Neuroprotective activity of ethanolic extracts of *Cassia auriculata* in experimentally induced Parkinsonism

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**ABSTRACT**

In the study designed to investigate the neuroprotective activity of *Cassia auriculata* in experimentally induced Parkinsonism. Experimentally induced parkinsonism by administering the haloperidol in either short term and long term model to observe changes in catalepsy and in behavioral parameters like muscle rigidity as measured using rota-rod test and Spontaneous loco motor activity measured using actophotometer test and studying the antioxidant properties. The results showed that polyphenols, flavanoids, tannins, saponins and alkaloids were presented in the Ethanolic extracts of *Cassia auriculata* leaves. These extract has antioxidant properties. In addition, the treatment with extract significantly showed anticataleptic action as well as reduction of muscle rigidity and increase the Spontaneous locomotor activity. It is concluded that the extract has antioxidants activity combat against oxidative stress induced progressive neurodegeneration and dopaminergic activity; might be responsible for the antiparkinsonism activity by modulation of central neurotransmission.

**Key words**: *Cassia auriculata*, haloperidol, muscle rigidity, rota-rod, actophotometer test.

**INTRODUCTION**

Parkinsonism describes a syndrome of Parkinson’s disease (PD) it is a chronic neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons of substantia nigra pars compacta in the ventral midbrain. The loss of dopaminergic neurons, leads to the reduction of dopamine being released into the striatum. These processes are then responsible for the clinical features of PD including difficulty in initiating movements, rigidity, bradykinesia and tremor[1]. *Cassia auriculata* Linn (Family: Caesalpiniaceae) commonly called as Senna, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant has been proved various activities like anthelmintic[2], hepatoprotective[3], antiinflammatory and analgesic[4], anticancer[5], diabetes[6], antipyretic activity[7]. In view of the above consideration, the present investigation was done to find the Neuroprotective activity of ethanolic extracts of *Cassia auriculata* in experimentally induced Parkinsonism.

**EXPERIMENTAL SECTION**

Wistar rats (180-250g) and Albino mice (15-30g) of either sex were used for this study. Animals procured from Bhaskar medical college, Hyderabad. The study was performed in accordance with the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals an authority regulating animal experiments.
Plant material
The fresh leaves of *Cassia auriculata* were collected from local areas of Ranga reddy, Hyderabad, India and authenticated by Botanist, Osmania University. The leaves were dried in shade and ground to get a coarse powder.

Preparation of extract
The Ethanol extract of *Cassia auriculata* leave (EECAL) was prepared by maceration method using Ethanol solvent for 72hrs at room temperature. A suspension of EECAL in 5% (w/v) Carboxy Methyl Cellulose was prepared for oral administration.

Preliminary phytochemical screening
A portion residue from extract was subjected for phytochemical analysis in order to see the presence of glycosides, polyphenols, saponines, flavonoids, tanins and steroids[8].

Chemicals
Haloperidol (Serenace, LPG Life Sciences) solution was diluted in saline. Ethanolic extract of *Cassia auriculata* leaves.

Models
Haloperidol is widely used to induce Parkinsonism. The study was performed using short term and long term models as given below[9].

Short term Model – Haloperidol (0.5 mg/kg i.p.) was administered for short term model. EECAL was administered orally 60 min before the haloperidol treatment. The study was started in the morning and readings were noted for each 10min interval after haloperidol treatment. The short term model showed duration of catalepsy for approximate 4 hours accordingly the observations were noted for 4 hours. Animals were divided in four groups each containing 6 mice’s.

Group 1:- Control- Haloperidol i.p.
Group 2:- EECAL 250- Treated with EECAL 250mg/kg p.o. Short term
Group 3:- EECAL 500- Treated with EECAL 500mg/kg p.o. Short term
Group 4:- EECAL 1000- Treated with EECAL 1000mg/kg p.o. Short term
Haloperidol solution was injected to animals in each group after 60min of EECAL treatment.

Long term model – The dose i.e. 4mg/kg s.c. was administered for long term Parkinsonism like conditions and to evaluate the effect of test compounds. EECAL was administered 60min before the haloperidol treatment. The study was of one day which started in the morning with dosing of EECAL and readings were noted for each 10min interval after haloperidol treatment. Animals were divided in four groups each containing 6 mice’s. The treatment scheme followed for each subgroup was as follows,

Group 1  Control-haloperidol i.p
Group 2  EECAL 250- Treated with EECAL250mg/kg p.o. Long term.
Group 3  EECAL 500- Treated with EECAL 500mg/kg p.o. Long term.
Group 4  EECAL 1000- Treated with EECAL 1000mg/kg p.o. Long term.

Parameters-
Scoring for catalepsy :-
0 - rat moved when placed on the table;
0.5 - rat moved only when touched or pushed;
0.5 - rat placed on the table with front paws set alternately on a 3 cm high wooden block, failed to correct the posture. Time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw;
1.0 - rat failed to correct posture when front paws are placed on a 9 cm high wooden block. Time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw.
Some modifications were made in the above reported scoring method. We scored the catalepsy at the interval of 10 minutes so as to explore the exact time at which catalepsy was developed and to observe the slight change in degree of induction of catalepsy with respect to time[10] (Schulz-Schaeffer WJ 2010). The scoring pattern was slightly modified and multiplied with time (in sec) taken to correct the posture by animal for each score. This was done to differentiate the intensity of catalepsy of animals showing same score.

**Tests for Locomotor activity using Actophotometer**

Albino mice (Swiss) were randomly divided in 2 sets of experiments having 4 groups (n=6). Out of 2 sets, one set for short term and another set for long term model. All the groups except control, were treated with haloperidol solution in the dose 0.5 mg/kg i.p. and 4 mg/kg s.c. after 60 min of EECAL treatment for short term model and long term model respectively. To see the locomotor activity, each mouse was placed individually in the activity cage for 5 minutes. The Spontaneous motor activity score for all the animals was noted after 1 hr the administration of control and test material[10].

**Tests for Muscle Relaxation using Rota rod test**

Albino mice (Swiss) were randomly divided in 2 sets of experiments having 4 groups (n=6). Out of 2 sets, one set for short term and another set for long term model. All the groups except control, were treated with haloperidol solution in the dose 0.5 mg/kg i.p. and 4 mg/kg s.c. after 60 min of EECAL treatment for short term model and long term model respectively. Animals remain on Rota-Rod (25 rpm) for 5 min or more after low successive trials are included in the study. After the administration of control and test material, the fall off time from the rotating rod was noted after 2 hrs. The difference in the fall off time between the control and the treated mice was taken as an index of muscle relaxation[11].

**Biochemical analysis**

Animals were euthanized 24 hours after haloperidol-EECAL treatment by decapitation under ether anaesthesia, brain was excised, washed with ice-cold saline solution (0.9 % NaCl), weighed and stored for the biochemical analyses. The brain was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10 % w/v for the bio chemical assays.

**Assay of TBARS**

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the brain tissues. The malondialdehyde (MDA) is one such carbonyl compound, which forms a characteristic chromogenic adduct with two molecules of TBA. The colourimetric reaction of TBA with MDA, a secondary product of lipid peroxidation, has been widely accepted for measuring lipid peroxidation. The total protein which was present in the homogenate was estimated [12].

**Assay of Glutathione**

This spectrophotometric procedure was based on the method of Ellman i.e. DTNB [5, 5'-dithiobis-(2-nitrobenzoic acid)] is reduced by –SH groups to form one mole of 2-nitro-5- mercaptobenzoic acid per mole of –SH, the units of the GSH activity which were determined were expressed in terms of nmoles µg/mg protein[13].

**Assay of SOD**

The assay of SOD was carried out, based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol, as described by Marklund and Marklund. The total protein which was present in the homogenate was estimated (Peterson GL et al., 1979). The units of the SOD activity which were determined were expressed in terms of milligrams of the total protein.

**Assay of Catalase**

In the UV range, Hydrogen peroxide shows a continuous increase in the absorption with decreasing wavelength. The decomposition of Hydrogen peroxide can be followed directly by the decrease in the absorbance at 240 nm. The difference in absorbance (ΔA) per unit time is a measure of the catalase activity. The units of the CAT activity which were determined were expressed in terms of nmol H$_2$O$_2$/mg protein [14].
**Statistical Analysis**
The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by tukey Kramer comparison test where P<0.01 was considered statistically significant.

**RESULTS**

**Catalepsy test**
Table 1 shows effect of different doses of EECAL on induction of catalepsy as observed in change in catalepsy score with respect to time in haloperidol induced catalepsy in short term and long term model. The results were summarized to depict the various parameters like onset and duration of overall catalepsy in short term and long term models respectively. The lowest dose of EECAL i.e 250 mg/kg showed slight changes in all the parameters in short term as well as long term model. Although there were some positive changes observed in short term as well as long term model groups at dose of 500 mg/kg and 1000 mg/kg of EECAL, the effects were significant. However at 500 mg/kg, the EECAL exhibited highly significant (P<0.01) changes in all the parameters of catalepsy.

**Table 1: - Effect of EECAL on onset and duration of overall catalepsy in haloperidol induced catalepsy – short term (0.5mg/kg i.p.) and long term (4mg/kg i.p.) model in Mice**

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUPS</th>
<th>Short term : Overall catalepsy (min)</th>
<th>Long term : Overall catalepsy (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Onset</td>
<td>Duration</td>
</tr>
<tr>
<td>1</td>
<td>Control + haloperidol</td>
<td>12±1.24</td>
<td>175.1±2.14</td>
</tr>
<tr>
<td>2</td>
<td>EECAL 250 + haloperidol</td>
<td>12.1±0.24*</td>
<td>161.2±1.57**</td>
</tr>
<tr>
<td>3</td>
<td>EECAL 500 + haloperidol</td>
<td>16.4±0.23*</td>
<td>139.2±2.51***</td>
</tr>
<tr>
<td>4</td>
<td>EECAL 1000 + haloperidol</td>
<td>18.1±2.56</td>
<td>134.1±1.52***</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01 - Individual readings were compared with readings of control. (n =6)

**Table 2: - Effect of EECAL on onset and duration of overall Spontaneous loco motor activity score in Actophotomotor – short term (1mg/kg i.p.) and long term (4mg/kg i.p.) model in Mice**

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUPS</th>
<th>Overall Spontaneous motor activity score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Short term duration</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control + haloperidol</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>EECAL 250 + haloperidol</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>EECAL 500 + haloperidol</td>
<td></td>
</tr>
</tbody>
</table>

* - P<0.05, ** - P<0.01 - Individual readings were compared with readings of control. (n =6)

**Table 3: - Effect of EECAL on onset and duration of overall Grip Strength (Sec) in rotarod – short term (1mg/kg i.p.) and long term (4mg/kg i.p.) model in mice**

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUPS</th>
<th>Overall Grip Strength (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Short Duration</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>120.5±1.2</td>
</tr>
<tr>
<td>2</td>
<td>Control + haloperidol</td>
<td>78.5±0.5**</td>
</tr>
<tr>
<td>3</td>
<td>EECAL 250 + haloperidol</td>
<td>89.2±1.8*</td>
</tr>
<tr>
<td>4</td>
<td>EECAL 500 + haloperidol</td>
<td>96.1±1.8**</td>
</tr>
</tbody>
</table>

* - P<0.05, ** - P<0.01 - Individual readings were compared with readings of control. (n =6)

**Fig-4:  Effect of haloperidol and EECAL on oxidative stress markers in long term model**

<table>
<thead>
<tr>
<th>S.No</th>
<th>GROUP</th>
<th>TBARS (nmoles MDA/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>SOB (Units/mg protein)</th>
<th>CAT (nmolesH2O2/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control+ haloperidol</td>
<td>9.5±0.42*</td>
<td>2.5±0.25*</td>
<td>8.2±0.14</td>
<td>18.15±0.54*</td>
</tr>
<tr>
<td>2</td>
<td>EECAL 250 + haloperidol</td>
<td>8.12±0.43*</td>
<td>2.4±0.45</td>
<td>7.5±0.14**</td>
<td>14.51±0.15</td>
</tr>
<tr>
<td>3</td>
<td>EECAL 500 + haloperidol</td>
<td>6.14±0.48</td>
<td>2.1±0.36*</td>
<td>6.1±0.71**</td>
<td>17.21±0.46*</td>
</tr>
<tr>
<td>4</td>
<td>EECAL 1000 + haloperidol</td>
<td>5.11±0.51*</td>
<td>1.9±0.49*</td>
<td>6.2±0.25*</td>
<td>19.19±0.41</td>
</tr>
</tbody>
</table>

* - P<0.05, ** - P<0.01 - Individual readings were compared with readings of control. (n =6)

**Behavioural Study of Mice after Haloperidol treatment**

**Actophotometer-- Test for locomotor activity**
The test results showed that, the locomotor activity was significant (P<0.01) decreased in haloperidol treated group when compared to control, where as administered two different doses of EECAL 250 and 500 mg/kg (p.o.) showed
dose dependent increase in locomotor activity compare to haloperidol treat group, that was significant (P<0.01) when compared to control in both short and long term model. (Table 2& Figure-2)

Table-5: Effect of haloperidol and EECAL on oxidative stress markers in short term model

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>TBARS (nmol MDA/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>SOB (Units/mg protein)</th>
<th>CAT (nmol H2O2/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control + haloperidol</td>
<td>7.91±0.14*</td>
<td>1.58±0.23*</td>
<td>6.85±1.25</td>
<td>13.02±0.45*</td>
</tr>
<tr>
<td>2</td>
<td>EECAL 250 + haloperidol</td>
<td>6.32±0.26</td>
<td>1.54±0.54**</td>
<td>5.84±0.41*</td>
<td>14.02±0.46</td>
</tr>
<tr>
<td>3</td>
<td>EECAL 500 + haloperidol</td>
<td>4.25±1.23*</td>
<td>1.15±0.12**</td>
<td>5.02±0.13*</td>
<td>16.55±0.15*</td>
</tr>
<tr>
<td>4</td>
<td>EECAL1000 + haloperidol</td>
<td>5.1±0.27*</td>
<td>1.32±0.41*</td>
<td>4.85±0.46*</td>
<td>19.14±0.43*</td>
</tr>
</tbody>
</table>

*- P<0.05, **- P<0.01 - Individual readings were compared with readings of control. (n = 6)

Fig-1: Effect of EECAL on onset and duration of overall catalepsy

Fig-2: Effect of EECAL on overall Spontaneous loco motor activity
Rotarod test -- Tests for Muscle relaxation
The test results showed that, haloperidol treated group highly significant (P<0.01) reduction in fall off time compared to control, where as administered two different doses of EECAL 250 and 500 mg/kg p.o. showed dose dependent increase in fall off time compared to haloperidol treat group, that was highly significant (P<0.01) when compared to control in both short and long term model. (Table 3 & Figure 3)

Figure 3: - Effect of EECA on onset and duration of overall Grip Strength (Sec) in rotarod –short term (1mg/kg i.p.) and long term (4mg/kg i.p.) model in mice

Fig-4: Effect of haloperidol and EECAL of long model on oxidative stress markers

Biochemical results
Effect of EECAL on TBARS Activity
The TBARS levels were found to be significantly increased (p<0.05) in the brain tissue of the haloperidol in both models treated animals as results as shown in [Fig-4,5].
Effect of EECAL on Glutathione Activity
There was a significant reduction (p<0.05) in the levels of GSH in the EECAL pretreated mice as compared to the haloperidol both models treated animals and dose dependent recovery on the haloperidol induced elevation of the glutathione levels in animals.

Effect of EECAL on SOD Activity
In this assay, the levels of SOD were significantly reduced (p<0.001) as compared to those in the haloperidol both models treated animals and dose dependent recovery on the haloperidol induced elevation of SOD levels in animals.

Effect of EECAL on Catalase Activity
There was a significant increase (p<.001) in the levels of catalase in the EECAL treated animals as compared to the haloperidol in short term model but no significant increase in the long term model.

DISCUSSION
Alcoholic extract of Cassia auriculata was assessed for the neuro pharmacological evaluation in Haloperidol treated rodents. Since haloperidol induced catalepsy had an underlying pathology of increased oxidative stress and as Cassia auriculata was high anti oxidant, the effect of Cassia auriculata was evaluated in haloperidol induced cataleptic mice. In the present study, the animals (haloperidol treated) showed decreased levels of glutathione and catalase and increased levels of lipid peroxidation products and super oxide dismutase as compared to the vehicle toxic control animals. This result was with that of previous studies which were done on the effects of haloperidol on the extra pyramidal symptoms and the markers of oxidative stress (GSH, SOD, CAT and TBARS), thus suggesting the possible induction of free radical generation by haloperidol treatment. Haloperidol (HP) is converted to potentially toxic (HHP+) metabolites which may play a role in the extrapyrimidal side effects in the patients who are treated with haloperidol[15]. Haloperidol-induced catalepsy occurs due to the blockade of dopamine and reduced dopaminergic transmission[16]. Spontaneous motor activity and motor coordination were decreased in Haloperidol treated group, it could be due to motor dysfunction and muscle relaxant effect. Oxidative stress to dopaminergic neurons of SNpc is believed to be one of the leading causes of neurodegeneration in PD. Antioxidants may play an important role in the prevention of PD and combat against oxidative stress induced progressive neurodegeneration by reactive oxygen species. According to our results, EECAL has been found that phyto constituents like alkaloids, polyphenols, saponins, flavonoids and tannins responsible for its antioxidant property. Medicinal plants like Mentha arvensis [17] and Gallic Acid Derivatives [18] have shown neuroprotective activity in Haloperidol induced PD due to their antioxidant property. Significant improvement of muscle relaxation and locomotor activity was observed by increased ambulatory movements, grooming, rearing and decreased latency period, ipsilateral rotations in ethanol extract of Cassia auriculata leaves treated animals.
CONCLUSION

From the present study, Cassia auriculata may prove to be a beneficial adjuvant in the treatment of drug-induced extra-pyramidal side effects and related disorders like PD. These behavioral parameters reveal an enhanced motor function, which is usually disturbed in Parkinsonism Disease. In conclusion, the present study showed that EECAL significantly reduced the duration of haloperidol induced catalepsy in rat along with muscle rigidity as shown by changes in rota-rod movement. Results particularly reduction in activity at 500 mg/kg indicating dose dependent activity, either rule out above hypothesis of dopamine release/dopaminergic activity or indicate possibility of additional mechanisms responsible for antiparkinsonian activity by EECAL.

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