS

Plant collection

The whole plant of *Ageratum conyzoides Linn.* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of September 2011 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

Whole plant of *Ageratum conyzoides Linn.* were shade dried, and the dried whole plant were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using Petroleum ether. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10%w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.

Animal Used

Albino Wistar rats, weighing 220–250 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25° C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee.

Acute Toxicity Study

The acute toxicity of Petroleum ether extract of whole plant *of Ageratum conyzoides Linn.* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at the 2000 mg/kg doses. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose was selected for further study [7].

PHARMACOLOGICAL STUDIES

Catalepsy induced by chronic haloperidol administration in experimental rats

Haloperidol (1.0 mg/kg, ip) was administered daily to the rats for a period of 20 days to induce catalepsy [8-10]. Plant extract and standard drugs were administered orally 30 min before to the haloperidol treatment. The animals were divided in to seven groups, each containing 6 animals.

Group I : The animals received 1% tween 20 (5 ml/kg, po) and served as control.

Group II : The animals received haloperidol (1.0 mg/kg, ip) and served as negative control.

Group III: The animals received haloperidol (1.0 mg/kg, ip) and treated with L-DOPA (100 mg/kg, po) suspended in 1% tween 20.

Group IV: The animals received haloperidol (1.0 mg/kg, ip) and treated with L-DOPA+Carbidopa (100+25 mg/kg, po) suspended in 1% tween 20.

Group V : The animals received haloperidol (1.0 mg/kg, ip) and treated with EEAC (200 mg/kg, po) suspended in 1% tween 20.

Group VI: The animals received haloperidol (1.0 mg/kg, ip) and treated with EEAC (400 mg/kg, po) suspended in 1% tween 20.

Group VII: The animals received haloperidol (1.0 mg/kg, ip) and treated with L-DOPA (100 mg/kg, po) EEAC (400 mg/kg, po) suspended in 1% tween 20.

Invivo pharmacological studies were carried out on last day of the experiment, and then the animals were sacrificed for biochemical parameters.

IN VIVO PHARMACOLOGICAL STUDIES

Effect of the EEAC and standard drugs L-DOPA, L-DOPA+Carbidopa on haloperidol induced catalepsy in rats (Block method 0-3.5 scale)

The effect of test drug and standard drugs on haloperidol induced catalepsy was studied by the following method [11].

Severity of catalepsy was measured every 30 min, thereafter up to a total duration of 3 hours. Catalepsy of an individual rat was measured in a stepwise manner by a scoring method as described below. The method assessed the ability of an animal respond to an externally imposed posture.

Stage I: The rat was taken out of the home cage and placed on a table. Rat moves freely no score was given.

Stage II : If the rat failed to move when touched gently on the back or pushed, score of 0.5 assigned.

Stage III: The front paws of the rat 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 I.

Stage IV: The front paws of the rat were placed alternately on a 3 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, StepII. Thus for an animal, the highest score was 3.5 (cut-off score) and that reflects in total catalepsy.

Effect of the EEAC and standard drugs L-DOPA, L-DOPA+Carbidopa on locomotor activity

The effect onlocomotor activity was measured for 10 min at every 30 min upto 3 hours using actophotometer [12].

The locomotor activity can be easily measured by using an actophotometer. It operates on photoelectric cells that are collected in circuit with a counter. When the beam of light falling on the photocell is cutoff by animal, a count is recorded. An actophotometer could have either circular or square arena in which animal moves.

Effect of the EEAC and standard drugs L-DOPA, L-DOPA+Carbidopa on exploratory behaviour (Head dipping)

The effect on exploratory behavior (head dipping)was measured for 10 min at every 30 min upto 3 hours using hole board [13].

The hole board made of plywood has the size (60 cm X 60 cm, 3 mmthick). The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed holes each of 5 cm in diameter. Each rat was acclimatized for 10 min and number of holes explored through head plunging acts during the total observation time period were noted. Care has to taken to avoid multiple events (two or more head plunging in quicker session). A fresh exploration was considered when the animal neatly plunged its head once and did something else in between like grooming, taking a short walk etc. before plunging its head for the next time. One animal at a time was tested for each activity.

Effect of the EEAC and standard drugs L-DOPA, L-DOPA+Carbidopa on exploratory behaviour (Line crossing)

The effect on exploratory behavior (line crossing) was measured for 10 min at every 30 min upto 3 hours using hole board [13].

The hole board made of plywood has the size (60 cm X 60 cm, 3 mmthick). The mat finished of the upper surface avoids reflections which might alter the behaviour of the animal. The board embodies 9 uniformly distributed lines. Each rat was acclimatized for 10 min and the number of line crossing acts, during the total observation period were counted. Care has to taken to avoid multiple events.

Statistical Analysis

The statistical analysis was carried out using analysis of variance (ANOVA),followed by dunett's test. P values < 0.05 considered as significant.

RESULTS

Acute Oral Toxicity Study

The acute oral toxicity study was done according to OECD guidelines 423 (acute toxic class method). A single dose of 2000 mg/kg body weight/po of the EEAC was administered to 3 female rats. Animals were observed for signs of toxicity for first 3 hours at 30 min time intervals. Thereafter animals were observed for 24 hours with continuous monitoring. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no signs of toxicity were observed. LD_{50} cut off dose per kilogram body weight was categorized as X (unclassified). The results are shown in Table -1.

***IN VIVO* PHARMACOLOGICAL ACTIVITY**

Effect of EEAC on Haloperidol Induced Catalepsy (0-3.5 SCALE)

The cataleptic scores are depicted in Table-2. There was a significant difference ($P<0.01$) between control group (I) and negative control group(II) in catalepsy. The EEAC treated groups shows significant anticataleptic action. EEAC at dose level of 400 mg/kg particularly, shows anticataleptic action comparable to standard drug treatment. There was a significant difference ($P<0.01$) between negative control group (II) and EEAC treated groups (V, VI, VII) in catalepsy. EEAC at dose level of 400 mg/kg, showed good anticataleptic action at 30, 150, 180 min after haloperidol challenge.

Effect of EEAC on Locomotor Activity

The changes in locomotor activity after haloperidol administration are shown in Table-3. There is a significant ($P<0.01$) decrease in locomotor activity in negative control group (II) when compared with the control group (I). EEAC 400 mg/kg treated animals showed improved locomotor activity when compared with negative control group ($P<0.01$). EEAC at a dose of 400 mg/kg potentiated the locomotor activity of standard drug.

All groups were showing significant difference ($P<0.01$) when compared with negative control group at all time intervals.

Effect of EEAC on Exploratory Behavior

The exploratory behaviour was expressed by head dippings and line crossings. Head dippings are shown in Table-4 and line crossings are shown in Table-5. Negative control group (II) indicated decrease in exploratory behaviour i.e. head dippings and line crossings when compared with control group. The results presented by the EEAC treated groups show significant ($P<0.01$ and $P<0.05$) increase in head dippings and line crossings when compared with negative control group at 90, 120, 150, 180 min after haloperidol challenge.

Table 1. Acute toxic class method (OECD 423 guideline)

S.No	Treatment	Dose	Weight of the animal in grams		Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
			Before test (1st day)	After test (14th day)				
1	EEAC	2 g/kg	190	195	No signs of toxicity	Nil	Nil	14 days
2	EEAC	2 g/kg	200	205	No signs of toxicity	Nil	Nil	14 days
3	EEAC	2 g/kg	190	1195	No signs of toxicity	Nil	Nil	14 days

Table 2. Effect of EEAC on catalepsy (0-3.5 scale)

Group	Catalepsy					
	30 min	60 min	90 min	120 min	150 min	180 min
I	0.00	0.00	0.00	0.00	0.00	0.00
II	$2.5\pm0.13^{a**}$	$3.0\pm0.18^{a**}$	$3.5\pm0.00^{a**}$	$3.5\pm0.00^{a**}$	$3.5\pm0.00^{a**}$	$3.5\pm0.00^{a**}$
III	$1.08\pm0.15^{b**}$	$1.67\pm0.10^{b**}$	$1.67\pm0.10^{b**}$	$1.5\pm0.12^{b**}$	$1.5\pm0.13^{b**}$	$1.67\pm0.10^{b**}$
IV	$0.83\pm0.10^{b**}$	$1.17\pm0.10^{b**}$	$1.33\pm0.10^{b**}$	$1.08\pm0.08^{b**}$	$1.08\pm0.08^{b**}$	$0.75\pm0.12^{b**}$
V	$2.0\pm0.13^{b*}$	2.5 ± 0.12^{bns}	$2.5\pm0.12^{b**}$	$2.08\pm0.15^{b**}$	$2.17\pm0.17^{b**}$	$2.16\pm0.10^{b**}$
VI	$1.33\pm0.10^{b**}$	$1.83\pm0.16^{b**}$	$1.83\pm0.10^{b**}$	$1.75\pm0.21^{b**}$	$1.67\pm0.17^{b**}$	$1.67\pm0.10^{b**}$
VII	$0.97\pm0.08^{b**}$	$1.33\pm0.17^{b**}$	$1.25\pm0.12^{b**}$	$1.25\pm0.12^{b**}$	$1.25\pm0.12^{b**}$	$1.00\pm0.29^{b**}$

The values are expressed as mean ± SEM of 6 animals.
Comparisons were made between: a-Group I with Group II
b-Group II with Group III, IV, V, VI and VII
Statistical significance test for comparison was done by ANOVA , followed by Dunnet's test.
**P<0.01,*P<0.05, ns- Non significant.

Table-3: Effect of EEAC on locomotor activity

Group	Locomotor activity					
	30 min	60 min	90 min	120 min	150 min	180 min
I	311.67±8.39	335.67±6.62	327.83±7.77	303.83±4.22	320.00±4.13	330.33±5.99
II	$27.33\pm1.11^{a**}$	$21.00\pm0.86^{a**}$	$21.00\pm2.79^{a**}$	$29\pm0.86^{a**}$	$33.67\pm3.13^{a**}$	$40.00\pm3.10^{b**}$
III	$75.33\pm5.28^{b**}$	$141.00\pm6.30^{b**}$	$158.67\pm6.06^{b**}$	$167.16\pm6.03^{b**}$	$178.5\pm6.71^{b**}$	$206.67\pm9.76^{b**}$
IV	$110.50\pm5.85^{b**}$	$210.67\pm7.22^{b**}$	$232.00\pm6.97^{b**}$	$252.83\pm6.47^{b**}$	$267.50\pm6.50^{b**}$	$300.00\pm7.44^{b**}$
V	40.17 ± 3.20^{bns}	$51.00\pm3.83^{b**}$	$78.17\pm3.99^{b**}$	$88.17\pm3.05^{b**}$	$89.00\pm2.73^{b**}$	$101.17\pm5.64^{b**}$
VI	$51.17\pm3.51^{b**}$	$97.67\pm5.81^{b**}$	$125.00\pm4.43^{b**}$	$142.17\pm5.24^{b**}$	$144.00\pm4.74^{b**}$	$150.17\pm3.80^{b**}$
VII	$97.17\pm4.47^{b**}$	$171.5\pm5.51^{b**}$	$201.5\pm3.31^{b**}$	$219.17\pm3.65^{b**}$	$350.83\pm4.19^{b**}$	$271.00\pm3.27^{b**}$

The values are expressed as mean ± SEM of 6 animals.
Comparisons were made between : a-Group I with Group II
b-Group II with Group III, IV, V, VI and VII
Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test.
**P<0.01,ns- Non significant.

Table 4. Effect of EEAC on exploratory behaviour-head dipping

Group	Head dipping					
	30 min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
I	7.83±0.30	7.5±0.43	8.17±0.54	7.33±0.42	6.33±0.42	6.33±0.21
II	0.5±0.22^{a**}	0.83±0.31^{a**}	0.17±0.17^{a**}	0.5±0.22^{a**}	0.33±0.21^{a**}	0.5±0.22^{a**}
III	1.17±0.17^{bns}	2.83±0.31^{b**}	5.83±0.31^{b**}	6.0±0.26^{b**}	5.33±0.42^{b**}	4.17±0.31^{b**}
IV	4.17±0.31^{b**}	6.83±0.31^{b**}	9.17±0.54^{b**}	8.83±0.31^{b**}	8.83±0.42^{b**}	7.33±0.21^{b**}
V	0.5±0.22^{bns}	1.33±0.21^{bns}	2.5±0.22^{b**}	3.17±0.31^{b**}	3.17±0.17^{b**}	2.83±0.31^{b*}
VI	0.83±0.17^{bns}	3.0±0.26^{b**}	4.17±0.31^{b**}	5.33±0.33^{b**}	4.50±0.22^{b**}	4.17±0.48^{b**}
VII	3.17±0.31^{b**}	4.83±0.48^{b**}	7.17±0.31^{b**}	7.83±0.60^{b**}	7.17±0.31^{b**}	7.0±0.36^{b**}

The values are expressed as mean ± SEM of 6 animals.
Comparisons were made between : a-Group I with Group II
b-Group II with Group III, IV, V, VI and VII
Statistical significance test for comparison was done by ANOVA , followed by Dunnet's test.
**P<0.01,*P<0.05, ns- Non significant.

Table 5. Effect of EEAC on exploratory behaviour-Line crossing

Group	Line crossing					
	30 min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
I	82.43±2.27	76.4±1.62	82.83±1.17	78.62±1.92	87.12±2.54	78.3±2.26 ^{a**}
II	7.2±0.36 ^{a**}	4.18±0.98 ^{a**}	2.23±0.56 ^{a**}	2.12±0.42 ^{a**}	3.2±0.68 ^{b**}	3.53±1.27 ^{b**}
III	15.32±1.18 ^{bns}	39.0±3.55 ^{b**}	54.23±1.04^{b**}	67.2±1.72 ^{b**}	72.0±2.67 ^{b**}	75.66±3.26 ^{b**}
IV	35.16±3.12 ^{b**}	59.0±1.40 ^{b**}	72.3±1.46 ^{b**}	78.32±4.25^{b**}	82.5±2.29 ^{b**}	82.17±5.22 ^{b**}
V	12.6±0.22 ^{bns}	22.5±3.45 ^{b*}	24.83±1.7 ^{b**}	35.23±1.2 ^{b**}	42.5±2.28 ^{b**}	35.5±2.06 ^{b**}
VI	15.4±0.29 ^{bns}	32.5±1.27 ^{b**}	42.0±2.06 ^{b**}	58.2±3.2 ^{b**}	67.0±2.29 ^{b**}	56.0±2.52 ^{b**}
VII	18.34±2.26 ^{b**}	48.17±3.23^{b**}	65.6±1.16 ^{b**}	78.33±2.7 ^{b**}	78.2±2.92 ^{b**}	75.0±2.83 ^{b**}

The values are expressed as mean ± SEM of 6 animals.
Comparisons were made between : a-Group I with Group II
b-Group II with Group III, IV, V, VI and VII
Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test.
**P<0.01,*P<0.05, ns- Non significant.

DISCUSSION & CONCLUSION

The present study revealed the anticataleptic effects of ethanolic extract of *Ageratum conyzoides L.* in haloperidol model of catalepsy in rats. Neuroleptic like haloperidol induced catalepsy in rats is used to evaluate the drugs for their antiparkinsonism effects. In this study the EEAC was screened for its effect in haloperidol induced catalepsy in rats. EEAC at dose of 400 mg/kg exhibited a pharmacological effect similar to that of standard drug (L-DOPA) and further potentiation was observed with L-DOPA+ carbidopa+EEAC combination therapy. Haloperidol a neuroleptic, upon long term administration causes oxidative stress, resulting from alterations of mitochondrial electron transport chain, has been responsible for its neurotoxicity [14-16].

Epidemiological studies have shown beneficial effects of flavonoids on neurodegeneration in particular. Flavonoids can protect the brain by their ability to modulate intracellular signals promoting cellular survival. Quercetin and its structurally related flavonoids showed a marked cytoprotective capacity in *in vitro* experimental conditions in models of predominantly apoptotic death. EEAC showing positive reaction for flavonoids in phytochemical screening. The neuroprotective action may be attributed to the presence of flavonoids in EEAC [17-19].

Antipsychotic effect of haloperidol is believed to be achieved by inhibition of dopeminergic transmission in rats has been proposed to be direct consequence of antagonism of dopamine D_2 receptors. Neuroleptics like haloperidol exerts multiple events on dopeminergic signaling and produce DA related behavioural changes and catalepsy. Glutamate receptors play a major role in the transmitter balance within the basal ganglia. N-methyl-D-aspartate (NMDA) receptor stimulation within the striatum acts behaviourally depressant. Antagonists of these receptors show stimulatory effects. In animal models of parkinsonism all NMDA receptor antagonists counteract parkinsonian symptoms or acts synergistically with L-DOPA. Non competitive NMDA receptor antagonists produce strong locomotion. In this study EEAC treated animals showed decreased levels of glutamate than negative control group. Decreased glutamate levels may result in improved locomotion [20-22].

It can be hypothesized from this study that ethanolic extract of *Ageratum conyzoides L.* ameliorates the symptoms of haloperidol induced catalepsy in rats. The

mechanism by which the amelioration takes place may be attributed to one or more pharmacological/biochemical mechanisms viz, EEAC may enhance the bioavailability of circulatory dopamine by up regulation of dopaminergic signaling and EEAC may enhance the bioavailability of L-DOPA by inhibiting DOPA-decarboxylase activity like that of carbidopa [23,24].

Ageratum conyzoides L. is a well known plant which is being used in Indian traditional system which has been reported to have a number of uses. But the role of ethanolic extract of this whole plant in the treatment of some CNS associated disorders has not been evaluated. Hence this Artilce is emphasized to explore the effect of ethanolic extract of this whole plant on haloperidol induced catalepsy in rats. In the present study the effect of EEAC on extrapyramidal symptoms such as rigidity, motor co-ordination and depression, key parameters found in Parkinson's disease was studied. In haloperidol induced catalepsy EEAC at the dose of 400 mg/kg exhibited significant anticataleptic activity. EEAC showed comparable anticataleptic actions with that of standard drug L-DOPA.

EEAC at a dose of 400 mg/kg significantly, reversed the haloperidol inhibited locomotor activity. The standard drugs L-DOPA, L-DOPA+ carbidopa and EEAC+L-DOPA+carbidopa combination also significantly reversed the haloperidol inhibited motor activity. EEAC at a dose of 400 mg/kg significantly, increase the exploratory behaviour like head dipping and line crossing in haloperidol administered rats. The standard drugs L-DOPA, L-DOPA+carbidopa and EEAC+ L-DOPA+carbidopa combination also significantly increased the exploratory behaviour in haloperidol administered rats.

From the observations of above studies it could be envisaged, that the protective effect of EEAC against symptoms of Parkinson's disease (catalepsy) may be due to regulation in neurotransmitters such as dopamine, serotonin, glutamate which are playing an important role in protection of catalepsy and antioxidant properties. Further studies have to be conducted on the various extracts and isolated principles of the plant for their effects on other CNS disorders.

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