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Original Research Article

ANTI-PARKINSONISM ACTIVITY OF *SARACA ASOCA* BY HALOPERIDOL INDUCED MODEL

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Abstract: Aim: The present study was designed to investigate the effects of different extracts of leaves of *Saraca asoca* in haloperidol induced Parkinsonism experimental model.
Methods: Swiss albino mice of either sex, weighing about 20-25 gm were used. Anti parkinsonism activity was done by haloperidol induced model using catalepsy score by Hole board test and catatonic score by block model and also evaluated in-vivo antioxidant, estimation of dopamine, nor adrenaline, adrenaline from brain tissue and histopathalogical studies of brain.
Results: The extracts of AESA, MESA & WESA at doses (200 mg/Kg, p.o.) revealed the anti-parkinsonism activity while the PESA non significant to Parkinsonism control. The anti-parkinsonism activity was confirmed by Catatonic score, Head dip, number of line crossing, anti-oxidant enzyme level, LPO, histology of brain and level of monoamines like dopamine etc. at the dose levels of (200 mg/Kg, p.o.). The extracts MESA & WESA significantly decreased the Catatonic score and increased the head dip & number of line crossing. The dopamine, nor adrenaline and adrenaline significantly increased with extracts treated group except PESA at a dose of 200 mg/kg. Extracts MESA & WESA (200 mg/Kg, p.o.) has shown significant morphological changes when compared to haloperidol administered group.
Conclusion: The results of the present work suggested that the MESA & WESA has a potent antiparkinsonism activity.

Key words: haloperidol, *Saraca asoca,* dopamine, Catatonic score, in-vivo antioxidant.

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Introduction: Parkinson's disease (PD) is a progressive, neurodegenerative disorder with cardinal motor features of tremor, bradykinesia, and rigidity [1]. This disease affects 2% of the worldwide population and 1.5 million Americans aged 65 and older [2] with the incidence increasing significantly with age [3].

In general, symptoms of Parkinson's disease appear when there is loss of nearly 50% of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) region resulting in decreased level of dopamine in brain [4]. The neuro-degeneration in Parkinson's disease progresses slowly after the onset and the current treatment strategies do not halt this. As plants are unexplored source of potentially useful drugs researchers are focusing to find therapies based on phytochemical constituents and food supplements for their neuroprotective or neurorestorative effects in Parkinson's disease [5].

The pathological hallmark of Parkinson's disease is the cell loss within *substantia nigra pars compacta* (SNpc) region [6] and the disease is charactrised by bradykinesia, rigidity, postural instability, orofacial dyskinesia, muscular stiffness and tremor. Other non motor complication includes sleep disorders and cognitive impairment [7], depression [8], mood fluctuations [9], psychosis and dementia [10], etc.

Herbal medicine has such an extraordinary influence that numerous alternative medicine therapies treat their patients with Herbal remedies, Unani and Ayurveda. Approximately 25 percent of all prescription drugs are derived from trees, shrubs or herbs. Nature has bestowed our country with an enormous wealth of medicinal plants therefore India has often been referred to as the medicinal garden of the world. So stand the medicinal plants *Saraca asoca* as one of the foremost plants utilized from antiquity till to date. Asoka or ashoka is a Sanskrit words which means "without sorrow" or which that gives no grief. Ashoka is one of the most legendary and sacred trees of India. It's universally known by its binomial name *Saraca asoca* (Roxb.) belonging family *Caesalpinaceae*.

The above statements clearly shows that parkinsonism is one of the major disorder in among the world population mainly older age people and current treatment with allopathic medication had so many disadvantages like ADR, Drug interaction etc [11- 14]. So the present study designed to evaluate the anti-parkinsonism activity in plant. The plant profile of *Saraca asoca* reviewed the plant had claimed the uses of CNS disorders but there is no scientific evidence for Anti-parkinsonism activity when review the literature of this plant. So the present study we are planned to provide scientific evidence for its anti-parkinsonism activity.

Materials and Method

Identification, Authentification and collection of plant materials: The leaves of *Saraca asoca* is collected from Chittoor district, Madanapalle region, Andhra Pradesh during the year of 2015. The plant was identified and authentified by Dr.K.Madhava chetty, Assistant Professor, Department of Botany of S.V.University, Tirupathi.

Preparation of extracts and phytochemical screening: The collected leaves of *Saraca asoca* were washed thoroughly and dried under shade for 15 days. The dried plants were made into a coarse powder using dry grinder. The powder leaves was passed through sieve no. 40 and stored in an air tight container at 25°C, used for further study. Powdered plant material (1.2 kg) were successively extracted using Soxhlet apparatus using the solvents in order of increasing polarity viz., petroleum ether (60-80°C), Acetone, methanol and water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by distilling the solvent in a rotary vacuum evaporator and evaporated to dryness. The yield was calculated with reference to the dried plant material [15]. The extracts of leaves of *Saraca asoca* was subjected to qualitative analysis for various phytoconstituents. [16].

Table : Data showing the % yield of dried leaf powder of *Saraca asoca* (Roxb)

Plant name	Part used	Method of extraction	Solvent	Colour of extract	Nature of extract	% yield of extract
Saraca asoca	Leaves	Hot percolation	Petroleum ether	Green	Semisolid	5.56
			Acetone	Brownish green	Semisolid	8.6
			Methanol	Dark brown	Semisolid	12.67
			Aqueous	Dark brown	Semisolid	10.45

Experimental Animals: All the experiments were carried out using Swiss Albino mice (25-30 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^0C and relative humidity of 30–70%. A 12 hrs day: 12 hrs night cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Sri Krishna Chaithanya College of pharmacy, Madanapalle, Andhra Pradesh.

Dose Determination: Acute toxicity Studies [17]: The acute toxicity study was carried out with extracts of SA as per OECD 423 Guidelines. Swiss albino mice with weight ranging (25-30g) were taken for the experiment. The animals were fasted overnight, provided with water after which extracts of SA was administered orally at a dose level of 5 mg/kg, p.o. intubation. If mortality was observed in animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 14 days.

Table: Study period and observation parameters

Initial once observation	First 30 min and periodically 24 hr
Special attention	First 1-4 hr after drug administration
Long term observation	Up to 14 days
Direct observation parameters	Tremors, convulsions, salivation, Diarrhea, lethargy, Sleep and coma.
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

Anti-Parkinsonism activity of different extracts of *Saraca asoca* in rats

Experimental study design: Swiss albino mice weighed about 25-30g were divided into seven groups of twelve mice each. Tardive dyskinesia test were recorded from twelve mice of each group than half the mice from each group used for catalepsy score and half the mice used for hole board test.

Group I: Normal control, received 1ml/kg of 1% v/v tween 80 orally

Group II :Negative control, received Haloperidol 1 mg/kg i.p.

Group III : Standard received combination of L-dopa and Carbidopa (100 & 10 mg/kg, i.p.).

Group IV: Received Petroleum extract of *Saraca asoca* 200 mg/kg (PESA) orally.

Group V : Received Acetone extract of *Saraca asoca* 200 mg/kg (AESA) orally.

Group VI : Received Methanol extract of *Saraca asoca* 200 mg/kg (MESA) orally.

Group VII: Received Water extract of *Saraca asoca* 200 mg/kg (WESA) orally.

Induction of Catalepsy

The treatment was continued for 15 days. On 15th day catalepsy was induced after 30 min treatment of respective group by the intra-peritoneal administration of Haloperidol at a dose of 1 mg/kg to all groups except Group I. All the behavioural studies were performed at room temperature in a calm room without any external interference [18]. Finally the animals are sacrificed by cervical dislocation and isolated the brain subjected to in vivo anti-oxidant activity, estimation of dopamine, nor adrenaline, adrenaline and Histopathological studies

Assessment of Behavioral Tests

Measurement of catalepsy by block Method

This scoring method is followed in 4 steps.

Step I: The mice was taken out of the home cage and placed on a table. If rat moved normally when placed on the table a score of 0 was assigned

Step 1I: The mice was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned.

Step III: The front paws of the rats were placed alternatively on a 3cm high block. If the mice failed to correct the posture within 15 sec a score of 0.5 is assigned for each paw and time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw

Step IV: The front paws of the mice were placed alternatively on a 9cm high block; if the mice failed to correct the posture within 15 sec a score of 1 is assigned for each paw and time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw [19, 20]. The following formula used to calculate catalepsy score.

Total Score =0.5 + (0.5 x Time in sec. of front right paw on 3 cm high wooden block) + (0.5 x Time in sec. of front left paw on 3 cm high wooden block) + (1 x Time in sec. of front right paw on 9 cm high wooden block) + (1 x Time in sec. of front left paw on 9 cm high wooden block)

Tardive dyskinesia test: Tardive Dyskinesia is referred to as Vacuous Chewing Movements (VCMs) in rodents. On the test day (15th day) mice were placed individually in a small (30× 20× 30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation cage before behavioral assessments. In the present study vacuous chewing movements are referred to as single mouth openings in the vertical plane not directed toward physical material. vacuous chewing movements occurred during a period of grooming, they were not taken into account. Mirrors were placed under the floor and behind the back wall of the cage to permit observation of oral dyskinesia when the animal was faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for a period of 5 min [21].

Hole board test: Head dipping is an exploratory behavior of the animals in the Hole Board test which is considered to be an indicator of anxiety. Mice were placed in a black Perspex box (50 x 50 cm, walls 30 cm high) with 16 equally spaced holes (3 cm diameter, 10 cm apart from each other) in the floor and the box was raised to a height of 25cms from the ground. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total no. of lines crossed and the number of head dippings were recorded after standard & Extracts of *Saraca asoca* administration. A head

dip was scored if both eyes disappeared into the hole [22].

Biochemical estimation

Preparation of Homogenate: Immediately after measurement of catalepsy on 15th day, the animals were sacrificed. The brains were removed; the forebrain was dissected, rinsed with isotonic saline, and weighed.

Reagents

1. 0.25 M sucrose solution: 85.87 gm of sucrose was dissolved in 1000 ml of distilled water.
2. 10 mM buffer solution: 1.2 gm of tris was dissolved in 900 ml of distilled water, pH was adjusted to 7.4 with 1M Hcl and dilute up to 1000 ml.

Procedure: Excised brain was cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly blotted with filter paper. The tissue was minced and homogenized in ice cold 10 mM tris HCL buffer (to pH 7.4) at a concentration of 10% (w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition was designed to disrupt as far as possible the ventricular structure of cells so as to release soluble protein and leave only membrane and nonvascular matter in a sedimentable form. It was then centrifuged in cooling centrifuge at 5000 rpm at 20ºC temperature and clear supernatant was separated and used to estimate superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO).

Preparation brain tissue for of epinephrine, nor-epinephrine and Dopamine

Tissue samples were homogenized in ice cold butanol to give a final concentration of 50 mg/ml. The homogenates were centrifuged at 800 rpm for 15 minutes at 4°C. Residue was discarded and to the supernatants 2.5 ml of distilled water and 2.5ml of n-heptane were added. The contents were thoroughly mixed and centrifuged at 1000 rpm for 5 minutes. The aqueous phase was separated and to this 200 mg of alumina was added followed by 1.5 ml of 2M sodium acetate and the PH was adjusted to 8.0 using 1N sodium hydroxide. The samples were again centrifuged at 1000 rpm for 5 minutes. [23]

Estimation of epinephrine, nor-epinephrine and Dopamine

The alumina was washed twice with 2ml of distilled water by vortexing the tube and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and walls of the tube were blotted with strips of filter paper. The monoamines were eluted by shaking the alumina with 2ml of 2N acetic acid. The tubes were centrifuged at 100 rpm for 5 minutes. The supernatant was transferred to another tube. To this 100 µl of EDTA was added and the PH was adjusted to 6.3, 100µl of iodine was added to the above tube and mixed thoroughly. The samples were allowed to stand at room temperature for 2 minutes then 200 µl of alkaline sulphite solution was added. The contents were shaken well and allowed to stand at room temperature for 2 minutes. The PH of the solution was adjusted to 5.4 with 5N acetic acid. The fluorescence of epinephrine was read in a Shimadzu Spectroflurimetry with excitation and emission wavelength of 410 nm and 500 nm respectively with a band width of 10/10 nm. After reading epinephrine the same samples were heated in a boiling water- bath for 2 minutes. The tube were cooled and fluorescence of nor-epinephrine was read with excitation and emission wavelength of 385 and 485 nm respectively with slit widths of 10/10nm.The samples were again heated for 5 minutes in a boiling water bath and cooled. The fluorescence of dopamine was read at excitation and emission wavelengths of 320 and 370 nm respectively with slit widths of 10/10nm.The amine content of each sample was calculated and the content was expressed as µg/gm wet wt of tissue.

Histopathological analysis [24]

Processing of isolated brain: A portion of the brain was immediately kept in 10% formalin to

fix the tissue after isolation. The brain were washed in running tap water, dehydrated in the descending grades of alcohol and finally cleared in xylene. The tissues were embedded in molten paraffin wax.

Embedding in paraffin: Hard paraffin wax was melted and poured in to square-shaped blocks. The brain were then dropped into the liquid paraffin quickly and allowed to cool.

Staining: The blocks were cut using microtome to get section of thickness 10µm. The section was dried completely before staining. Eosin an acidic stain and hematoxylin a basic stain, was used for staining and observed under an electronic microscope for histopathological changes.

Observation: All the slides were observed under an electronic microscope for the changes in Histopathological characteristic and photographs were taken.

Statistical Analysis: Results of the above experiments were expressed as Mean ± SEM, and the difference between means was analyzed by one way analysis of variance (ANOVA) using graph pad prism 5 software followed by Dunnett's test, with $p < 0.05$, $p < 0.01$, & $p < 0.001$ being considered as statistical significant .

Results:

Preliminary Phytochemical screening test of different extracts of Leaves of *Saraca Asoca*

Preliminary phytochemical screening of petroleum ether, acetone, methanol and water was investigated. Primarily petroleum ether contain glycosides, steroids and triterpenoids; acetone extract contains alkaloids, glycosides, tannin and steroids; methanol extract showed the presence of tannins, triterpenoids, saponin, flavonoids and glycosides; water extract contain alkaloids, saponin, tannins, flavonoids and glycosides;

Table: Preliminary phytochemical screening of different extracts of Leaves of *Saraca Asoca* (Roxb.).

S.No	Constituents	Petroleum ether	Acetone	Methanol	Aqueous
1	Alkaloids	-	+	-	+
2	Steroids	+	+	-	-
3	Carbohydrates	-	-	-	-
4	Glycosides	+	+	+	+
5	Fixed oils & Fats	-			
6	Phenolic Compounds	-	+	+	+
7	Proteins & amino acids	-	-	-	-
8	Saponins	-	-	+	+
9	Tannins	-	+	+	+
10	Gums & mucilage	-	-		-
11	Flavonoids	-	-	+	+
12	triterpenoids	+	-	+	-

Where + =present, - =absent

Acute toxicity

Table: Observation parameters in acute toxicities of different extracts of *Saraca asoca*

PARAMETERS	OBSERVATION			
	PESA	**AEPSA**	**MESA**	**WESA**
Tremors	Not observed	Not observed	Not observed	Not observed
Convulsions	Not observed	Not observed	Not observed	Not observed
Salivation	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal
Diarrhea	Feces Normal	Feces Normal	Feces Normal	Feces Normal
Lethargy	Normal	Little laziness	Normal	Little laziness
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Respiratory	Normal	Normal	Normal	Normal
Circulatory	Normal	Normal	Normal	Normal
Autonomic and CNS	No observed changes	Slight depression	Slight depression	Slight depression
Somatomotor activity	No observed changes	less motor activity	less motor activity	less motor activity

It was observed that the petroleum ether, acetone, methanol and water extracts of leaves of *Saraca asoca* (Roxb.) were not lethal to the mice even at the 2000 mg/kg doses. Hence LD50 of extracts found to be 2000 mg/kg. Therefore, 1/10th (200mg/kg) of this dose consider as ED50 and selected for further study.

Haloperidol induced catalepsy

It was observed that haloperidol alone treated group, significantly increased the catalepsy 15th day as compared to control group. In Levodopa & carbidopa treated group, significant decrease in catalepsy score ($p<0.001$) was seen 15th day, as compared to haloperidol treated group. PESA 200 mg/kg pretreated groups did not cause any significant change in catalepsy score on 15th day. But on 15th day, AESA & WESA 200 mg/kg pretreated groups showed significant decrease in catalepsy score ($p<0.05$& $p<0.01$) when compared to haloperidol (as shown in table). Whereas no significant difference in catalepsy score was seen when MESA 400 mg/kg treated group compared to levodopa & carbidopa treated group.

Table: Effect of different extracts of *Saraca asoca* on haloperidol induced catalepsy in mice

Groups	Treatment	Catalepsy score (sec) in minutes on 15th day						
		0	**30**	**60**	**90**	**120**	**150**	**180**
I	Vehicle	19.5±0.3	19.25± 0.4	18.90±0.4	16.71±0.1	12.5±0.5	15.24±0.1	18.30±0.3
II	Haloperidol	15.23±0.1	278.5±4.3	301.6±0.5	328.6±0.4	284.2±3.4	246.63±3.4	238.73±1.8
III	LD & CD+HP	18.20±0.2	86.40±5.2***	119.4±2.4***	147.1±0.1***	114.82±0.47**	78.5±0.41***	42.6±3.1***
IV	PESA+HP	16.43±0.1	254.3±0.4^{ns}	268.3±3.1*	282.3±0.62*	247.15±1.94*	228.5±1.9^{ns}	223.14±2.7^{ns}
V	AESA+HP	19.67±0.62	214.8±3.1*	232.5±2.4*	248.5±2.3*	219.3±1.4*	186.5±3.2**	135.4±1.4**
VI	MESA+HP	19.29±0.4	115.5±5.2**	149.7±3.8***	168.27±1.0***	128.5±3.2**	96.28±1.4***	64.5±0.62***
VII	WESA+HP	17.25±0.1	157.0±1.5**	184.5±1.5**	201.3±2.7**	183.47±2.7**	142.8±0.92**	108.5±3.1**

Values are Mean ± SEM from 6 animals in each group. Significant difference at *p<0.05, **p<0.01 ***p<0.001 when treated group compared to negative control. ^{ns}- Non significant.

Tardive dyskinesia: Haloperidol (1 mg/kg, i.p.) treatment Group II increased vacuous chewing movements (VCM). AESA, MESA & WESA treated Group 200 mg/kg for a period of 15 days significantly ($p< 0.05$, $p<0.001$ & $P<0.01$) inhibited haloperidol-induced VCMs. [Table]. PESA (200 mg/kg) administered Group did not produce any significant change in VCMs, when compared with the Group II. Whereas no significant difference in no. of VCMs was seen when MESA 200 mg/kg treated group compared to combination of levodopa & carbidopa treated group.

Table: Effect of different extracts of *Saraca asoca* on tardive dyskinesia in haloperidol treated mice

Groups	Treatment	Vacuous Chewing Movements in 5 min on 15th day
I	Vehicle	9.52±1.32
II	Haloperidol	46.21±3.74
III	LD & CD+HP	12.64±2.14***
IV	PESA+HP	39.47±1.97^{ns}
V	AESA+HP	32.15±4.12*
VI	MESA+HP	19.28±3.94***
VII	WESA+HP	21.80±2.51**

Values are Mean ± SEM from 6 animals in each group. Significant difference at *p<0.05, **p<0.01 ***p<0.001 when treated group compared to negative control. ^{ns}- Non significant.

Hole board test

It was observed that haloperidol alone treated group, significantly decreased the no. of head dips and line crossings 15th day as compared to control group. In combination of Levodopa & carbidopa treated group, significant increase in no. of head dips and line crossings (p<0.001& p<0.01) was seen on both 15th day, as compared to haloperidol treated group. PESA 200mg/kg, pretreated groups did not cause any significant change in no. of head dips and line crossings on 15th day. But on 15th day, AESA, WESA & MESA 200 mg/kg pretreated groups showed significant increase in no. of head dips and line crossings (p<0.05 & p<0.01) when compared to haloperidol (as shown in table).

Table: Effect of different extracts of *Saraca asoca* on hole board test in haloperidol treated mice

Groups	Treatment	Number of head dips on 15th day	No of times of Line crossings on 15th day
I	Vehicle	45.48±1.9	32.18±1.8
II	Haloperidol	13.27±2.6	10.94±2.4
III	LD & CD+HP	48.26±1.3***	29.61±3.4**
IV	PESA+HP	12.59±1.6^{ns}	12.27±1.8^{ns}
V	AESA+HP	23.52±2.4*	19.82±2.6*
VI	MESA+HP	36.92±3.1**	27.52±1.9**
VII	WESA+HP	32.26±2.4**	25.37±1.1**

Values are Mean ± SEM from 6 animals in each group. Significant difference at *p<0.05, **p<0.01 ***p<0.001 when treated group compared to negative control. ^{ns}- Non significant.

Biochemical estimation

Haloperidol treated animals exhibited decreased levels of protective antioxidant enzymes such as SOD, CAT and increased level of LPO suggesting a possible free radicals generation. Treatment with standard, MESA & WESA 200 mg/kg showed significantly (p<0.01) increased levels of protective enzymes such as SOD, CAT and attenuated the increase level of LPO suggesting its possible antioxidant action. Whereas the AESA shows p<0.05 significant changes in haloperidol induced PD. But PESA revealed no significant changes was observed compared to haloperidol treated group.

Table: Effect of different extract of *Saraca asoca* on biochemical alterations in haloperidol treated rats

Groups	Treatment	SOD (U/mg of tissue)	CAT (U/mg of tissue)	LPO (µM/mg of wet tissue
I	Vehicle	15.65±0.06	10.52±0.2	3.84±0.03
II	Haloperidol	1.28±0.4	3.47±0.57	25.37±0.64
III	LD & CD+HP	4.2±0.2**	8.54±0.62**	2.73±0.09**
IV	PESA+HP	1.4±0.2^{ns}	3.26±0.09^{ns}	21.95±0.02^{ns}
V	AESA+HP	2.4±0.08*	5.81±0.18*	16.86±0.04*
VI	MESA+HP	3.81±0.04**	8.68±0.24**	5.29±0.43**
VII	WESA+HP	3.25±0.01**	7.52±0.48**	8.64±0.09**

Values are Mean ± SEM from 6 animals in each group. Significant difference at *p<0.05, **p<0.01 ***p<0.001 when treated group compared to negative control. ^{ns}- Non significant.

Estimation of brain epinephrine, nor-epinephrine and dopamine

Effect on brain epinephrine level : Epinephrine, level was significantly decreased in haloperidol treated group as compared to control vehicle group, while their level was significantly (p<0.05 & p<0.001) increased with acetone, methanol and water extracts of *Saraca asoca* at 200 mg/kg as compared to haloperidol group, not significantly increased of epinephrine level with petroleum ether extract when given with HP. However, MESA & WESA at a dose of 200 mg/kg had no significant activity as compared to normal cotrol group.

Effect on brain nor-epinephrine level : Results of the present study showed that nor-epinephrine level was significantly (P<0.001) altered by haloperidol treated group, while it was significantly increased at a dose of 200mg/kg with acetone, methanol and water extracts of SA as compared to haloperidol group. Plant extract PESA alone at a dose of 200 mg/kg significantly no changes compared to HP treated group. The effect of MESA & WESA extracts exhibited as like as standard.

Effect on dopamine level in the brain: The result of the present study revealed that the dopamine level was significantly (p<0.001) decreased in the haloperidol treated animals as compared to control group and their level was significantly (P<0.001) increased at 200 mg/kg with methanol and water extracts of SA as compared to haloperidol group. The Acetone extract of *Saraca asoca* showed significant (p< 0.05) increase of dopamine level at a dose of 200 mg/kg dose as compared to haloperidol treated group (Table). Whereas there is no observed change in dopamine level with PESA treated group.

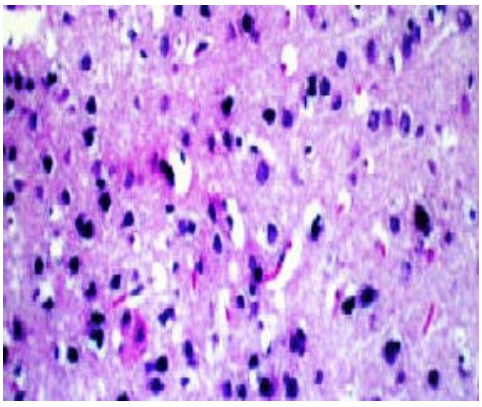
Table: Effect of *Saraca asoca* leaves on brain monoamines level in haloperidol treated mice.

Groups	Treatment	µg/g brain tissue		
		Epinephrine	Nor-epinephrine	Dopamine
I	Vehicle	4.58±0.09	5.43±0.05	5.38±0.04
II	Haloperidol	1.94±0.12	2.14±0.41	1.96±0.08
III	LD & CD+HP	3.52±0.06***	5.25±0.03***	4.55±0.05***
IV	PESA+HP	1.48±0.06^{ns}	2.16±0.05^{ns}	1.92±0.55^{ns}
V	AESA+HP	2.10±0.04*	3.02±0.06*	2.83±0.05*
VI	MESA+HP	3.48±0.13***	4.82±0.09***	4.61±0.08***
VII	WESA+HP	3.15±0.09***	4.39±0.13***	4.28±0.19***

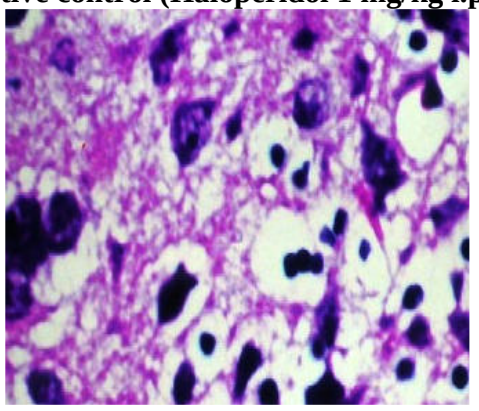
Values are Mean ± SEM from 6 animals in each group. Significant difference at *p<0.05, **p<0.01 ***p<0.001 when treated group compared to negative control. ^{ns}- Non significant.

Histological study: Mice treated with 1%v/v tween 80 showing a normal histopathological architecture of brain. Mice treated with Haloperidol alone showing Pericellular oedema, revealed alterations in brain histology in the form of typical necrosis, nucleus shrinkage or disappearance and cellular edema. Mice treated with combination of L-dopa and Carbidopa (100mg + 10mg/kg by i.p) followed by Haloperidol showing mild pericellular oedema. Mice treated with PESA revealed alterations in brain histology in the form of typical necrosis, disorganization of the typical layered appearance of the cerebral cortex, nucleus shrinkage or disappearance and edema. Mice treated with AESA revealed mild congestion and degeneration of tissue. Mice treated with 200 mg/kg body weight of MESA & WESA respectively for showing mild diffuse gliosis in cerebral cortex.

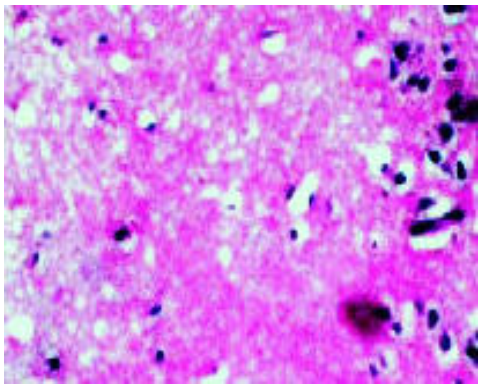
Normal Control



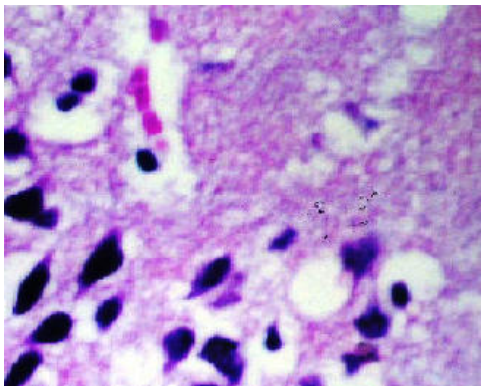
Negative control (Haloperidol 1 mg/kg i.p.)



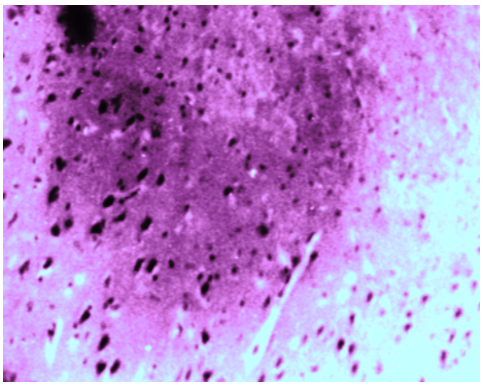
Standard



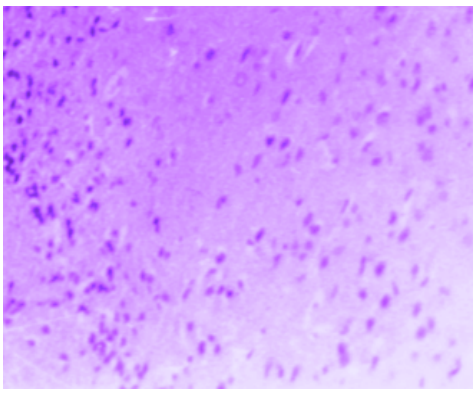
PESA extract of *Saraca asoca*



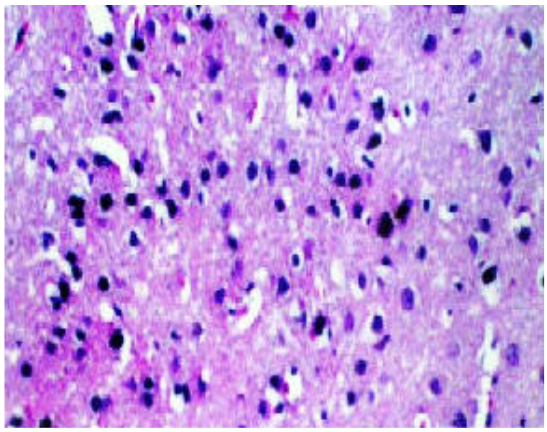
AESA extract of *Saraca asoca*



MESA extracts of *Saraca asoca*



WESA extracts of *Saraca asoca*



Discussion: Parkinson's disease is a chronic neurodegenerative disorder characterized by degeneration of dopamine producing neurons in the substantia nigra, caudate nucleus and putamen leading to resting tremor, bradykinesia, shuffling gait, flexed posture and rigidity. While the cause of the degeneration is not known, oxidative stress plays a vital role. Oxidative stress may arise from the metabolism of dopamine with the production of potentially harmful free radical species [25]. Compared to the rest of brain, the substantia nigra pars compacta is exposed to a higher rate of reactive oxygen species formation and to higher levels of oxidative stress. This may be related to the energy metabolism of these cells or to their high content of dopamine [26].Various studies have reported oxidative stress changes in the brain of Parkinson's disease patients [27]. Haloperidol, a neuroleptic drug, induces tardive dyskinesia's which is due to a blocking of post synaptic striatal dopamine D2 receptors and many studies have shown reactive oxygen species as a cause of Haloperidol induced toxicity[28,29]. Neuroleptics like haloperidol neurotoxicity has been linked to a blocking of post synaptic striatal dopamine D2 receptors and studies have proposed reactive oxygen species as cause of haloperidol induced toxicity [30]. Drugs which attenuate haloperidol induced motor disorders might reduce the extrapyramidal signs of Parkinson's disease.

In the present study, three behavioral assessment parameters catalepsy score, tardive dyskinesia, hole board tests were used to assess haloperidol induced Parkinson disease in mice. The mice when pretreated with *different extracts of Saraca asoca* for 15 days, significantly decreased the catalepsy score in block model and vacuous chewing movements (VCMs) in tardive dyskinesia test and this effect is comparable to that of levodopa & carbidopa group. The above findings of behavioral tests are similar with other previous studies [31,32].

The mice when pretreated with AESA, MESA & WESA (200 mg/kg, p.o.) for 15 days, significantly increased the no. of dips and line crossings in hole board test, and this effect is comparable to that of levodopa & carbidopa group. The above findings of behavioral tests are similar with other previous studies done on different parkinsonian animal models induced by MPTP and haloperidol [33-36]. *Saraca* is an important medicinal plant that plays a significant role in protection from oxidative stress. A present studies have shown that *Saraca Acetone, methanol and water extracts* has significant increase in anti-oxidant enzyme level such as CAT and SOD. It has been hypothesized that antioxidants may be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals [37]. Inquiries into the role of neuro-inflammation in Parkinson's disease have coincided with increasing interests in determining whether anti-

inflammatory medications may be helpful in preventing PD. Experimental evidence and animal models in particular support a preventative role for nonsteroidal anti-inflammatory drugs (NSAIDs) in Parkinson's disease. For example, studies have demonstrated that anti-inflammatory drugs such as acetylsalicylic acid are protective against haloperidol –induced striatal dopamine depletion in mice. Recently, involvement of inflammatory process has been also reported in the pathogenesis of Parkinson's disease [38]. It is widely accepted that inflammation and oxidative stress are interrelated. Oxidative stress can increase inflammatory activity and conversely, inflammation is known to cause oxidative stress [39].

Earlier studies have also emphasized the anti-inflammatory properties of *saraca* in rats. Previous studies show that *ethanol* extract was found to have anti-inflammatory property. *Saraca leaf* is known to be rich in Phenolic compound such as gallic acid is known to exert anti-oxidant activity [40,41]. The present anti-oxidant studies of SA increase protective enzymes such as SOD, CAT, and decrease the LPO suggesting its possible antioxidant action. Oxidation of dopamine by MAO-B and aldehyde dehydrogenase generates hydroxyl free radicals (.OH) in presence of ferrous ions (basal ganglia are rich in iron) [42]. So to find out oxidative stress at various levels the defensive antioxidant enzymes in rat brain were measured. SOD is the most important enzymes in the antioxidant defense system of the body. The major function of superoxide dismutase (SOD) is to catalyze the conversion of superoxide anion radicals to H2O2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. Catalase (CAT), which is present in all mammalian cells, is responsible for the removal of H2O2. LPO is the measure of the excessive oxidation of the lipids in the body indicating increased superoxide production. Oxidative stress cause depletion Dopamine neurotransmitter was more affected in Parkinson's disease [43, 44] where as other brain amines like norepinephrine, epinephrine and serotonin were much less affected than dopamine in haloperidol treated group [45]. Norepinephrine was less altered in parkinsonian patient this is in accordence with earlier reported studies [46]. Altered norepinephrine might contribute to some aspects of intellectual dysfunction in PD [47].

Dopamine is the precursor to norepinephrine, norepinephrine is a precursor to the hormone epinephrine. Norepinephrine and epinephrine are antistress chemicals in the body obviously there is great stress from Parkinson's disease. Further, epinephrine is involved in increasing the power of muscles and prolonging the action of muscle, by its ability to activate the release of glucose from glycogen. Thus optimizing the ability of epinephrine may help achieve more muscle control, perhaps reducing motor symptoms of Parkinson's disease [48].

The antioxidant studies of SA support anti-oxidant properties of gallic acid, terpenoids and flavanoids are responsible for the anti-Parkinson effect by neuroprotective from oxidative free radicles. The neuroprotective effect of extracts of SA was confirmed by increased level of dopamine, epinephrine and nor-epinephrine in brain tissues. The AESA, MESA, WESA extracts of *Saraca asoca* revealed the presence of terpenes, saponins, glycosides, flavonoids, tannins, and phenolic compounds. Terpenes and Flavonoid possess MAO inhibitory activity [49, 50]. The anti-parkinsonism effect of AESA, MESA and WESA extracts of *Saraca asoca* may be synergy of a number of phytoconstituent components viz. glycoside, terpenoid, Phenolic compound, flavonoids, tannic acid.

Conclusion: In the present study we concluded that the acetone, methanol and water extracts of *Saraca asoca* was found to possess a therapeutic effect against Parkinson's disease in haloperidol induced PD model in mice among the three extacts the MESA & WESA has potentially equal to Standard. Whereas

Petroleum ether extracts has no effect on Haloperidol induced PD and it was confirmed by Catalepsy score, tardive dyskinesia test, hole board test and antioxidant studies. Further studies are encouraged to identify and isolation the chemical constituents from MESA & WESA responsible for Anti-Parkinson's activity. Also clinical studies to prove this effect is also needed for its applicability in humans for treatment of Parkinson's disease.

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