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IN VITRO AND *IN VIVO* EVALUATION OF ANTI-PARKINSONISM AND ANTI-OXIDANT ACTIVITIES OF NEW ISATIN DERIVATIVES

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system. It results from the death of dopamine generating cells in the substantia –nigra, a region the mid brain, the cause of the cell death is unknown. Isatin derivatives shows maximum anti-parkinsonian activity, in – vitro and in – vivo anti-oxidant activity. Schiff bases of Isatin derivatives were investigated for in-vitro antioxidant activity. The following tests such as Nitric oxide scavenging activity, DPPH scavenging activity and acute oral toxicity studies were performed and investigated for Anti-parkinson's activity. The following tests such as, Assessment of behavioral and biochemical parameters, Determination of acetylcholine levels, Determination of Acetyl cholinesterase activity were performed, and also investigated for in-vivo antioxidant activity. Estimation of superoxide Dismutase, Estimation of Lipid Peroxidation, Estimation of reduced glutathione levels were performed. Isatin derivative test compounds when given at a dose of 30mg/kg body weight administered orally to rat they showed maximum anti parkinsonian activity, decreased tremors, salivation and lacrimation.

KEY WORDS

Isatin, Anti-parkinson's activity, in – vitro and in – vivo anti-oxidant activity.

INTRODUCTION

Isatin was chemically known as 1H- indole-2,3-dione. It was discovered by **Erdmann** and **Laurent** in 1840 as a product of oxidation of indigo with nitric and chromic acids. Isatin has been known for about 150 years and has been recently found, like oxindole and endogenous polyfunctional heterocyclic compounds, to exhibit biological activity in mammals. Isatin is a synthetically versatile substrate, used for the synthesis of variety of heterocyclic compounds, such as indoles and quinolines and has a raw material for drug synthesis. Isatin and its derivatives demonstrate diverse biological and pharmacological activities including Anti- convulsant, Anti–neoplastic, Anti-microbial, Anti-HIV, Anti-viral, Anti-inflammatory, Anti-psychotrophic, Anti-alzheimer's, Anti-arthritic, Anti-bacterial, Anti-diuretic, Anti-hypertensive, Anti-anaphylactic asthama, Anti-fungicidal, Anti-fertility, Anti-pyretic[14].

Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system. It results from the death of dopamine generating cells in the substantia nigra, a region the mid brain; the cause of the cell death is unknown. It is also called as Parkinson's, idiopathic parkinsonism, primary parkinsonism, PD or paralysis agitans. Early in the course of the disease, the most obvious symptoms are movement related, including shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, cognitive and behavioural problems may arise with dementia commonly occuring in the advanced stages of the disease. Other symptoms include sensory, sleep and emotional problems. PD is more common in the elderly occuring after the age of fifty[1].

Oxygen sustains life. However around 5% of the inhaled oxygen is converted into reactive oxygen species[3]. Despite the existence of endogenous defense mechanism against ROS, it has been observed that whenever the level of cellular antioxidant system goes down or when the reactive oxygen species reach abnormally high levels, oxidative damage to cells occurs finally leading to pathological conditions.

Some of the well-known consequences of generation of free radicals *in-vivo* are DNA strand break, nucleic acid base modifications, protein modification and lipid peroxidation[4,6]. ROS and free radicals also formed in the body as a result of normal metabolic reactions, exposing to ionizing radiation and by the influence of many xenobiotics. These are responsible for the causation of several diseases[2].

Antioxidants which can scavenge free radicals have an important role in biological system and their use is implicated in prevention of various diseases. Human body has an inherent mechanism to reduce the free radicals induced injury by enzymatic or non-enzymatic methods. When the normal level of antioxidant defense mechanisms is not sufficient for the eradication of free radicals induced injury, administration of antioxidants has a protective role to play. Several antioxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress[10].

2. MATERIALS & METHODS

2.1: Experimental animals: Sprague dawley rats, weighing 180-200gms were used for the experiment. All the animals were acclimatized for a period of one-week, free access to food and water, maintained at 28±2^0 c, 12h light/day cycles supplied by Mahaveer enterprises private limited, Hyderabad. Care and maintenance were carried out as per the guidelines. (The protocol for the animal study in prescribed proforma-B was approved by the Institutional Animal Ethics Committee (IAEC) under reg. no. 1505/po/a/11 CPCSEA)

2.2: Chemicals used: Sodium nitroprusside, acetylcholine, 5,5 dithios 2 nitro benzoic acid, phenazinne methosulpahte, nitro blue tetrazolium, pyridine, n-butanol and Sodium dodecyl sulpahte were procured from SD Fine Chemicals Limited. Ellman's, Trichloroacetic acid, Benzatropine and Hydroxylamine hydrochloride were gift sample from Merck Lab, Hyd. 1,1-diphenyl-2-picryl hydroxide, Thiobarbituric acid and Ferric chloride were procured from Nice chem Ltd, cochi. Ethanol was obtained from Qualinkems Ltd, cochi, and Oxotreomorine and MPTP were obtained from sigma Aldrich.

3. METHODOLOGY:

3.1: *In-vitro* Antioxidant Activity:

3.1.1: Nitric oxide scavenging activity: Nitric oxide scavenging activity was measured by spectrophotometer method. Sodium nitro prusside (5Mm) in phosphate buffered saline was mixed with different concentrations of Schiff bases of isatin derivatives (25-800µg/ml) dissolved in normal saline solution and incubated at 25^{0}c for 30min. A control without test compound but with equivalent amount of nitroprusside was taken. After 30min 1.5ml of incubated solution was taken and diluted with 1.5ml griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthyl ethylene diamine dihydrochloric acid was measured at 546nm. Vitamin E used as reference standard[11].

3.1.2: 1,1-Diphenyl-2-Picryl hydrazyl (DPPH) radical scavenging activity: DPPH scavenging activity was measured by spectophotometric method.0.1 mM solution of DPPH was prepared in ethanol and 1ml of this solution was added to 3 ml of Schiff bases of isatin derivatives in normal saline at different concentrations (25-800µg/ml).Equal amount of saline was added to the control. The mixture was shaken well and incubated at room temperature for 30 min. The absorbance was read at 517 nm using a spectrophotometer. Vitamin-E was used as reference standard[12].

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Test}} \times 100$$

Statistical Analysis: Data was statistically evaluated, and results were presented as mean±SEM, using statistical package INSTAT version. The values were considered significant when ($P<0.05$).

3.1.3: Acute oral toxicity studies: The procedure was followed by using OECD guidelines (Organization of Economic Cooperation Development) 423 (Acute Toxic Class Method)[8].

The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number

of animals while allowing acceptable database scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg bodyweight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

Twelve rats weighing 180-220gms were used for study the starting dose level of isatin test compounds was 30mg/kg body weight. Dose volume was administered 0.1 ml/10gm bodyweight to the rat which were fasted overnight with water ad libitum. Food was withheld for a further 3-4 hrs after administration of drug. Bodyweight of the rat before and after termination were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic, central nervous system somato-motor activity and behavioral pattern were observed, signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted the onset of toxicity and signs of toxicity were also noted if any.

3.2: Anti-Parkinson's Activity:

3.2.1: Assessment of behavioral and biochemical parameters:

Experiment protocol: Different groups of Sprague drawly rats of both sex weighing 100-200 gm were used. They were dosed orally 30 mg per kg with the test compounds T-1, T-2, T-3, T-4, T-5, T-6, for respective group animals given orally, 1 hr prior the administration of 0.5 mg/kg oxotremorine s.c. standard drug bezatropine (5mg/kg) was given to respective group animals, I her prior the administration of 0.5 mg/kg oxotremorine s.c. Tremor was scored after oxotremorine dosage in 10 s observation period every 15 min for 1h. Salivaiton and lacrimation were scored 15 and 30 min after oxotremorine injection[7].

Scoring was given as below:	
Absent	**0**
Slight	**1**
Medium	**2**
Server	**3**

Experimental design: Animals were divided into 9 groups each group comprising of 6 animals, all the animals were marked as head, body, tail, head body, head tail, and colorless. Animals were weighed and dose for each animal was calculated. Animals were divided as Group I to Group IX.

Group I: Normal group, treated with 0.1% CMC.

Group II: Disease induced group, treated with oxotremorine (0.5 mg/kg b.wt) (S.c.).

Group III: Test group, treated with test compound I orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group IV: Test group, treated with test compound II orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group V: Test group, treated with test compound III orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group VI: Test group, treated with test compound IV orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group VII: Test group, treated with test compound V orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group VIII: Test group, treated with test compound VI orally (30 mg/kg b.wt), 1 hr prior to oxotremorine injection (s.c.)

Group IX: Standard group treated with the standard drug benzatropine orally (5mg/kg b.wt), 1 hr prior to oxotremorine injections (s.c)

Different parameters like tremors, salivation and lacrimation were observed and observation values were tabulated. After the observational period all the animals were sacrificed, brains where isolated and different biochemical parameters were determined.

Determination of different biochemical parameters were determined.

Preparation of brain tissue homogenate:

After the observation period, animals were anaesthetized, decapitated and brains are isolated. Brains of all the marked animals of different groups were isolated separately and homogenized with ice cold buffer solution (P^H 7.4). The homogenized tissue was used for estimation of different biochemical parameters. After the assessment of different parameters animals are scarified and brains were isolated and evaluated for biochemical studies:

3.2.2: Determination of acetylcholine levels:

Procedure: Tissue homogenates are placed in boiling water for 15min to terminate the AchE activity and also to release the bound Ach. 0.5ml tissue homogenate+1ml of alkaline hydroxylamine hydrochloride+1ml of 50%Hcl were added, Contents were mixed and centrifuged at 100rpm for 5min and supernatant was collected. To the supernatant 0.5ml of

0.37M of ferric chloride is added, intensity of colour was read at 540nm, against blank and compared with standard values. Ach were expressed as µmol of Ach/mg protein[9].

3.2.3: Determination of Acetyl cholinesterase activity: Reaction mixture comprising of 3ml of 0.1ml phosphate buffer (pH 8), 20µL of 0.075M acetylthiocholine iodide, 100µl of 0.01M 5,5-dithiobis -2-nitro benzoic acid and 100µl of tissue homogenate. The reaction mixture is then incubated for 30 min at room temperature. Colour absorbance is read at 412nm using spectrophotometer. For the determination of pseudocholinesterase activity, the substrate butyrl thiocholine iodide was added instead of acetylcholine iodide and the activity of butyrl cholinesterase (BuchE) was estimated and subtracted from the total cholinesterase activity to obtain the specific activity of AchE. Enzyme activity was expressed as µmol of Ach hydrolyzed/mg protein/hr[9].

Statistical analysis:

Data were presented as mean±SEM, data was analyzed by one-way ANOVA followed by Dunnets multiple comparison test using statistical package INSTAT. The values were considered significant when ($P < 0.05$)

3.3: *In-vivo* antioxidant activity:

Experimental protocol: The animals were divided into 9 groups each consisting of six rats. Group I served as vehicle control and received 0.1% CMC Group II to received 20 mg/kg MPTP four injections (i.p) at 2 hours intervals (Gerhard vogel's) Group III, IV, V, VI, VII, VIII received test compounds T-1, T-2, T-3, T-4, T-5, T-6 respectively at a dose of 30 mg/kg P.O on the first day 30 minutes prior to first injection of MPTP and daily for another six days of the experimental period.

Experimental design: Animals were divided into nine groups, each group consisting of 6 animals. All the animals in each group and marked as head, body, tail, head body, head tail, and colourless. Animals were weighed and dose for each animal was calculated.

Group I: Control group treated with 0.1%CMC.

Group II: Disease induced group, treated with MPTP (20 mg/kg b.wt) (i.p).

Group III: Test group treated with test compound I (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group IV: Test group treated with test compound II (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group V: Test group treated with test compound III (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group VI: Test group treated with test compound IV (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group VII: Test group treated with test compound V (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group VIII: Test group treated with test compound VI (30 mg/kg b.wt) orally for 7 days and 1st day 30 min prior to MPTP injection (i.p).

Group IX: Standard group treated with the standard drug L-dopa orally (200mg/kg) for 7 days and on 1st day 30 min prior to MPTP injection (i.p).

After the experimental period (after 7 days of treatment) the animals were fasted overnight and sacrificed by cervical, decapitation the brains were excised immediately, and the brain tissue was homogenized in ice cold buffer solution (pH7.4). The homogenized tissues were used for estimation of different biochemical parameters.

3.3.1: Estimation of superoxide Dismutase:

Procedure:

1. Assay mixture compromising of 0.1ml of sample+1.2ml of sodium pyrophosphate buffer (*pH* 8.3,0.052M) + 0.1ML phenazine methosulphate (186µM) + 0.3ml of 300µM nitro blue tetrazolium+0.2ml NADH(750µM)
2. Reaction was started by addition of NADH, incubated at 30°c for 90 secs. Then reaction was stopped by the addition of 0.1ml glacial acetic acid.
3. Reaction mixture was stirred vigorously with 4ml of n-butanol, allowed to stand for 10min, Centrifuged and n-butanol layer was separated. Colour intensity was measured at 560nm and SOD was expressed as nM/mg protein[13].

3.3.2: Estimation of Lipid Peroxidation:

Procedure:

1. The tissues are homogenized in 0.1M buffer pH 7.4 with a teflon glass homogenizer. LPO in the homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of ohkawa[3].
2. 0.2ml of tissue homogenate ,0.2ml of 8.1% sodium dodecyl sulphate (sds),1.5ml of 20%acetic acid and 1.5Ml 8% TBA were added.

To volume of the mixture was made upto 4ml with distilled water and then heated at 95°c on a water bath for 60 min using glass balls as.

3. After incubation the tubes were cooled at room temperature and final volume was made upto 5ml in each tube.5ml of butanol: pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation of 3000rpm for 10min.
4. The organic layer was taken, and its OD read at 532nm against an appropriate blank without the sample. The levels of lipid peroxidase were expressed as n moles of thiobarbituric acid reactive substances(TBARS)/mg protein using an extinction coefficient of $1.56\times05M1cm^{-1}$

3.3.3: Estimation of reduced glutathione (GSH):

Procedure:

1. Tissue homogenate (in 0.1 M phosphate buffer pH7.4) was taken. The procedure was followed initially as described by Ellman et al(Brigelius-Flohe,1989), the homogenate was added with equal volume of 20%trichloroacetic acid(TBA) containing 1mM EDTA to precipitate the tissue proteins
2. The mixture was allowed to stand for 5min prior to centrifugation for 10min at 200rpm.The supernatant was then transferred to a new set of test tubes and ellman's reagent (5,5'-dithio bis-2-nitrobenzoic acid) was added in 0.3M phosphate buffer with 1%of sodium citrate solution.
3. All the test tubes were made upto 2ml and measured at 412nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Statistical analysis:

Data were presented as mean±SEM, data was analyzed by one-way ANOVA followed by Dunnets multiple comparison test using statistical package INSTAT. The values were considered significant when ($P< 0.05$).

4. RESULTS AND DISCUSSION

4.1: Acute Toxicity Results:

4.1.1: *Invitro* antioxidant activity:

4.1.1.1: Nitric oxide scavenging activity: Schiff bases of Isatin derivatives showed promising free radical scavenging effect against nitric oxide induced release of free radicals in concentration dependent manner. IC50 values of Schiff bases of isatin derivatives are.

Table1: Nitric oxide scavenging activity

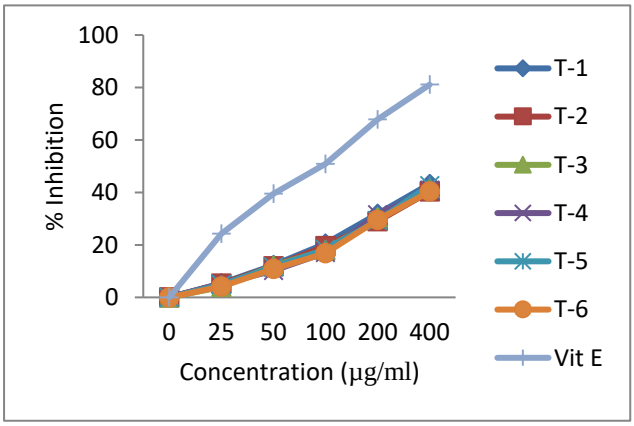
Concentration (µg/ml)	% Inhibition						
	T-1	T-2	T-3	T-4	T-5	T-6	Vitamin E
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
25	5.3±0.04	5.2±0.04	4.0±0.03	5.1±0.08	4.8±0.09	4.15±0.02	24.2±0.16
50	12.3±0.08	11.8±0.02	12.0±0.02	10.2±0.01	11.5±0.01	10.86±0.2	39.46±0.02
100	20.4±0.06	19.5±0.06	18.0±008	17.0±0.05	18.3±0.09	16.8±0.04	50.82±0.01
200	31.8±0.08	29.0±0.02	30.5±0.06	31.5±0..04	29.3±0.02	29.5±0.08	67.82±0.01
400	43.2±0.06	40.3±0.01	41.5±004	40.8±0.05	42.6±0.08	40.3±0.02	81.05±0.01
800	56.1±0.02	55.5±50.03	51.6±0.03	52.8±0.06	54.9±0.02	53.1±0.01	96.18±0.01
IC_{50} and confidence interval	637±0.07	640±0.04	639±0.07	643±0.03	641±0.02	645±0.04	142.19±0.02

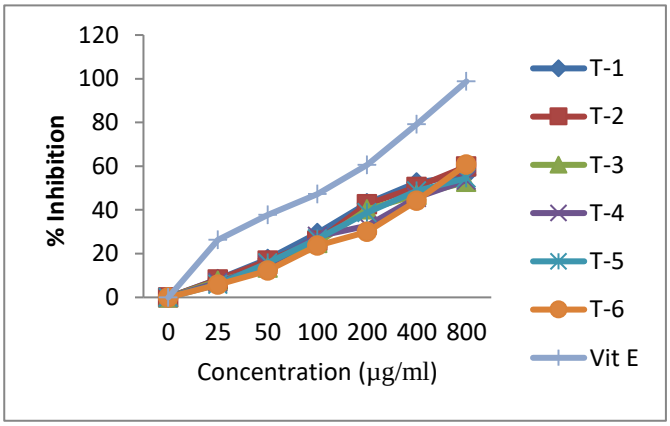
4.1.1.2: DPPH SCAVENGING: Schiff bases of Isatin derivatives showed promising free radical scavenging effect in the reduction of DPPH in the concentration dependent manner at a concentration of 25-800µg/ml. The IC_{50} values of the test compounds were found to be release of free radicals in concentration dependent manner. IC50 values of Schiff bases of isatin derivatives.

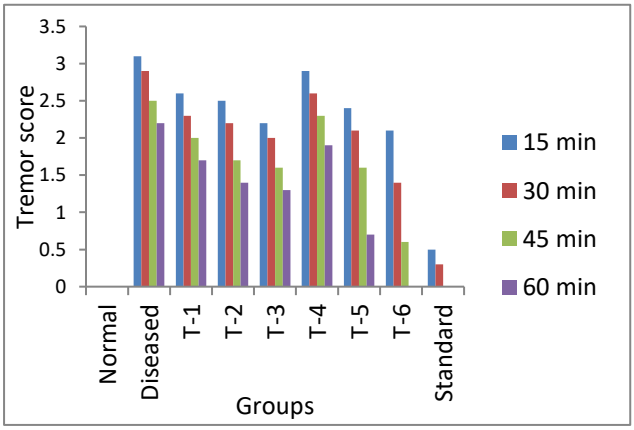
Table2: Free radical scavenging activity

Concentration (µg/ml)	% Inhibition						
	T-1	T-2	T-3	T-4	T-5	T-6	Vitamin E
0	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
25	8.27±0.04	8.10±0.03	7.5±0.02	6.8±0.03	5.9±0.03	5.85±0.08	26.30±0.09
50	17.5±0.05	16.8±0.09	13.6±0.03	14.9±0.02	15.8±0.01	12.31±0.13	37.66±0.02
100	29.35±0.07	25.9±0.08	24.8±0.01	27.9±0.09	26.8±0.03	23.74±0.04	47.30±0.03
200	43.05±0.04	42.6±0.04	40.2±0.12	32.8±0.13	38.7±0.02	30.08±0.09	60.60±0.10
400	52.49±0.5	50.5±0.02	46.8±0.03	45.7±0.09	48.7±0.01	44.14±0.12	79.19±0.08
800	55.98±0.03	59.9±0.01	52.8±0.02	53.6±0.08	54.5±0.07	60.76±0.05	98.77±0.03
IC_{50} and confidence interval	459.7±0.50	530.9±0.011	472.3±0.02	531.6±0.218	542.7±0.32	568.6±0.228	164.71±0.018

4.1.1.3: Behavioral parameters: When compared with group I, group II showed maximum tremors, salivation and lacrimation. When compared with group II Group III, IV, V, VI, VII, VIII showed less tremors, salivaition and lacrimation. Group IX showed very significant decrease in tremors, salivation and lacrimation when compared to all other groups.







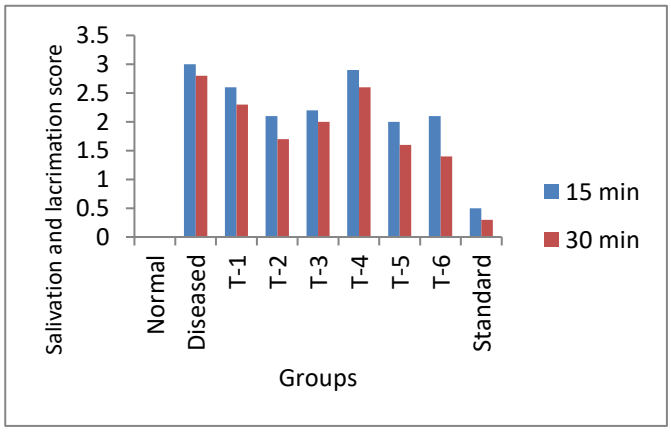


Table 4: Acetylcholine levels of different treatment groups.
(Values are MEAN ± SEM of 6 independent analysis)

TREATMENT GROUP	ACH
Group – I	2.11±0.05
Group – II	10.26±0.14
Group – III	4.34±0.07
Group – IV	5.31±0.01
Group – V	4.82±0.05
Group – VI	5.98±0.09
Group- VII	6.50±0.03
Group – VIII	7.23±0.05
Group – IX	3.16±0.01

When compared with Group I, Group II is significant, because Ache levels are increased when compared with Group II, Group III, IV, V, VI, VII, VIII, IX are significant because Ach levels are significantly decreased. When compared with Group IX Group III, IV, V, VI, VII, VIII are less significant, because Ach levels are not significantly decreased.

Table 5: Acetyl cholinesterase levels of different treatment groups
(Values are Mean ± SEM of 6 independent analysis)

TREATMENT GROUP	AchE
Group – I	6.57±0.07
Group – II	1.01±0.03
Group – III	1.98±0.08
Group – IV	1.52±0.09
Group – V	1.76±0.03
Group – VI	1.89±0.05
Group- VII	1.63±0.02
Group – VIII	1.23±0.03
Group – IX	3.73±0.22

When compared with Group I, Group II is significant, because AchE levels are decreased when compared with Group II, Group III, IV, V, VI, VII, VIII, IX are significant because AchE levels are significantly increased. When compared with Group IX Group III, IV, V, VI, VII, VIII are less significant, because AchE levels are not significantly increased.

Table 6: Superoxide dismutase levels of different treatment groups
(Values are Mean ± SEM of 6 independent analysis)

TREATMENT GROUP	SOD
Group – I	10.23±0.05
Group – II	22.52±0.17
Group – III	13.71±0.15
Group – IV	14.92±0.05
Group – V	14.01±0.09
Group – VI	15.95±0.03
Group- VII	15.06±009
Group – VIII	16.74±0.20
Group – IX	11.77±0.14

When compared with Group I, Group II is significant, because SOD levels are increased when compared with Group II, Group III, IV, V, VI, VII, VIII, IX are significant because Ach levels are significantly decreased. When compared with Group IX Group III, IV, V, VI, VII, VIII are less significant, because Ach levels are not significantly decreased.

Table 7: Glutathione levels of different treatment groups
(Values are Mean ± SEM of 6 independent analysis)

TREATMENT GROUP	GSH
Group – I	15.67±0.18
Group – II	4.11±0.07
Group – III	11.93±0.15
Group – IV	10.62±0.18
Group – V	7.93±0.21
Group – VI	8.73±0.15
Group- VII	9.98±0.09
Group – VIII	7.62±0.08
Group – IX	14.14±0.15

When compared with Group I, Group II is significant, because GSH levels are decreased when compared with Group II, Group III, IV, V, VI, VII, VIII, IX are significant because Ach levels are significantly increased. When compared with Group IX Group III, IV, V, VI, VII, VIII are less significant, because Ach levels are not significantly increased.

Table 8: Lipid peroxidation levels of different treatment groups
(Values are Mean ± SEM of 6 independent analysis)

TREATMENT GROUP	LPO
Group – I	7.67 ±0.22
Group – II	24.13 ±0.2
Group – III	12.34 ±0.16
Group – IV	13.68 ±0.17
Group – V	14.530.19
Group – VI	15.05 ±0.03
Group- VII	15.98 ±0.09
Group – VIII	16.24 ±0.06
Group – IX	9.68 ±0.21

When compared with Group I, Group II is significant, because LPO levels are increased when compared with Group II, Group III, IV, V, VI, VII, VIII, IX are significant because Ach levels are significantly decreased. When compared with Group IX Group III, IV, V, VI, VII, VIII are less significant, because Ach levels are not significantly decreased.

Fig5: Acetyl choline levels of different treatment gps

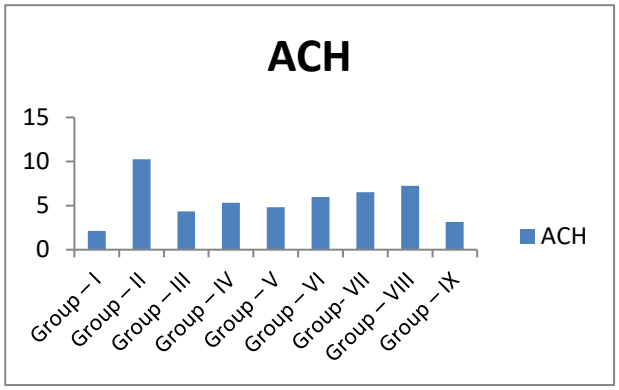


Fig6: Acetyl cholinesterase levels of different treatment gps

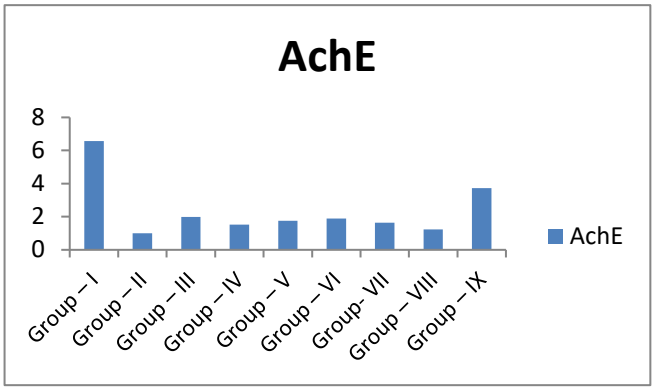


Fig7: Superoxide dismutase levels of different treatment gps

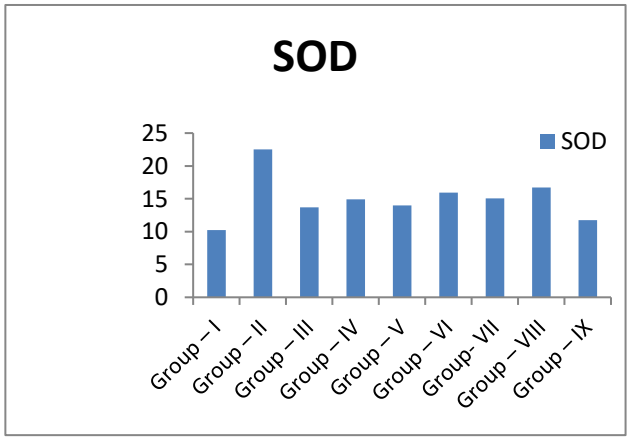
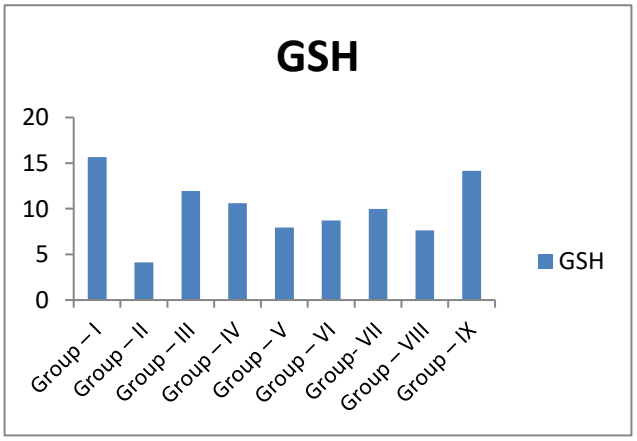


Fig8: Glutathione levels of different treatment gps



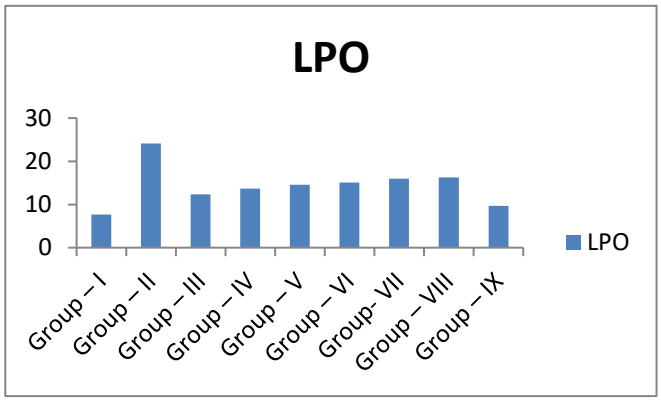


Fig9: Lipid peroxidation levels of different treatment groups.

Indigenous drug system can be a source of a variety of new drugs, which can provide relief from pain, fever, and various other disorders and diseases of the human body, but their claimed reputation has to be verified on a scientific basis. The present investigation revealed those Isatin derivatives as a significant and anti-parkinsonin activity. Recent studies suggest that neroudegenaration and subsequent loss of neurons is due to the liberation of free radicals. The free radicals have been implicated in the path physiology of various

clinical disorders like parkinsonism, cancer, inflammation etc., normally intracellular anti-oxidants protect the tissue from injury by free radicals.

Therefore, development of antioxidant drug could be beneficial as adjunct to anti-parkinsonism therapy. Phytochemical screening revealed the presence of phenols, which could be responsible for anti-parkinsonian and anti-oxidant activity.

5. CONCLUSION:

The following conclusions have been drawn from the above results.

- Isatin derivative test compounds when given at a dose of 30mg/kg body weight administered orally to rat they showed maximum anti parkinsonian activity.
- Isatin derivative test compounds decreased tremors, salivation and lacrimation,
- The present study has made an attempt to demonstrate the anti-parkinsonian and antioxidant activities of isatin derivative.
- Further this was evidenced by various in – vitro and in – vivo anti-oxidant assay methods like DPPH, NO, GSH, LPO, SOD scavenging activities isatin derivatives.

6. PROTOCOL:

The protocol for the animal study in prescribed proforma-B was approved by the Institutional Animal Ethics Committee (IAEC) of Talla Padmavathi College of Pharmacy under Reg. No. 1505/po/a/11 CPCSEA.

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