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# Evaluation of Neuroprotective Effect of Ethyl Acetate Extract of *Sarcostemma acidum* on 6-Hydroxydopamine Induced Parkinson Disease in Wistar Rats

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**Keywords:** Parkinson Disease, *Sarcostemma acidum,* Acute-toxicity, Dopamine neurotransmitter, Antioxidants, and Histopathological studies. PWP- Person With Parkinson disease

**ABSTRACT**

**Parkinson's Disease** – A Central nervous system disorder that affects movements, often including tremors. Nerve cell damage in the brain causes dopamine levels to drop, leading to the symptoms of Parkinson's disease. Hence, the total burden of PD includes direct medical costs of $25.4 billion and $26.5 billion in indirect and non-medical costs, including an indirect cost of $14.2 billion, non-medical costs of $7.5 billion, and $4.8 billion due to disability income received by PWPs. More than 10 million people worldwide are living with PD. So the drug Selegiline 5mg/kg is an irreversible inhibitor of MAO-B, used as a standard drug here. Herbal medicines are back into prominence nowadays because the synthetic medicines which once had universal acceptance, are now known to cause side effects and fewer clinical benefits. Here *Sarcostemma acidum* is an Indian traditional medicinal plant categorized as soma plants belongs to the family Asclepiadaceae, it is widely used for the treatment of a variety of diseases and disorders due to its various medicinal properties and easy availability. *Sarcostemma acidum* has psychopharmacological activity, antifertility activity, hepatoprotective activity, anti-oxidant activity, anti-ulcer activity, anti-microbial activity, *in vitro* thrombolytic activity, invitro anti-acne activity, anthelmintic activity, insect antifeedant, and growth-regulating activities. Acute oral toxicity was done according to the OECD Guidelines 423, no signs of toxicity were observed. Results of EASA from the behavioral assessment showed a beneficial effect on muscle coordination and locomotor activity. Dopamine level got increased by EASA extract. Similarly, the anti-oxidants such as superoxide dismutase, lipid peroxidase, and Catalase were found to be most effective in this study. Finally, Histopathological studies confirmed the effect of EASA extract against 6-OHDA treated Rats. So the present work concludes that ethyl acetate extract of *Sarcostemma acidum* has a significant effect against 6-OHDA induced Parkinsonism in Wistar rats.

## INTRODUCTION

Parkinson's disease (PD) is a typical neurodegenerative disorder, characterized by symptoms including rest tremors, postural instability, gait abnormality, bradykinesia, and rigidity. The major pathological change of Parkinson's disease is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc).

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[1]. Treatment includes Dopamine agonists, MAO-B inhibitors, COMT inhibitors, Amantadine, and Anticholinergic drugs[2].

Currently, there are two surgical treatments available for people living with PD — deep brain stimulation (DBS) or surgery performed to insert a tube in the small intestine, which delivers a gel formulation of carbidopa/levodopa (Duopa™). Other therapies may be used to help with Parkinson's disease symptoms. They include physical, occupational, and speech therapies, which help with gait and voice disorders, tremors and rigidity, and decline in mental functions. Other supportive therapies include a healthy diet and exercises to strengthen muscles and improve balance[3].

MAO-B inhibitors might slow the progression of PD, offering neuroprotection. When tested in humans in the 1980s, Selegiline was shown to delay the need for levodopa by nine months, suggesting neuroprotection. However, this benefit may simply have been from the antiparkinson symptom effect of selegiline[4].

When synthetic drugs fail to be effective or show serious side effects, it is the herbal medicine that brings relief and it has the healing therapy to cure the diseases of mankind[5].

*Sarcostemma acidum* is an Indian traditional medicinal plant. It is widely used by the folk people in the treatment of a variety of diseases and disorders. It is found widely in India, Pakistan, Europe, etc. In India Bihar, Bengal, Madhya Pradesh, Tamil Nadu, Maharashtra, and Kerala[6]. There are 40 different types of ailments that were treated using this plant by the Ethnic communities[7]. For example, it is used in wounds and cuts[8], chronic ulcer[9], bone fracture[10], arthritis and joints[11], etc. The Plant *Sarcostemma acidum* has psychopharmacological activity[12], antifertility activity[13], hepatoprotective activity[14], antioxidant activity[14], anti-ulcer activity[14], anti-microbial activity[14], *in-vitro* thrombolytic

activity[15], invitro anti-acne activity[15], antihelmintic activity[16], *in-vitro* anti-inflammatory activity[17], insect antifeedant and growth-regulating activities[18].

In the plant *Sarcostemma acidum,* Stem decoction contains- sucrose, terpenes, phytosterol, and saponins[19].In warm places, it has sucrose, malic acid, succinic acid, alkaloids, phytosterols, tannins, alpha and beta amyrins, beta sitosterol[20]. Powdered leaves contain alkaloids, phenolics, triterpenes, tannins, flavonoids, saponins, and carbohydrates[21]. Chinese origin contains sacidumlignan-A, B, C, and D, degraded derivatives of lignans such as sacidumol-A, B, perforatic acid, pinoresinol, 9 alpha hydroxyl pinoresinol, peucinin, and 7 o methyl ether[22]. Ethanol extract contains reducing and non-reducing sugar[19]. Chloroform extract contains steroids and triterpinoids[19]. The dried flower contains rare flavonol glycoside[23]. Methanol solvent shows fatty acids, carotenoids, tannins, saponins, coumarins, and anthracene glycosides[24]. Hexane extract contains alkaloids, steroids, carotenoids, tannins, and anthrocyanins[24].

## MATERIALS AND METHODS

The whole plant of *Sarcostemma acidum* was collected and authenticated by Prof. P. Jayaraman, Ph.D., Director, Institute of herbal botany, Plant anatomy and research Centre, Chennai, Tamil Nadu, India. The collected leaves were washed, shade dried and coarsely powdered at room temperature was extracted with ethyl acetate using soxhlet apparatus for 12 hours. The percentage yield of extract was calculated and stored at 4°C until use for further analysis.

## EXPERIMENTAL ANIMALS (WISTAR ALBINO RATS)

The Wistar albino rats weighing 150-200gm were used for this study. The Wistar albino rats were procured from the animal house of C. L. Baid Metha College of Pharmacy, Thuraipakkam, and Chennai-97. They were housed six per cage under standard laboratory conditions at a temperature of 17-23°C with 12:12 hours light and dark circle. The animals were provided with standard animal feed, water, and *libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC).

**IAEC Approval No: 17/321/PO/Re/S/01/CPCSEA dated 14/3/20.**

## ACUTE TOXICITY STUDIES

The procedure was followed according to OECD guidelines (Organization of economic corporation and development) 423 (acute toxic class method).

Female Wistar albino rats are selected which are weighed 150-250gms were used for the study. The starting dose level of EASA was 2000mg/kg body weight p.o as most of the crude extracts possess. Dose-volume administered was 1ml/150gm bodyweight to fasted rat with 0.9% normal saline solution. Food was withheld for a further 3-4 hrs, after administration (p.o) of drugs and observed for the signs of toxicity.

Bodyweight of rats before and after administration were noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous system, motor activity, and behavior pattern were observed and also signs of tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma was noted. The onset of toxicity and signs of toxicity also noted.

## EXPERIMENTAL DESIGNS

### Acute oral toxicity

According to OECD Guidelines 423, three Female Wistar rats for Acute toxic class method-2000mg/kg p.o.

### Grouping

Each group consists of 6 male Wistar rats. Group I was treated with Control, Group II was treated with Intracranial inj. of 4µg of 2µl 6-OHDA, Group III were treated with Intracranial inj. of 4µg of 2µl 6-OHDA and selegiline 5mg/kg p.o. Group IV was treated with Intracranial inj. of 4µg of 2µl 6-OHDA and EASA 200mg/kg p.o. and Group V were treated with Intracranial inj. of 4µg of 2µl 6-OHDA and EASA 400mg/kg p.o.

## 6-OHDA INDUCED PARKINSON'S MODEL

Group, I rats was treated with saline solution. Groups II, III, IV and V rats were treated with 2µl of 4µg 6-OHDA in 0.02%ascorbic acid and saline were infused on the 21$^{st}$ day in the left striatum of the brain mounted in stereotaxic apparatus at a rate of 0.5µl/min by using 10µl HAMILTON Syringe. Group IV and V animals were pretreated with 200mg/kg b.w. and 400mg/kg b.w p.o respectively of EASA Extract orally for 21 days. After one week of the

last dose, the parameters such as muscle coordination in the Rota rod apparatus, locomotor activity in Actophotometer are to be analyzed. After the Assessment, animals were sacrificed for neurochemical analysis such as dopamine content, superoxide dismutase, Lipid peroxidase, and Catalase activity. Also, Histopathological examinations were done in the substantia nigra of the brain immediately after euthanization of experimental animals.[25]

**ASSESSMENTS**

**BEHAVIOURAL ANALYSIS**

**ASSESSMENT OF MUSCLE COORDINATION**

Muscle coordination and balance were assessed using a rotarod apparatus. In total, four training trials per day with an interval trial time of one hour were performed. Rats falling off during a training trial were put back on the rotating rod. Following the training days, a one-day test of three trials was performed using two-speed levels (10 and 25rpm) mode of apparatus over 5-min. Animals are placed individually in separate lanes on a rod rotating at 5 rpm such that animals may walk forward to keep balance. The rotating rod is allowed to rotate at 10rpm and animals from each group were placed individually on the rod and the fall off time was recorded for each animal. A similar procedure was followed by increasing the speed to 25rpm. Total 5 groups were involved and their average hanging duration was concluded using MEAN±SEM[26].

**ASSESSMENT OF LOCOMOTOR ACTIVITY**

The spontaneous locomotor activity of each group of the animals was measured using an actophotometer with infrared-sensitive photocells. Before the locomotor test, each animal was placed individually in the actophotometer cage for 2 mins for habituation. Thereafter, locomotor activity was recorded for 5 mins for animals from groups I, II, III, IV, and V. During this period, locomotor activity and immobility time were recorded in secs. The difference in the activity was recorded considering control group I and after treatment groups i.e., II, III, IV, and V[27].

**BODYWEIGHT**

Body Weight was measured.

## NEUROCHEMICAL ANALYSIS

### ESTIMATION OF DOPAMINE

Dopamine was estimated by the method of Zhang et al (2012) [28].

### ESTIMATION OF SUPEROXIDE DISMUTASE

Superoxide Dismutase was estimated by the method of Misra et al (1967) [29].

### ESTIMATION OF LIPID PEROXIDATION

It was estimated by the method of Ohkawa et al in 1979 [30].

### ESTIMATION OF CATALASE

The activity of catalase was estimated by the method of Sinha (1972)[31].

## HISTOPATHOLOGICAL ANALYSIS

The animals from each group were anesthetized using inhalation of chloroform. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice-cold normal saline and fixed in 10% formalin saline. Paraffin-embedded sections were taken 100µm thickness and processed in alcohol-xylene series and stained with Hematoxylin-Eosin dye. The sections were examined microscopically for histopathological changes in the cortex zone.

## STATISTICAL ANALYSIS

Data were analyzed using one-way ANOVA followed by Dunnett's test and expressed as Mean± Standard Error of Mean (SEM). Statistical Analyses were performed using Graph Pad Prism version 9.0, for windows. Differences between mean values of different groups were considered statistically significant at (P<0.0001)****(P<0.001)***(P<0.01)**(P<0.05)*, ns- non significant.

## OBSERVATIONS

## PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The preliminary phytochemical screening on EASA revealed the presence of various phytoconstituents such as alkaloids, carbohydrates, proteins, and tannins.

## ACUTE TOXICITY STUDIES

**Table No. 1: Results of Acute Toxicity Studies**

| S. no | Treatment group | Dose | Weight of animal (gm) | | Signs of toxicity | Onset of toxicity | Reversible or irreversible | Duration |
|---|---|---|---|---|---|---|---|---|
| | | | Before test (on day 1) | After test (on day 14) | | | | |
| 1 | EASA | 2gm/kg | 150 | 155 | Nil | Nil | Nil | 14 days |
| 2 | EASA | 2gm/kg | 125 | 130 | Nil | Nil | Nil | 14 days |
| 3 | EASA | 2gm/kg | 155 | 160 | Nil | Nil | Nil | 14 days |

## EFFECT OF EASA ON ROTAROD AND ACTOPHOTOMETER

**Table No. 2: Effect of EASA on Rotarod and Actophotometer by 6-OHDA induced Parkinson's Model.**

| S.no | Groups | Fall of time (secs) | | Locomotor Index (count/min) | Immobility Time (Time in Secs) |
|---|---|---|---|---|---|
| | | 10rpm | 25rpm | | |
| 1 | Group I | 55.667±5.007 | 35.667±3.882 | 386.33±5.186 | 107.167±4.400 |
| 2 | Group II | 15.500±2.950 a**** | 11.667±2.160 a*** | 188.833±5.862 a**** | 241.500±18.516 a*** |
| 3 | Group III | 48.167±2.639 a* b**** | 35.000±2.449 $a^{ns}$ b*** | 368.833±15.869 $a^{ns}$ b*** | 105.167±10.710 $a^{ns}$ b** |
| 4 | Group IV | 33.333±2.251 a*** b*** c** | 23.333±1.633 a**b***c*** | 323.833±19.482 $a^{ns}$ b** c** | 205.167±17.704 a**b*c** |
| 5 | Group V | 42.333±2.658 a** b**** $c^{ns}$ | 29.500±2.739 $a^{ns}$ b*** c** | 338.333±11.543 a* b*** $c^{ns}$ | 117.667±13.778 $a^{ns}$ b** $c^{ns}$ |

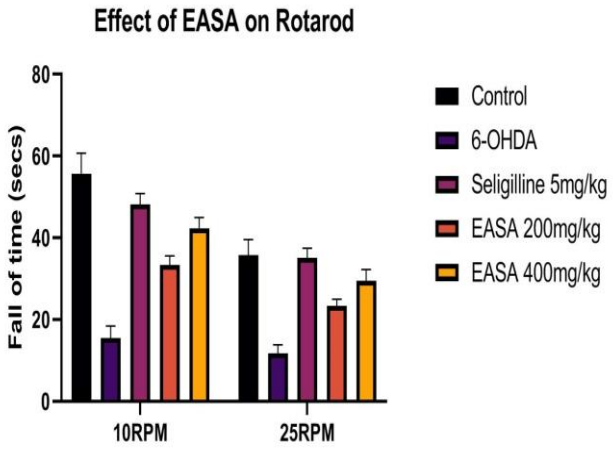


**Figure No. 1: Effect of EASA on Rotarod by 6-OHDA induced Parkinson's Model.**

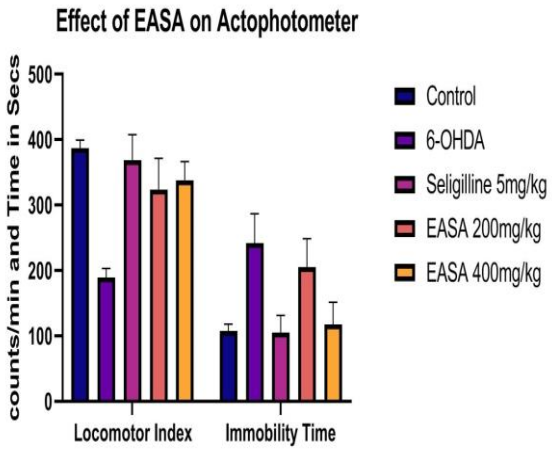


**Figure No. 2: Effect of EASA on Actophotometer by 6-OHDA induced Parkinson's Model.**

## EFFECT OF EASA ON BODY WEIGHT

**Table No. 3: Effect of EASA on Bodyweight by 6-OHDA induced Parkinson's Model**

| S.no | Groups | Treatment | 1st DAY (gm) | 14th DAY (gm) | 21st DAY (gm) |
|---|---|---|---|---|---|
| 1 | Group I | Control | 95.000±4.282 | 125.000±12.583 | 143.333±13.081 |
| 2 | Group II | 6-OHDA | 98.333±4.773 $a^{ns}$ | 135.833±6.635 a** | 155.000±4.472 a*** |
| 3 | Group III | Selegiline 5mg/kg | 98.333±6.540 $a^{ns}b^{ns}$ | 145.833±4.729 a***b* | 153.333±4.216 a**$b^{ns}$ |
| 4 | Group IV | EASA 200mg/kg | 102.500±4.787 a*$b^{ns}c^{ns}$ | 135.833±10.117 a*$b^{ns}$c* | 147.500±7.500 a*b*c* |
| 5 | Group V | EASA 400mg/kg | 101.667±4.014 a*$b^{ns}c^{ns}$ | 133.333±4.014 a*b*c* | 150.000±5.627 a**b*$c^{ns}$ |

**Values are indicated as Mean±SEM.** (One-way Analysis of Variance followed by Dunnett's t-test).

Group, I is compared with Group II, III, IV, and V is considered as 'a'.

Group II is compared with Group III, IV, and V is considered as 'b'.

Group III is compared with Group IV and V is considered as 'c'.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett's multiple comparisons tests where,**** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05) and ns is non-significant.


**Figure No. 3: Effect of EASA on Bodyweight by 6-OHDA induced Parkinson's Model.**

**EFFECT OF EASA IN NEUROTRANSMITTER AND ANTIOXIDANT ACTIVITY**

**Table No. 4: Effect of EASA on Dopamine Neurotransmitter and on Superoxide Dismutase by 6-OHDA induced Parkinson's Model**

| S.no | Groups | Dopamine level (ng/L) | Superoxide dismutase (nmoles/min/mg/protein) |
|---|---|---|---|
| 1 | Group I | 367.308±4.454 | 23.847±0.995 |
| 2 | Group II | 126.382±0.489<br>a**** | 10.963±0.454<br>a**** |
| 3 | Group III | 323.438±1.573<br>a****b**** | 24.152±0.973<br>$a^{ns}$b*** |
| 4 | Group IV | 135.260±0.494<br>a****b*c**** | 16.322±0.349<br>a****b***c**** |
| 5 | Group V | 320.457±0.636<br>a****b****$c^{ns}$ | 23.080±0.861<br>$a^{ns}$b***$c^{ns}$ |

**Values are indicated as Mean±SEM.** (One-way Analysis of Variance followed by Dunnett's t-test)

Group, I is compared with Group II, III, IV, and V is considered as 'a'.

Group II is compared with Group III, IV, and V is considered as 'b'.

Group III is compared with Group IV and V is considered as 'c'.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett's multiple comparisons tests where,**** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05) and ns is non-significant.

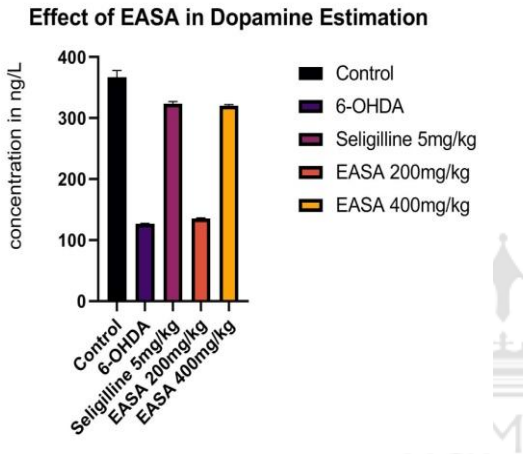


**Figure No. 4: Effect of EASA on Dopamine Neurotransmitter by 6-OHDA induced Parkinson's Model**

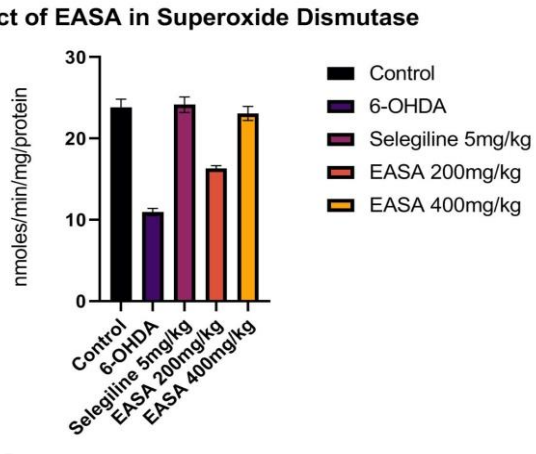


**Figure No. 5: Effect of EASA on Superoxide Dismutase by 6-OHDA induced Parkinson's Model**

**EFFECT OF EASA IN LIPID PEROXIDASE AND CATALASE**

**Table No. 5: Effect of EASA on Lipid Peroxidation and catalase by 6-OHDA induced Parkinson's Model.**

| S.no | Groups | Lipid Peroxidase (nmoles/min/protein) | Catalase nmol/mg/protein |
|---|---|---|---|
| 1 | Group I | 1.240 ±0.090 | 29.067±0.484 |
| 2 | Group II | 3.012±0.057 a**** | 18.327±0.473 a**** |
| 3 | Group III | 1.307±0.049 $a^{ns}$b**** | 27.650±0.609 a**b**** |
| 4 | Group IV | 2.223±0.031 a****b****c**** | 20.983±0.249 a****b***c**** |
| 5 | Group V | 1.447±0.094 $a^{ns}$b****$c^{ns}$ | 26.487±0.516 a**b****$c^{ns}$ |

**Values are indicated as Mean±SEM.** (One-way Analysis of Variance followed by Dunnett's t-test)

Group, I is compared with Group II, III, IV, and V is considered as 'a'

Group II is compared with Group III, IV, and V is considered as 'b'

Group III is compared with Group IV and V is considered as 'c'.

The Statistical significance test for comparison was done by One-way ANOVA followed by Dunnett's multiple comparisons tests where,**** is (P<0.0001),*** is (P<0.001), ** is (P<0.01),* is (P<0.05) and ns is non-significant.

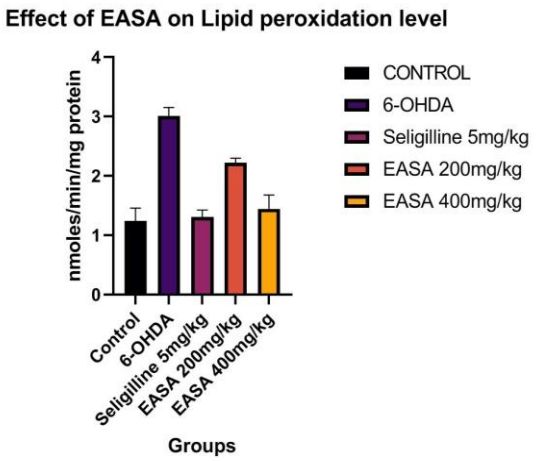


**Figure No. 6: Effect of EASA on Lipid Peroxidation by 6-OHDA induced Parkinson's Model**

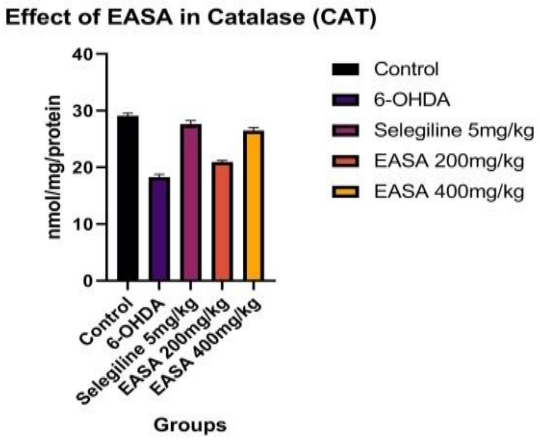


**Figure No. 7: Effect of EASA on Catalase by 6-OHDA induced Parkinson's Model.**

## HISTOPATHOLOGICAL ANALYSIS IN RAT BRAIN

Group I was observed to be Neuroparenchyma with no significant pathological findings and Group II was found to be Neuroparenchyma with focal neuronal aggregates. Congested blood vessels and mild gliosis were noted. Similarly, Group III was found to be Neuroparenchyma with mild neuronal disarray with focal neuronal aggregates. Group IV was observed as the Neuroparenchyma shows mild disarray alone. And Group V was found to be No significant pathological findings noted.

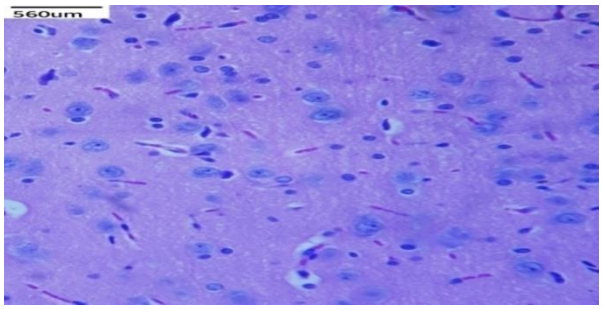


**Figure No. 8: GROUP-I**

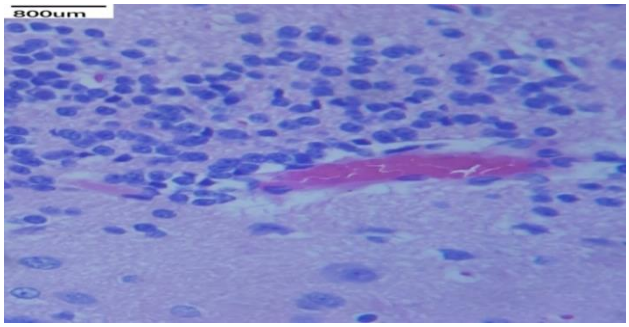


**Figure No. 9: GROUP-II**

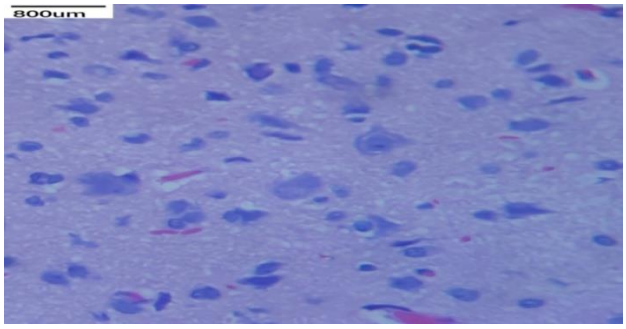


**Figure No. 10: GROUP-III**

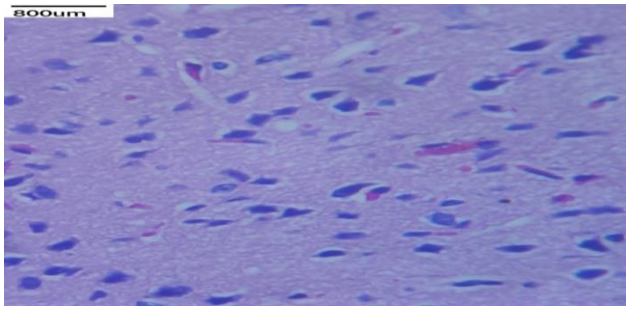


**Figure No. 11: GROUP-IV**

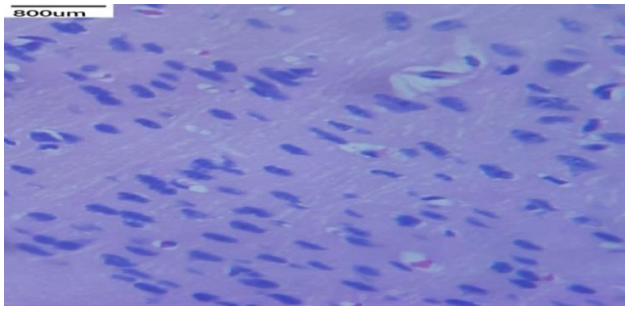


**Figure No. 12: GROUP-V**

## RESULTS

### EXTRACTION YIELD

The Ethyl acetate extract of the whole plant of *Sarcostemma acidum* extract was dark brown and was stored in the refrigerator until use. The percentage yield was found to be 28%w/w.

### PRELIMINARY PHYTOCHEMICAL ANALYSIS

The Preliminary phytochemical evaluation of the EASA Plant showed the presence of carbohydrates, proteins, alkaloids, tannins, and steroids and showed the absence of flavonoids, glycosides, amino acids, triterpenoids, gums, and mucilages.

### ACUTE TOXICITY STUDIES

The acute oral toxicity study was done according to the OECD guidelines 423 (acute toxic class method). A single administration. A single administration of 2000mg/kg b.w/ p.o of EASA was administrated to three adults female Wistar rats and observed for 7 days. There were no considerable changes in body weight before and after treatment and no signs of toxicity were observed. **Results are shown in Table 1.**

**EFFECT OF EASA ON ROTAROD BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS**

**IN 10RPM**

When Group I were compared with Group II ($p<0.0001$), II($p<0.05$), IV($p<0.001$), V($p<0.01$) fall of time(sec) were significantly decreased and Group II were compared with Group III and V ($p<0.0001$), IV($p<0.001$) fall of time(sec) were significantly increased. Similarly, Group III was compared with Group IV ($p<0.01$) fall of time(sec) were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 2 and figure 1.**

**IN 25RPM**

When Group I were compared with Group II ($p<0.001$), IV($p<0.01$) fall of time(sec) was significantly decreased and Group III and V were non-significantly decreased and Group II were compared with Group III, IV, and V ($p<0.001$) fall of time(sec) were significantly decreased. Similarly, Group III was compared with Group IV ($p<0.001$) fall of time(sec) were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 2 and figure 1.**

**EFFECT OF EASA ON ACTOPHOTOMETER BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS.**

**LOCOMOTOR INDEX**

When Group I were compared with Group II ($p<0.0001$), V($p<0.05$) locomotor index were significantly decreased and Group III and IV were non-significantly decreased and Group II were compared with Group III, IV, and V ($p<0.001$) locomotor index were significantly increased. Similarly, Group III was compared with Group IV ($p<0.001$) locomotor indexes were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 2 and figure 2.**

**IMMOBILITY TIME**

When Group I were compared with Group II ($p<0.001$), IV ($p<0.01$) immobility time were significantly increased and Group III and V were non-significantly decreased and increased respectively and Group II were compared with Group III and V ($p<0.001$) and Group IV

(p<0.05) immobility time were significantly decreased. Similarly, Group III was compared with Group IV (p<0.001), and Group V(p<0.01) immobility time was significantly increased. **Results are shown in table 2 and figure 2.**

**EFFECT OF EASA ON BODY WEIGHT BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS.**

**ON 1$^{ST}$ DAY**

When Group I was compared with Group II, the III body weight was non-significantly increased and Group IV (p<0.01), V(p<0.05) body weight were significantly increased and Group II were compared with Group III, IV, and V body weight were non-significantly increased. Similarly, Group III was compared with Group IV (p<0.001) body weight was significantly increased and V was non-significantly increased. **Results are shown in table 3 and figure 3.**

**ON 14$^{th}$ DAY**

When Group I were compared with Group II (p<0.01), III(p<0.001), IV and V(p<0.05) body weight were significantly increased and Group II were compared with Group III (p<0.01), V (p<0.05) body weight were significantly decreased and Group IV were non-significantly decreased. Similarly, Group III was compared with Group IV and V (p<0.05) body weight was significantly decreased. **Results are shown in table 3 and figure 3.**

**ON 21$^{st}$ DAY**

When Group I were compared with Group II (p<0.01), III and V (p<0.01) and Group IV (p<0.05) body weight were significantly increased and Group II were compared with Group III body weight were non-significantly decreased and Group IV and V (p<0.05) body weight were significantly decreased. Similarly, Group III was compared with Group IV (p<0.05) body weight were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 3 and figure 3.**

**EFFECT OF EASA ON NEUROTRANSMITTER (DOPAMINE) LEVEL BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS.**

When Group I were compared with Group II, III, IV, and V (p<0.0001) dopamine level were significantly decreased and Group II were compared with Group III, V (p<0.0001) and Group

IV ($p<0.05$) dopamine level were significantly decreased. Similarly, Group III was compared with Group IV ($p<0.0001$) dopamine levels were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 4 and figure 4.**

**EFFECT OF EASA ON SUPEROXIDE DISMUTASE(SOD) LEVEL BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS.**

When Group I were compared with Group II ($p<0.0001$) SOD level were significantly decreased, Group IV ($p<0.0001$) were significantly increased and Group III and V were non-significantly decreased and Group II were compared with Group III, IV, V ($p<0.0001$) SOD level were significantly increased. Similarly, Group III was compared with Group IV ($p<0.0001$) SOD levels were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 4 and figure 5.**

**EFFECT OF EASA ON LIPID PEROXIDATION(LPO) LEVEL BY 6-OHDA INDUCED PARKINSON'S DISEASE IN WISTAR RATS.**

When Group I was compared with Group II, IV ($p<0.0001$) LPO levels were significantly increased and Group III and V were non-significantly increased and Group II was compared with group III, IV, and V ($p<0.001$) LPO level were significantly decreased. Similarly, Group III was compared with Group IV ($p<0.0001$) LPO levels were significantly increased and Group V were non-significantly increased. **Results are shown in table 5 and figure 6.**

**EFFECT OF EASA ON CATALASE(CAT) LEVEL BY 6-OHDA INDUCED PARKINSON DISEASE IN WISTAR RATS.**

When Group I were compared with Group II, IV ($p<0.0001$) and Group III, V ($p<0.01$) CAT level were significantly decreased and Group II were compared with Group III, V ($p<0.0001$) and Group IV ($p<0.001$) CAT level were significantly increased. Similarly, Group III was compared with Group IV ($p<0.0001$) CAT levels were significantly decreased and Group V were non-significantly decreased. **Results are shown in table 5 and figure 7.**

**DISCUSSION**

Parkinson's disease (PD) is a typical neurodegenerative disorder, characterized by symptoms including tremors at rest, postural instability, gait abnormality, bradykinesia, and rigidity. The major pathological change of Parkinson's disease is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc).

More than 10 million people worldwide are living with PD. The incidence of Parkinson's disease increases with age, but an estimated four percent of people with PD are diagnosed before age 50. Men are 1.5 times more likely to have Parkinson's disease than women. The total burden of PD includes direct medical costs of $25.4 billion and $26.5 billion in indirect and non-medical costs, including an indirect cost of $14.2 billion (PWP and caregiver burden combined), non-medical costs of $7.5 billion, and $4.8 billion due to disability income received by PWPs. The Medicare program bears the largest share of excess medical costs, as most PD patients are over age 65. Projected PD prevalence will be more than 1.6 million with a projected total economic burden surpassing $79 billion by 2037.

In Parkinson's disease, the dopamine level is gradually decreased and gets worse. Neurons get degenerated in the substantia nigra of pars compacta in the brain. Some enzymes such as monoamine oxidase, catechol-O-methyltransferase, etc. This methylates the catecholamine such as dopamine, epinephrine, and norepinephrine converted to 3,4-dihydroxy phenylacetaldehyde (DOPAL), 3-hydroxy-4-hydroxyphenyl glycolaldehyde.

The mechanism for neuronal death in Parkinson's disease includes protein aggregation in Lewy bodies, Lewy bodies may be found in the midbrain (within the substantia nigra) or within the cortex, disruption of autophagy both micro and macroautophagy, mitophagy is autophagy-dependent degradation of mitochondria. This function is important to preserve the integrity of these organelles and to limit the reactive oxygen species produced by the mitochondria, changes in cell metabolism or mitochondrial function, neuroinflammation, and blood-brain breakdown resulting in vascular leakiness. Many brain cells of people with Parkinson's contain Lewy bodies, unusual clumps of the protein alpha-synuclein. Abnormal functions of alpha-synuclein and its relationship to genetic mutations that impact Parkinson's disease.

Dopamine is metabolized mainly by MAO-B. Selegiline increases dopamine content in the central nervous system by inhibiting MAO-B. Also inhibits the uptake of dopamine and noradrenaline into the presynaptic nerve and increases the turnover of dopamine. Selegiline significantly potentiates the pharmacological effects of levodopa. These favorable characteristics have been applied in the treatment of Parkinson's disease using Selegiline both with levodopa and alone. Unlike earlier MAO-inhibitors, Selegiline does not potentiate the hypertensive effects of tyramine. This is due to the selectivity to MAO-B, leaving intestinal MAO-A intact, and also because Selegiline inhibits the uptake of tyramine into neurons.

Selegiline (1-deprenyl) is an irreversible inhibitor of monoamine oxidase-Selegiline can prevent Parkinsonism caused by MPTP in animals; similar findings have been reported with other toxins like 6-OHDA that destroys noradrenergic nuclei. Furthermore, Selegiline reduces oxidative stress caused by the degradation of dopamine and increases free radical elimination by enhancing superoxide dismutase and catalase activity. These findings may be important when considering the possible neuroprotective effects of Selegiline.

It is rightly accepted that the nature has best answers to all the diseases affecting the human body from time to time. When synthetic drugs fail to be effective or show serious side effects, it is the plant medicine that brings relief. Many of the plant species distributed throughout the world have some pharmacological action on the body. Herbal treatment is the natural form of healing therapy to cure the diseases of mankind. Nowadays, herbal medicines are back into prominence because synthetic medicines, which once had universal acceptance, are now known to often cause side effects.

Here *Sarcostemma acidum* contains many chemical constituents such as malic acid, succinic acid, reducing sugar-sucrose**,** tannins, alkaloids, phytosterols, etc. The tannins play a vital role in Parkinson's disease. Tannins mostly precipitate in the solvent of Ethyl Acetate. Tannins act as monoamine oxidase inhibitors. Monoamine oxidase inhibitors slow down the enzyme that breaks down dopamine in the brain.

In the present study, tannins are the most important constituents in the plant of *Sarcostemma acidum* against Parkinson's disease. It reduces the symptoms of Parkinson's disease and also regenerates the neurons by increasing the level of dopamine by breakdown the enzyme Monoamine oxidase in the substantia nigra of pars compacta in the brain.

Previous phytochemical investigations revealed the presence of flavonoids, tannins, alkaloids, steroids, phenols, and Saponins. Also found that tannins could act as a Monoamine oxidase inhibitor in recent research works. Hence we expect that the ethyl acetate extract of *Sarcostemma acidum* has a curative effect on one of the most common neurodegenerative diseases called Parkinsonism[32].

Results of EASA from the behavioral assessment showed a potential beneficial effect in muscle coordination and locomotor activity.

Dopamine level has been increased by preventing the formation of DOPAC metabolized by the enzyme Monoamine oxidase-B. The inhibition of monoamine oxidase is present in the extract of EASA.

Superoxide dismutase, an enzyme that catalyzes the dismutation (or partitioning) of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. Superoxide is produced as a by-product of oxygen metabolism and if not regulated, causes many types of cell damage. EASA treated significantly increased than 6-OHDA induced animals[33].

Lipid peroxidation causes cell membrane destruction and cell damage. The presence of a high concentration of oxidizable fatty acids and iron significantly contributes to ROS production. Furthermore, the abundance of polyunsaturated fatty acids (PUFAs) and redox-active transition metal ions in the brain in addition to its high oxygen usage makes it highly susceptible to oxidative damage. 6-OHDA significantly elevated the malondialdehyde (MDA) levels in the brain indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. EASA treatment significantly reversed these alterations causing a significant decrease in MDA levels suggesting its protective effects against 6-OHDA induced oxidative damage.

Catalase oxidation reactions occur in the presence of hydrogen peroxide ($H_2O_2$) to form acetaldehyde. It's a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). EASA treated animals showed an increase in catalase than the 6-OHDA treated animals[34].

Histopathological study of animal's brain proved damaging in 6-OHDA treated group which showed neuronal death. While groups treated with EASA protected neurons by reversing the damage induced by 6-OHDA.

The mechanism of monoamine oxidase inhibitors reveals that the level of dopamine gets increased by breaks down the enzyme monoamine oxidase. Tannins can act as monoamine oxidase inhibitors. So, the above mechanism suggests that the plant extract can have better action against Parkinson's disorder.

**CONCLUSION:**

The present study demonstrated that the whole plant of Ethyl Acetate Extract of *Sarcostemma acidum* (EASA) contains tannins that act as a key role in showing the potential effect of

Neuroprotective activity by inhibiting Monoamine Oxidase B which is compared to a standard drug – Selegiline (5mg/kg) and also had Anti-oxidant activity which enhances the protective affect neurons. Thus, it proved that *Sarcostemma acidum* has a significant Neuroprotective effect against 6- Hydroxy Dopamine induced in Wistar rats.

Further studies are required to explore the molecular mechanism of Neuroprotection of herbal extract *Sarcostemma acidum.*

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