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EVALUATION OF NEUROPROTECTIVE ACTIVITY OF MEDICINAL PLANT EXTRACT

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ABSTRACT

Neuroprotection aims to protect neurons or slow disease progression and secondary injuries by faltering or at least slowing the loss of neurons Despite differences in symptoms or injuries associated with CNS disorders. The term Neurodegeneration is a combination of two words- “neuro” stand for nerve cells and “degeneration” stand for progressive damage. Thus, in the strict sense of the word, neurodegeneration corresponding to any pathological condition primarily affecting neurons. Among the many of different neurodegeneration disorder, so far, including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and Amyotrophic lateral sclerosis (ALS). In practice, neurodegeneration disease represents a large group of neurological disorder with pathological expression and heterogenous clinical affecting specific subset of neurons.

KEYWORDS: - Parkinson disease (PD), Amyotrophic lateral sclerosis, Central Nervous System, Catechol Omethyl transferase, Catechol Omethyl transferase.

1. INTORDUCTION

Neuroprotection aims to protect neurons or slow disease progression and secondary injuries by faltering or slowing the loss of neurons. Excitotoxicity is the pathological process by which neurons are damaged and killed by the overactivations of receptors for the excitatory neurotransmitter glutamate, such as the NMDA receptor and AMPA receptor. Neuroprotective treatment often targets oxidative stress and excitoxicity – both of which are highly associated with Parkinson’s disease. Neuroprotection refers to the relative preservation of neuronal structure and function Oxidative stress is essentially an imbalance between the production of

free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants In the case of an ongoing insult (a neurodegenerative insult) the relative preservation of neuronal integrity implies a reduction in the rate of neuronal loss over time, the prevalence of Parkinson's Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 1% of the population over 55 years of age. Disease increases with increasing age, and this age specific prevalence is remarkably similar in the majority of countries. The main symptoms of PD are tremor, catalepsy, rigidity, bradykinesia, hypokinesia, impaired balance, gait disturbance and postural instability. This disease is characterized by the loss of 50–70% of the dopaminergic neurons in the substantia nigra pars compacta (SNc) and the presence of intracytoplasmic inclusions called Lewy bodies (LB), mainly found α-synuclein and ubiquitin. The main symptoms of PD are tremor, catalepsy, rigidity, bradykinesia, hypokinesia, impaired balance, gait disturbance and postural instability. The exact causes of PD remain unidentified however oxidative stress, inflammation, mitochondria dysfunction, and excitoxicity have been suggested to play a significant role in the pathogenesis and progression of the disease. Even after being the second most common neurodegenerative disease, the current therapies remain purely symptomatic and there is no treatment available which can either reverse or halt the progression of disease. Therefore treatment of PD should focus more on interfering with the underlying neuropathology, i.e. dealing with the loss of dopaminergic neurons in the Substantia Nigra pars compacta.

1.2. Factor causing of PD

1.2.1 Advancing age

The risk continues to increase as one gets older. Many of the researcher assumes that people with Parkinson's have neural damage from genetic or environmental factors that get worse as they age.

1.2.2 Sex

Males are more likely to get Parkinson's then females. Possible reasons for this may be that males have greater exposure to other risk factors such as toxins exposure or head trauma. It has been theorized that oestrogen may have neuro-protective effect.

1.2.3 Family history

Having more than one close relatives with the disease increases the likelihood that you will get it, but to a minimal degree.

1.3 Statistical data

1.3.1 Smoking, drinking, vitamins and the risk of Parkinson's disease.

Table 1.3.1: Smoking, drinking, vitamins and the risk of Parkinson's disease.

Risk factor	Risk	Study period	Study design	Study population	Country	Description
Cigarette smoking	Lower	1992–2001	Prospective	M = 63,348; F = 79,977	U.S.	N = 413 had definite or probable PD during follow-up period 31
Coffee consumption	Lower	1981–1998	Prospective cohort	N = 13,979	U.S.	No. of PD = 395; control = 2320
Coffee	Lower	1982–1987	Cross-sectional	N = 29,335	Finland	PD associated with amount of coffee consumed daily
Tea	Lower	1992–1997	Cross-sectional	-	-	-
Cigarette smoking	Lower	1990–1995	Case-controlled	PD = 190; control = 190	Italy	-
Coffee/tea	-	-	retrospective	-	-	No difference
Cigarette smoking	Lower	2002–2003	Case-controlled	PD = 114; control = 205	China (Beijing)	Reduced risk for PD among those who had ever smoked
Cigarette smoking	Lower	1994–1998	Case-controlled	PD = 377; control = 377	India	Reduced risk for PD among smoker ≤ 20 yr
Well-water drinking	-	-	-	-	New Delhi	≤ 10 yr exposure, no significant effect

1.3.2 Systemic diseases and the risk of Parkinson's disease

Table 1.3.2: Systemic diseases and the risk of Parkinson's disease.

Risk factor	Risk	Study period	Study design	Study population	Country	Description
Gout	Lower (in men only)	1995–2001	Prospective	PD = 1052 control = 663	U.S.	Previous history of gout, lower risk of PD
Initiation of medications	Lower	-	-	-	-	Initiation of anti-gout medicine, lower risk of PD:
Hypertension	Not associated	1976–2000	rospective	F = 121,046	U.S. (Boston)	Self-reported history
Hypertension	Lower	1981–1998	Prospective cohort	N = 13,979	U.S	No. of PD = 395; control = 2320 for current users of antihypertensive drug

1.3.3 Environmental/genetic factors and the risk of Parkinson's disease

Table 1.3.3: Environmental/genetic factors and the risk of Parkinson's disease.

Risk factor	Risk	Study period	Study design	Study population	Country	Description
Previous head injury	Higher	Cross-sectional	Case-controlled	N = 140; control = 147	U.S	OR = 6.23; CI = 2.58–15.07
Higher levels of physical activity	Lower	1986–2000	Prospective cohort	M = 48,574, F = 77,254	U.S.	Greater baseline physical activity associated with a lower risk of PD
Family history of PD	Higher	1998	Case-controlled	PD = 136; control = 272	Italy	OR = 41.7; 95% CI = 12.2–142.5
Patient with ≥ 3 children	Higher	1981–1998	Prospective cohort case-controlled	N = 13,979	U.S	Risk increase with increased number of children
Herbicide/pesticide exposure	-	1990–1995	Retrospective control	PD = 190, control = 190	Italy	No significant difference

Table 1.5: Characteristic of Parkinson tremor Vs Essential tremor.

Characteristic	Parkinson's disease	Essential tremor
Age at onset (years)	55–75	10–80
Tremor frequency (Hz)	4–6	5–10
Family history	+/–	++
Tremor characteristics	Supination–pronation	Flexion–extension
Influencing factors		
Rest	Increases	Decreases
Mental concentration	Decreases	Increases
Action	Decreases	Increases
Writing	Decreases (micrographia)	Increases (tremulous)
Walking	Increases	Decreases
Postural tremor	Re-emergent	Without latency
Limb tremor	Asymmetric	Symmetric
Kinetic tremor	+/–	Yes
Distribution other than limbs	Face, jaw, lips, chin	Head, voice
Neuroimaging-dopaminergic system	Marked dopaminergic deficit	Mild dopaminergic deficit
Neuropathology	Nigrostriatal degeneration, Lewy bodies	Mild cerebellar degeneration, Lewy bodies in the substantia nigra.

Mid-brain sonography	Marked hyper-echogenicity	Mild hyper-echogenicity
Treatment	Anticholinergics, amantadine, dopaminergic drugs, deep brain stimulation	Alcohol, beta-blockers, primidone, topiramate, gabapentin, botulinum toxin, deep brain stimulation

2. MATERIALS AND METHODS

2.1. Collection and Authentication of plant material

The seed of *Brassica Nigra Linn*. were collected from Medico of herbal products and raw materials, Dombiwali, Mumbai, *Brassica nigra* seeds were authenticated at Guru Nanak Khalsa college, Matunga, Mumbai. *Brassica Nigra* is commonly known as black mustard. The black mustard seeds were wrapped, packed in brown paper and brought to pharmacology lab in Dr.L.H.Hiranandani College of Pharmacy, Ulhasnagar, for further research work.

2.2. Extraction procedure

Processing of the Black Mustard seeds to obtain Hydro-Alcoholic *Brassica nigra* extract.

Extraction: Percolation

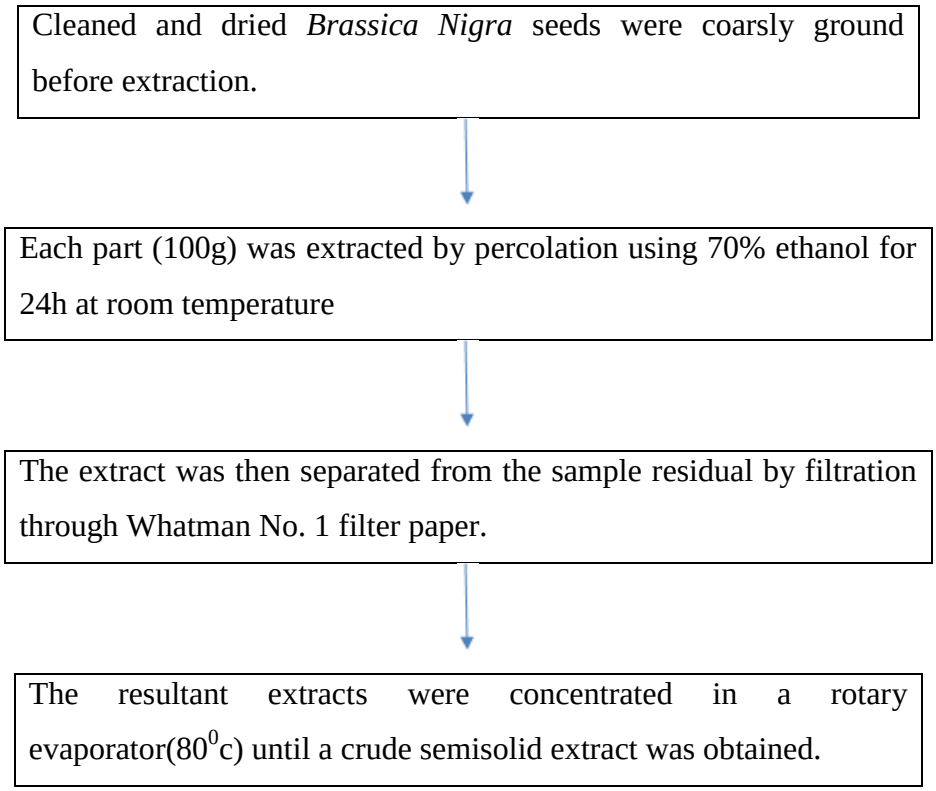


Fig. 5.1: Steps involved in the extraction of bassica nigra seeds.

2.3. Purification

The crude distilled to get 1/3rd of solution using the Soxhlet apparatus at 70^0 c for 3hrs ethanol was recovered to concentrate crude extraction and then the solution is kept overnight at room

temperature 25°C to precipitate. The solution and the obtained particles are dried in the oven overnight at 60°C. Water was added in the Soxhlet apparatus, and allowed to evaporate at lower temperature.[11]

2.4. Gallic acid test

To detected Gallic acid we take extract (1 ml of ethanol extract) mixed with 10 ml of distilled water and filtered. Ferric chloride reagent (3drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic.

2.5. Isolation of gallic acid

Gallic acid was isolated using the organic solvent ethyl acetate in a separating funnel. The material was removed and water was added to it. This was heated to about 60 – 70^{o}C. Ethyl acetate was added to the extract in a separating funnel. Gallic acid being soluble in organic solvent was extracted into the organic phase. Gallic acid was separated from ethyl acetate by rotary vacuum evaporation. The pressure and temperature at which this separation was done was 200mbar and 70^{o}C. Diethyl ether was added to it and was evaporated in a rotary evaporator to obtain pure Gallic acid.[12]

2.6. Identification of compounds

2.6.1. Preliminary phytochemical screening

A. Test for carbohydrates

Benedict's test –extract was mixed with few drops of Benedict's reagent and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

B. Test for alkaloids

- Dragendroff’s test: To 2-3 ml of filtrate, add few drops of Dragendroff’s reagent. Orange brown ppt indicate the presence of alkaloid.
- Hager's Test: Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.
- Wagner’s test**:** To 2-3 ml of filtrate, add few drops of Wagner’s reagent. Reddish brown ppt indicate the presence of alkaloid.

C. Test for flavonoids

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red colour indicating the presence of flavonoids.

D. Test for Steroids and Triterpenoids

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

E. Test for Tannins and Phenolic compound

To 2-3 ml of crud extract add few drop of 5% $fecl_3$ solution. Deep blue black colour formed indicates the presence of tannins and phenolics compound.

2.6.2 Identification of gallic acid

A. Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC), is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a quick, inexpensive microscale technique that can be used to verify a substance's identity. Isolated Gallic acid compound was compared with standard Gallic acid using TLC method.

Procedure: Readymade activated TLC plate was used. The extract was dissolved in respective solvent and spotted over an activated plate (1cm above from the bottom). It was then kept in previously saturated developing chamber containing mobile phase, and allowed to run $3/4^{th}$ of the height of the plate. The developing plate was removed and air dried and observed under ultraviolet light.

Standard Solution: Gallic acid + Ethanol

Test solution: Isolated Gallic acid + Ethanol

Mobile phase: Ethyl acetate: Formic acid (9:1)

Formula:

$$R_f = \frac{\text{distance of the spot on the TLC-plate}}{\text{distance of the solvent front}}$$

TLC the solvent system was selected as ethyl acetate and formic acid in the ratio 9:1. The Rf value of standard gallic acid and the isolated gallic acid was calculated and compared.[13]

A. Fourier Transform Infra Red (FTIR) Spectroscopy

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. Because the analyst requires a frequency spectrum (a plot of the intensity at each individual frequency) in order to make an identification, the measured interferogram signal cannot be interpreted directly. A means of "decoding" the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the Fourier transformation. This transformation is performed by the computer which then presents the user with the desired spectral information for analysis. The isolated Gallic acid compound was compared with standard Gallic acid by using FTIR (Fourier Transform Infrared Spectroscopy). FTIR study was carried out to analyze the functional groups present in the compound. Sample was fixed in Potassium bromide disc and was scanned between 400-3500cm^{-1}.[14]

B. High Performance Liquid Chromatography (HPTLC)

The high performance thin layer chromatographic analysis of isolated Gallic acid compound was done to compare with standard gallic acid compound on HPTLC plate.

Procedure

Standard solution: Dissolve 10 mg of gallic acid in 10 ml of ethanol.

Test solution: Take 36.8 mg of sample and diluted to 10 ml with ethanol, sonicate to dissolve and filter.

Chromatographic condition

HPTLC plate: HPTLC plate 10 × 10 cm silica gel 60 with fluorescence indicator (Merck).

Developing solvent: Toluene: Ethyl acetate: Formic acid(6 : 4: 1).

Spray reagent: 1% $FeCl_3$ solution.

Visualization: R_f value of the resolved bands were recorded, under UV 254 nm.

2.7 Pharmacological studies

Animals: Albino swiss mice:

Species: Mice

Strain: Albino Swiss Mice.

Weight: 18-25 g

Gender: Male

Numbers to be used: 60

2.8 Animals house conditions

Albino Swiss Mice 18-25gm were procured from Bharat serum and vaccines limited, plot no. A-371 372, road no.27 Wagle industrial estate, near bus depot, thane 400604, Maharashtra, registration number 103/99/CPCSEA. The animals were brought to animal house of Dr L.H. Hiranandani college of pharmacy, opposite to Ulhasnagar railway station, CHM campus, Ulhasnagar-03. These animals were acclimatized in animal house under standard husbandry conditions, i.e. room temperature of 24 ± 100C, relative humidity 45-55% and 12:12 hr light/dark cycle. The institutions animal house is registered with Govt. of India, having registration number 879/ac/05/CPCSEA and confirms to the CPCSEA guideline for the use and care of experimental animal research. The animals were housed in standard propylene cages with wire mesh top and husk as bedding. The animals had free access for food and water supplied ad libitum under strict hygienic condition. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. The approval of the Institutional animal ethical committee (IAEC) of Dr L.H. Hiranandani college of pharmacy was taken prior to the start of experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA) and with the protocol no.: IAEC/PCOL-05/2014.

2.9 Acute toxicity studies

Acute toxicity study of test drug was performed on Albino Swiss mice weighing around 18-25 gms as per OECD Guidelines 423. Animals were fasted overnight and then test drug (Isolated Gallic acid compound) were provided with single oral dose of 2000mg/kg. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a

total of 14 days. The parameters observed were tremor, convulsion, sedation, salivation, urination and death.[10]

Table 5.1: Dose for acute toxicity studies.

Group	Dose	No. of animals
Test	2000mg/kg	6

2.10 In vitro study of antioxidant activity

A. Metal chelation assay

Principle: The antioxidant present in plant extract forms a coordinate complex with metals ion (chelating agent) and inhibit the transfer of electron. Thus, oxygen reaction reaction is arrested and no radicle are produced. Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complexes decreased. Measurement of colour reduction therefore allows the estimation of the chelating activity of the coexisting chelators. The transition metal ion Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ion.

Procedure

1. 1 ml of IGA extract and Standard EDTA (0-5 mg/ml) was diluted with 3.75 ml of distilled water separately.
2. The above solutions were mixed with $FeCl_2$ (2 mm, 0.1 ml) and 4,41-[3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzenesulphonic acid (ferrozine) (5 mm, 0.2 ml).
3. After 10 minute the absorbance at 562 nm was determined.

Evaluation: The percentage of metal chelating activity was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

B. Reduction power determination

Principle: The yellow color of the test solution change to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicle (antioxidant)

causes the conversion of ferricyanide. Therefore, formation of pearls Prussian blue at 700nm. A higher absorbance at 700nm indicate a higher reducing power.

Procedure

Different amounts of each extracts (25-800 µg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K3 Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50^{o} C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

2.11 Haloperidol induced catalepsy in mice

Parameters to be evaluated are as follows

A. Behavioral changes

- Muscles rigidity using Rotarod test
- Locomotor activity using Actophotometer

B. Biochemical parameters

- Super oxide scavenging activity.
- Glutathione estimation.
- Lipid peroxidation estimation.

The main objective of this study was to evaluate the neuroprotective effect of IGA in Haloperidol induced catalepsy.

Principle

Haloperidol is a neuroleptic which causes extrapyramidal side effect like catalepsy, akinesia (Immobility), bradykinesia (slow movement), muscular rigidity and tremor. Chronic treatment with haloperidol increases free radicle production and oxidative stress and causes decrease in the activity of antioxidant defence enzyme, superoxide dismutase (SOD) and catalase. Catalepsy is the inability to change uncomfortable, imposed body posture.

Mechanism

Catalepsy caused by haloperidol can be exploited for the study of PD. haloperidol at the dose commonly used in clinical practice causes EPS as they occupy more than 80% of dopamine D2 receptor. Dopamine D2 receptor is only a target for the action of neuroleptics, but its blockade change the activity of many neuronal pathway, transmitting impulses from the striatum, through numerous relay station to effector, which are muscles in case of extrapyramidal disturbance.

Preparation of drug

Two dose of IGA 100mg/kg (low dose) and 500mg/kg (high dose) were prepared by suspending IGA in the required volume of distilled water. Dose of Trihexyphenidyl Hydrochloride 0.1mg/kg was prepared by suspending it in saline. Vehicle containing 0.5% sodium carboxy methyl cellulose (CMC) in water was used as control. All the solution was freshly prepared everyday prior to the administration.

Study design

Swiss albino mice weighing between 18-25gm were randomly divided into 5 groups of 6 animals each (n=6) as follows:

Groups	Dose	No. of animals (Mice)
Vehicle control	0.5% Na CMC	6
Disease control	2mg/kg	6
Standard + Haloperidol	0.1mg/kg + 2mg/kg	6
Test 1+ Haloperidol	100mg/kg +2mg/kg	6
Test 1+ Haloperidol	500mg/kg +2mg/k	6

Dosing schedule

The animals in the respective groups were administrated IGA suspending in purified water and the standard drug group received Trihexyphenidyl Hydrochloride p.o, daily one hour prior to the administration of haloperidol for 14 days.

Induction

Induction of catalepsy was done by the administration of haloperidol (2mg/kg) in filtered distilled water p.o once daily for 14 days.

A. Behavioral parameters

- **Locomotor activity using actophotometer**

One of the cardinal features of PD is bradykinesia which refers to slowness of movement, which will result in a decrease in locomotor activity. Assessment of locomotor activity was done using an actophotometer. An actophotometer consist of infrared sensor and a digital counter. The animal's movements will interrupt the infrared beam, which will get recorded and displayed digitally. This principle is used to count total locomotor activity of an animal. Before subjecting the animal to cognitive task they were individual placed in actophotometer and the total activity count was registered for 5 min. The locomotor activity was expressed in terms of total counts/5min per animal. In the present study the locomotor activity was assessed on the 4^{th}, 8^{th}, 14^{th}, 24hr after haloperidol administration.

- **Rotarod apparatus**

The rotarod test is used to assess motor coordination and balance in rodents. Mice have to keep their balance on a rotating rod. It is measured the time (latency) it takes the mouse to fall off the rod rotating at different speeds or under continuous acceleration (e.g. from 4 to 40 rpm).

On the day of testing, mice should be kept in their homes cages and acclimate to the testing room for at least 15 min (Acclimation phase). For ease of identification at later trials, mark the mice, using non-toxic ink, with respective strips at the base of the tail before testing. Turn on the rotarod apparatus. Record the latency at which each mouse fall off the rod. If a mouse is clinging on the rod completes a full passive rotation stop the timer for that mouse by pushing down the lever and record the latency. Remove the mouse and place it back in its home cage.be very careful not to disturb the other mice that are still running in the adjacent lanes. Also take note of passive rotation on the data sheet.

B. Biochemical parameters

Evaluation of antioxidant enzymes as follows

On 14^{th} day after behavioural assessments, animals were sacrificed by cervical dislocation and brain were removed. The cerebellum was discarded and the remaining brain tissue was weighed and preserved at -20^{0}C in deep freezer till further analysis. The known weight of brain tissue was homogenized for the estimation of antioxidant enzymes as follows.

- **Super oxide scavenging activity.**

The super oxide radicles were generated in a system with phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH). The NADH-phenazine methosulphate – nitro blue tetrazolium formazan inhibition reaction can be adopted for the rapid, simple, sensitive and reliable assay. A blank was prepared with all the chemicals. In the PMS/NADH-NBT system; super oxide anions reduce NBT to a blue formazan compound. Acetic acid in the concentration was used to arrest the formazan formation. The addition of acetic acid instead of trichloroacetic acid to stop the reaction helps to dissolve the protein so that a subsequent centrifugation step can be avoided. The colour reaction reaction of super oxide radicals and NBT was detected on spectrophotometer at 560nm. The enzyme activity was expressed as change in optical density per milligram protein per minute.

- **Glutathione estimation**

Rotruck and his co-workers measured the activity of glutathione peroxidase. The reaction mixture contained 0.2 ml of 0.4 M tris HCl buffer, 0.2 ml standard glutathione, 0.1ml of 0.2 M H_2O_2. The content was incubated at 37^0C for 10 minutes. The supernatant was assayed for glutathione content by Ellman's reagent (19.8 mg of 5, 5'-dithiobis nitrobenzoic acid (DTNB)in 100ml of 0.1% sodium nitrate). GPx activity was expressed as µg of GSH utilized /minute/mg protein.

- **Lipid peroxidation**

Lipid peroxidation was estimated calorimetrically in brain tissue by quantifying TBARS. The estimation of TBARS the supernatant of the tissue homogenate was treated with tertiary butanoltrichloroaceticacid-hydrochloricacid, (TBA-TCA-HCL) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed colour was red at 535 nm using a UV spectrophotometer against a reagent blank.

The lipid in the cell membrane are highly susceptible to peroxidative damage and are broken into number of units to form malondialdehyde. This react with TBA to form Thiobarbituric Acid Reacting Substance (TBARS) which has a pink colour with absorption maxima at532 nm.[10]

2.12 Reserpine induced behavioural change in mice

Parameters to be studied are as follows

The main objective of this studies was to evaluate the neuroprotective effect of IGA in Reserpine induced catalepsy.

Principle

The administration of RES 5mg/kg intraperitoneally for five consecutive days in mice it irreversibly blocks the uptake and storage of norepinephrine and dopamine into synaptic vesicles by inhibiting the vesicular monoamine transporter (VMAT) as the consequence of this several striatal dopamine depletion is generated along with spine and glutamatergic synapse of striatopallidal medium spiny neurons pruning. The change in neurochemical balance generated by reserpine are associated with behavioural deficits predominantly in motor activity.

Preparation of drug

Dose of IGA 500mg/kg were prepared by suspending IGA in the required volume of 0.5% sodium carboxy methyl cellulose (CMC) solution in water. Dose of Trihexyphenidyl HCL0.1mg/kg was prepared by suspending it in saline. Vehicle containing 0.5% sodium carboxy methyl cellulose (CMC) in water was used as control. All the solution was freshly prepared everyday prior to the administration.

Study design

Swiss albino mice weighing between 18-25gm were randomly divided into 4 groups of 6 animals each (n=6) as follows:

Groups	Dose	No. of animals (mice)
Vehicle control	0.5% Na CMC	6
Disease control	5mg/kg	6
Standard + Reserpine	0.1mg/kg +5mg/kg	6
Test + Reserpine	500 mg/kg +5mg/kg	6
Total		24

Dosing schedule

The animals in the respective groups were administered IGA suspending in 0.5% sodium CMC orally and the standard drug group Trihexphenidyl HCL p.o, daily one hour prior to the administration of Reserpine for 7 days.

Induction

Mice were treated with Reserpine for 5 consecutive days to generate acute DA depletion. Reserpine was dissolved in 1% glacial acetic acid at a concentration of 2.5mg/ml. This solution was diluted with H_2O to obtain a final concentration of 0.1% glacial acetic acid and 250 µm/ml Reserpine. This Reserpine solution was injected i.p. at a concentration of 5mg/kg.

Behavioural parameters

A. Locomotor activity using actophotometer

One of the cardinal features of PD is bradykinesia which refers to slowness of movement, which will result in a decrease in locomotor activity. Assessment of locomotor activity was done using an actophotometer. An Actophotometer consist of infrared sensor and a digital counter. The animal's movements will interrupt the infrared beam, which will get recorded and displayed digitally. This principle is used to count total locomotor activity of an animal. Before subjecting the animal to cognitive task they were individual placed in actophotometer and the total activity count was registered for 5 min. The locomotor activity was expressed in terms of total counts/5min per animal. In the present study the locomotor activity was assessed on the Reserpine administration.

B. Bradykinesia

In the impaired ability to initiate movements test, the mouse was held by the tail so that he is standing by his forelimbs and moving on his own. The number of steps taken with both forelimbs was recorded for 30 s. The existence of bradykinesia (movements in parkinsonian mice are slower than observed in healthy controls) was measured by placing the animal's forepaws on a horizontal wooden bar(0.7 cm in diameter), 4 cm above the table top. The time until the mouse removed both forepaws from the bar was recorded, with a maximum cut off time of 3 min.[10]

3 RESULT AND DISCUSSION

The present study dealt with phytochemical and pharmacological evaluation of the Isolated Gallic acid compound from the seeds of *brassica nigra*. The Gallic acid was evaluated for phytochemical and pharmacological characteristics at doses (100 mg/kg & 500 mg/kg).

3.1. Identification of compounds

3.1.1. Identification of Gallic acid in IGA

Table 4.1: Phytochemicals identification.

Sr. No	Constituents	Test / Reagents	Observation	Inference
1.	Carbohydrates	Molish's test, Fehling's test	Positive	Carbohydrates presents
2.	Flavonoids	Conc. H_2SO_4, NaOH	Positive	Flavonoids presents
3.	Alkaloids	Dragendroff's test, Hager's test Wagner's test	Positive	Alkaloids Presents
4.	Tannins and phenolic compound	5% $FeCl_3$, Lead acetate, Acetic acid, Dil. Iodine soln., Dil.HNO_3	Positive	Tannins and phenolic compound Presents
5.	Steroids	Salkowski test	Positive	Steroids Presents
6.	Test for gallic acid	Dissolve 0.1 g of gallic acid in 20 ml of concentrated sulphuric acid (=0.5% (w/v) solution)	Positive	Gallic acid present

3.2. Description and Melting point of gallic acid

Gallic acid is a colourless or slightly yellow crystalline compound. white hygroscopic crystals. odourless white solid.

3.2.1 Solubility

1 G Dissolves In: 87ml of Water, 3ml of Boiling Water, 6ml or Alcohol, 100 mi of Ether, 10ml of Glycerol, 5ml of Acetone and practically insoluble in Benzene, Chloroform, Petroleum Ether.

3.2.2 Decomposition

Thermal decomposition products include carbon dioxide and carbon monoxide. When heated to decomposition it emits acrid smoke and irritating fumes.

3.2.3 Stability

Stability Stable, but may discolour upon exposure to light. Hygroscopic. Incompatible with strong oxidizing agents, strong bases, acid chlorides, acid anhydrides.

3.2.4 Melting point: 252^{0}C, Density: 1.694.

3.2.5 Identification by Thin Layer Chromatography (TLC)

Fig. 4.1: TLC Plate.

S= Standard gallic acid

T = Test (isolated) gallic acid

3.2.6 IR profiling of the isolated gallic acid compound

FTIR study was carried out to analyze the functional group present in the compound.

Sample was fixed in potassium bromide disc (Kbr and was scanned between 400 to 3500 cm^{-1}

The functional group were recovered by using the FTIR spectroscopy.

The IR showed 3385- 3258 cm^{-1} i.e. H-bond hydroxyl group, 1678 cm^{-1}i.e C=O oxide carbonyl, 1523 cm^{-1} aromatic ring.

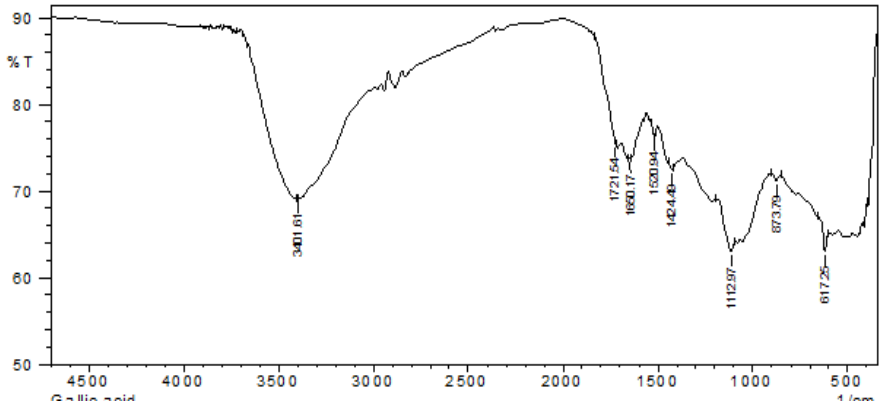


Fig. 4.2: IR spectra of standard Gallic acid.

Quantification of Isolated GALLIC ACID

100 gm of seed ground powder found 8.6 gm of GALLIC ACID

Therefore, % Yield (C) = TG × 100 / TS

Where, TG – Total GALLIC ACID

Ts – Total Seed powder

% Yield (C) = 8.6 × 100 / 100

% Yield (C) = 8.6 %

3.3 Acute toxicity study

A = Absent & N = Normal

Table 4.2: Observation table for toxicity study.

Time period	Observation					
Days (min)	Tremor	Convulsion	Sedation	Lethargy	Urination	Other behavioral change
Day 1						
30 min	**A**	**A**	**A**	**A**	**N**	**N**
60 min	A	A	A	A	N	N
90 min	A	A	A	A	N	N
120 min	A	A	A	A	N	N
150 min	A	A	A	A	N	N
180 min	A	A	A	A	N	N
210 min	A	A	A	A	N	N
240 min	A	A	A	A	N	N
Day 2	A	A	A	A	N	N
Day 3	A	A	A	A	N	N
Day 4	A	A	A	A	N	N
Day 5	A	A	A	A	N	N
Day 6	A	A	A	A	N	N
Day 7	A	A	A	A	N	N
Day 8	A	A	A	A	N	N
Day 9	A	A	A	A	N	N
Day 10	A	A	A	A	N	N
Day 11	A	A	A	A	N	N
Day 12	A	A	A	A	N	N
Day 13	A	A	A	A	N	N
Day 14	A	A	A	A	N	N

The test drug was found safe at 2000mg/kg bodyweight. Slight grooming was observed for first four hours. After 24 hrs all animals were observed normal. There was no death of animals during or after 14 days. The main purpose of this study was to identify safe therapeutic dose as well as to observe behavioural changes and toxicity.

3.4. To determine Antioxidant Activity of Test Drug: In-Vitro models of Antioxidant activity

3.4.1 Reduction power determination: In the in vitro Reduction power determination, the yellow colour of the test solution change to various shade of green and blue in depending manner upon the reducing power of each compound. The percent inhibition of isolated Gallic acid at 20 µg/ml conc. showed 71.64 % and that of standard ascorbic acid at 20 µg/ml conc. was 82.83 %. The IC50 value of Test group (Isolated Gallic Acid) was found to be 12.56 µg/ml and correlation coffecient was calculated from the graph and found to be 0.9858. The IC50 value of Standard group was found to be 11.91µg/ml and correlation coffecient was calculated from the graph and found to be 0.9796.

Table 4.3: Percent inhibition of Reduction power determination Test group (IGA).

Concentration (µg/ml)	% inhibition of isolated compound	IC $_{50}$ value
5	25.37	12.56µg/ml
10	38.05	
15	56.71	
20	71.64	

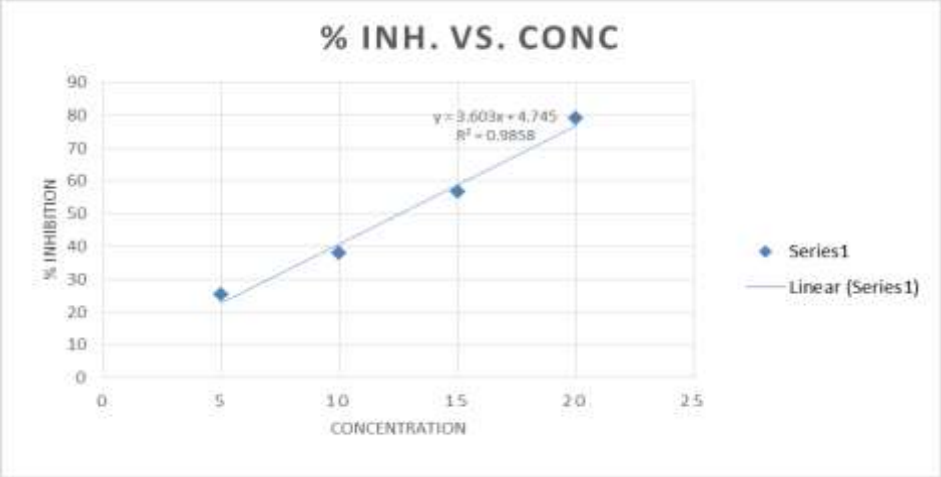


Fig. 4.4: Percent inhibition of Reduction power determination Test group (IGA).

Table 4.4: Percent inhibition of Reduction power determination Ascorbic Acid.

Concentration (µg/ml)	% inhibition of Ascorbic Acid	IC $_{50}$ value
5	23.13	11.91
10	45.52	
15	64.17	
20	82.83	

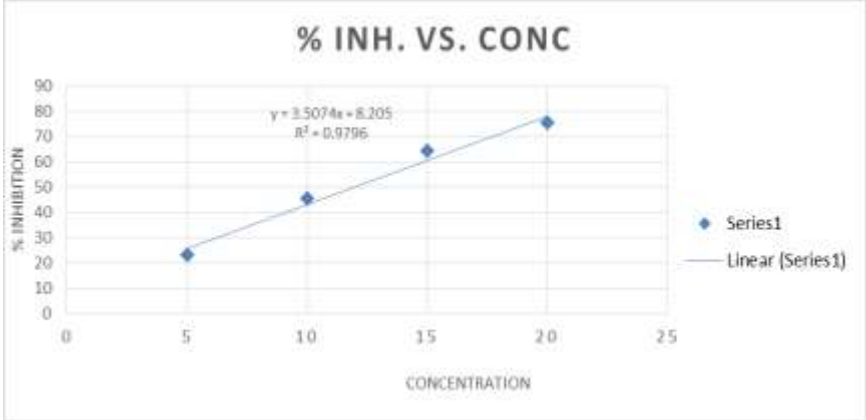


Fig. 4.5: Percent inhibition of Reduction power determination Ascorbic Acid.

X Axis: Concentration µg/ml

Y Axis: % Inhibition of Reduction power determination

- In the invitro Reduction power determination of a compound is related to its electron transferability and may serve as a significant indicator of its potential antioxidant activity. The percent inhibition of IGA at 20 µg/ml conc. showed 71.64% and that of standard ascorbic acid is 82.83 %.
- From the above observation, IGA inhibit free radicle dependent concentration upto 20µg/ml and thus inhibit oxidative mechanism that lead to degenerative disease.

3.5 Metal chelation assay

The antioxidant present in plant extract forms a coordinate complex with metal ion (chelating activity) and inhibit the transfer of electron. Thus, Oxygen reaction is arrested and no free radicles are produced. The percent inhibition of isolated Gallic acid at 5 µg/ml conc. showed 68.05 % and that of standard EDTA at 5 µg/ml conc. was 74.02%. The IC50 value of Test group (Isolated Gallic Acid) was found to be 1.90 µg/ml and correlation coffecient was calculated from the graph and found to be 0.4444. The IC50 value of Standard group EDTA was found to be 0.8 µg/ml and correlation coffecient was calculated from the graph and found to be 0.4552.

Table 4.5: Percent inhibition of Metal chelation assay Test group (IGA).

Concentration (µg/ml)	% inhibition of isolated compound	IC $_{50}$ value
0	0	1.90
0.05	30.65	
0.1	41.62	
0.5	54.45	

1	59.25	
2.5	64.72	
5	68.05	

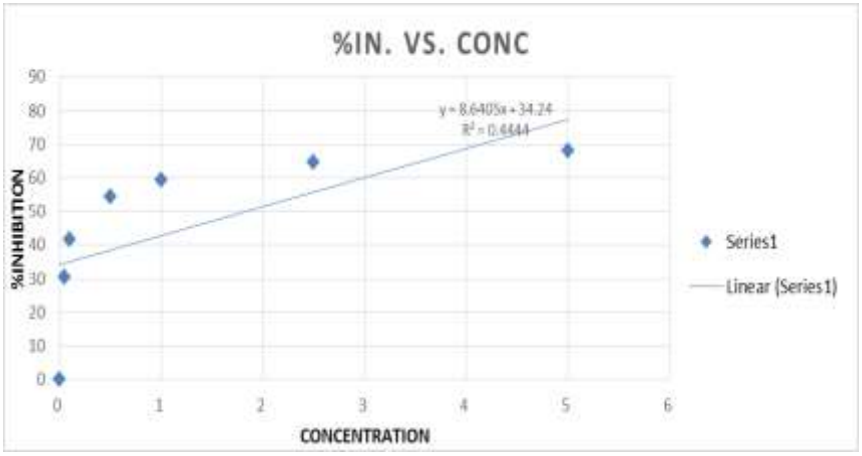


Fig. 4.6: Percent inhibition of Metal chelation assay Test group (IGA).

Table 4.6: Percent inhibition of Metal chelation assay EDTA.

Concentration (µg/ml)	% inhibition of EDTA	IC $_{50}$ value
0	0	0.8
0.05	37.15	
0.1	42.15	
0.5	58.72	
1	63.2	
2.5	68.97	
5	74.02	

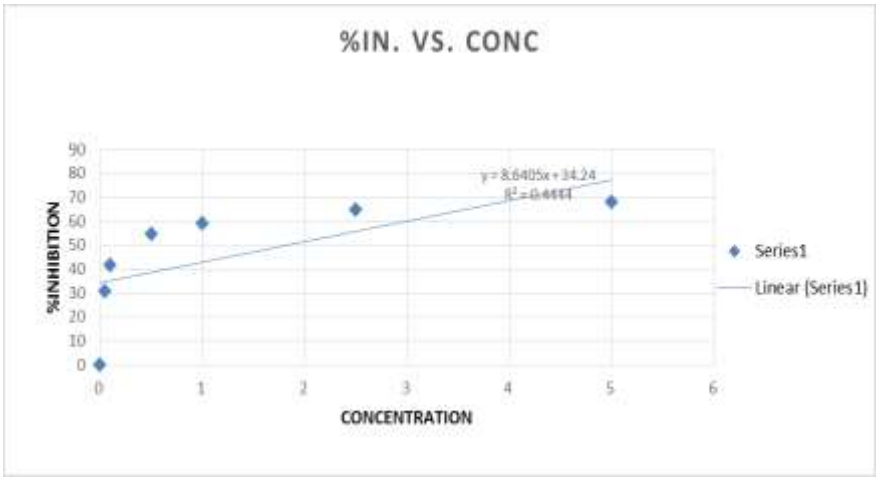


Fig. 4.7: Percent inhibition of Metal chelation assay EDTA.

X Axis: Concentration µg/ml

Y Axis: % Inhibition of Metal chelation assay

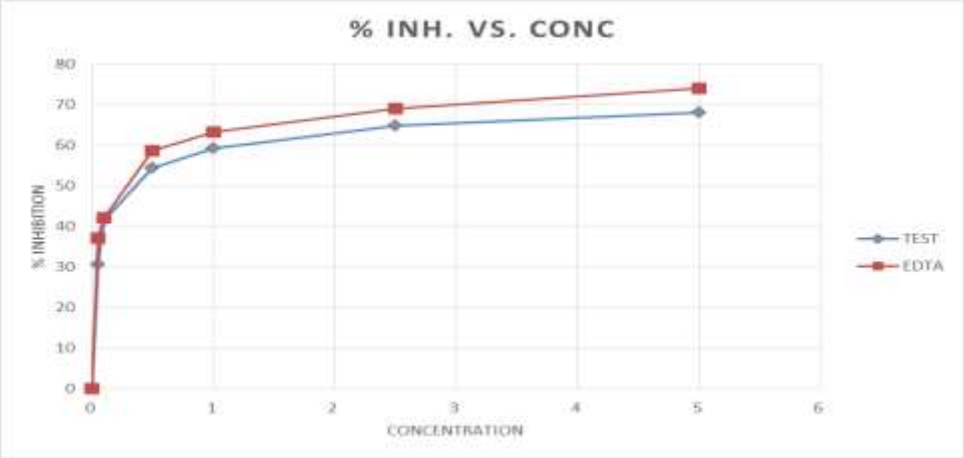


Fig: 4.8: Graphical representation of effect of IGA & EDTA in Metal chelation assay.

- In the in vitro Metal chelation model, It reduce the concentration of the transition metal that catalyser lipid per oxidation. IGA showed a strong metal chelating activity on neuroprotective activity. The percent inhibition of IGA at 5 µg/ml conc. showed 68.05 % and standard EDTA showed 74.02%.

3.5 In-vivo models

3.5.1 Haloperidol induced catalepsy in mice

- Catalepsy was caused with haloperidol will be administered to mice daily for a period of 14 days and tested for behavioural parameter as follows:
- Behavioural parameters: actophotometer, rota rod apparatus.
- Biochemical parameters: Superoxide scavenging activity, Glutathione determination, Lipid peroxidation estimation.

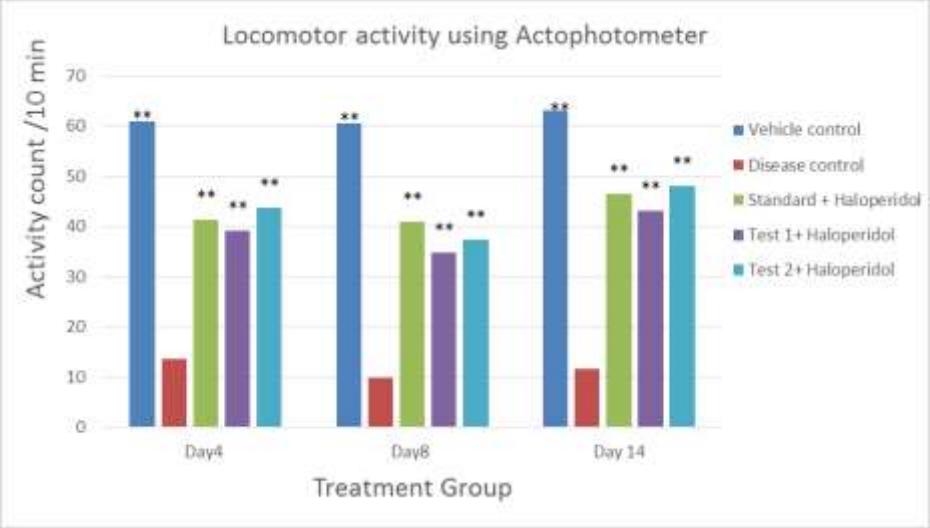
Behavioural parameters

3.5.1.1 Locomotor activity using actophotometer

Table 4.7: Locomotor activity using actophotometer.

Day	Vehicle control	Disease control	Standard + Haloperidol	Test 1+ Haloperidol	Test 2+ Haloperidol
Day 4	60.83±3.87**	13.66±1.20	41.33±1.83**	39.16 ±1.53**	40±2.12**
Day 8	60.5±4.65**	9.83± 0.79	41.00±2.43**	34.83±1.93**	37.33±1.78**
Day 14	63.00±3.41**	11.66±0.76	40.83±1.85**	38.83±3.40**	37.83±2.53**

Value were expressed as Mean± SEM for 6 mice in each group. Significance was determined bu One-Way ANNOVA followed by Dunnett's multiple comparison tests. *: $p<0.01$ when compared with vehicle control group, **: $p<0.01$ when compared with Positive control group.



Graph 3.1: Graphical representation of locomotor activity using actophotometer.

DISEASE CONTROl group (Haloperidol 2mg/kg) showed significance difference in locomotor activity as compared to negative control group. Standard group (trihexyphenidyl HCL 0.1 mg/kg & Haloperidol 2mg/kg) and Test 2 group showed a significance difference when compared to disease control group. Test group 1 did not show significance effect.

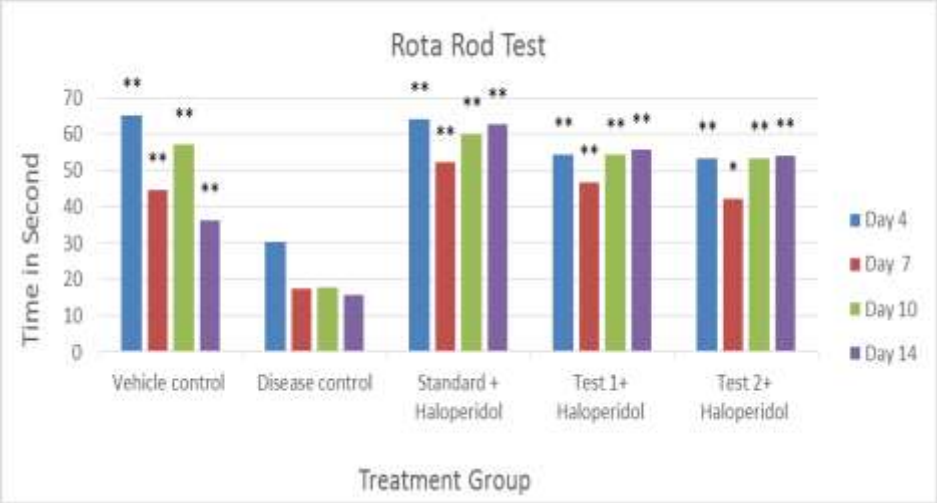
From above result it is seen that Test 2 show locomotion in Parkinson disease induced mice as compared to disease control Haloperidol group.

3.5.1.2 Muscles rigidity by rotarod

Table 4.8: Muscles rigidity by rotarod.

Day	Vehicle control	Disease control	Standard + Haloperidol	Test1+ Haloperidol	Test 2+ Haloperidol
Day 4	65.13±5.60**	30.42±2.95	64.18±6.90**	54.34±4.41**	53.33±2.41**
Day 7	44.74±5.60**	17.50±1.73	52.14±9.56**	46.86±1.68**	42.21±3.75*
Day 10	57.09±4.37**	17.91±0.95	60.14±4.13**	54.24±2.46**	53.40±4.86**
Day 14	35.27±2.40**	15.31±1.39	61.46±3.56**	57.26±2.12**	56.28±3.82**

Value were expressed as Mean±SEM for 6 mice in each group. Significance was determined by One-Way ANNOVA followed by Dunnett's multiple comparison tests. *: $p<0.01$ when compared with vehicle control group, **: $p<0.01$ when compared with Positive control group.



Graph 3.2: Graphical representation of locomotor activity using Rota Rod apparatus.

Haloperidol (2mg/kg) treatment group showed significant reduction in the rota rod activity of muscles rigidity on all assessment day as compared to control group. Treatment with IGA Test drug (100mg/kg and 500mg/kg) showed significant increase in activity on all assessment day.

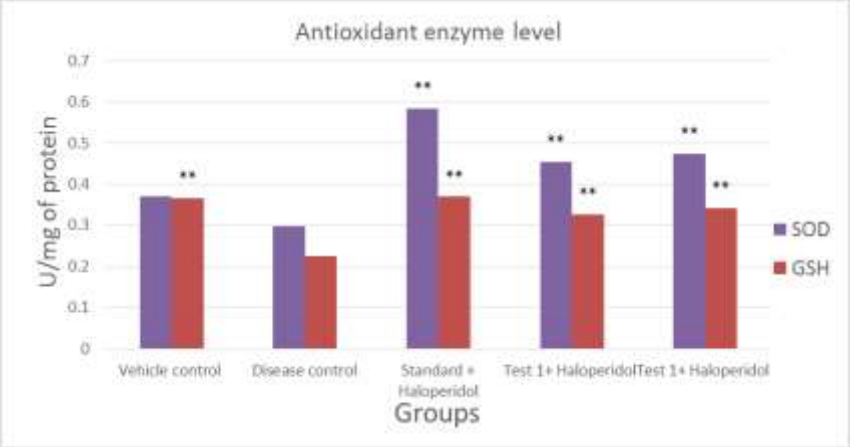
Disease control group showed significance reduction in rota rod activity of muscles rigidity on all assessment day as compared to vehicle control group. From the above observation it is found that treatment with IGA (Test1 & Test2) showed significant increases in activity on all assessment day.

3.5.1.3 Biochemical estimations

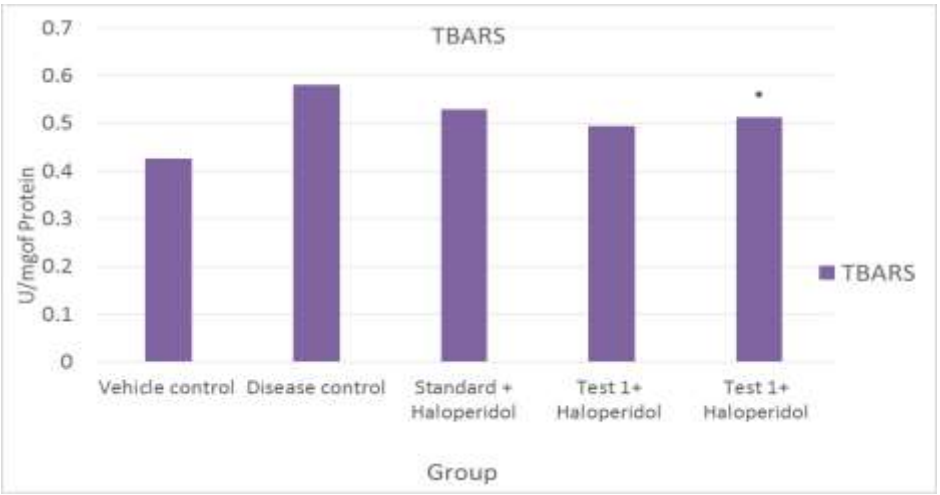
Table 3.9: Biochemical estimations.

Parameter	Sod	Tbars	GSH
Vehicle control	0.37±0.03	0.43±0.05	0.37±0.02**
Disease control	0.30±0.03	0.45±0.06	0.22±0.02
Standard + Haloperidol	0.58±0.03**	0.68±0.10	0.37±0.02**
Test 1+ Haloperidol	0.45±0.05*	0.57±0.06	0.33±0.03**
Test 1+ Haloperidol	0.4746±0.03**	0.74±0.06*	0.34±0.02**

- Value were expressed as Mean± SEM for 6 mice in each group. Significance was determined by One-Way ANNOVA followed by Dunnett's multiple comparison tests. *: $p<0.01$ when compared with vehicle control group, **: $p<0.01$ when compared with Positive control group.



Graph 3.3: Graphical representation of Antioxidant enzyme level (SOD & GSH).



Graph 3.4: Graphical representation of Antioxidant enzyme level (TBARS).

In biochemical estimations, brain level of antioxidant enzymes (Superoxide scavenging activity (SOD), Glutathione estimation (GSH) were found to increases significantly ($p<0.05$) in experimental drug group, Standard drug group and vehicle control group as compared to Disease control group and Lipid peroxidation (TBARS) were found to be decreased significantly ($p<0.05$) in experimental drug group, Standard drug group and vehicle control group as compared to Disease control group.

3.5.2 Reserpine induced behavioural change in mice

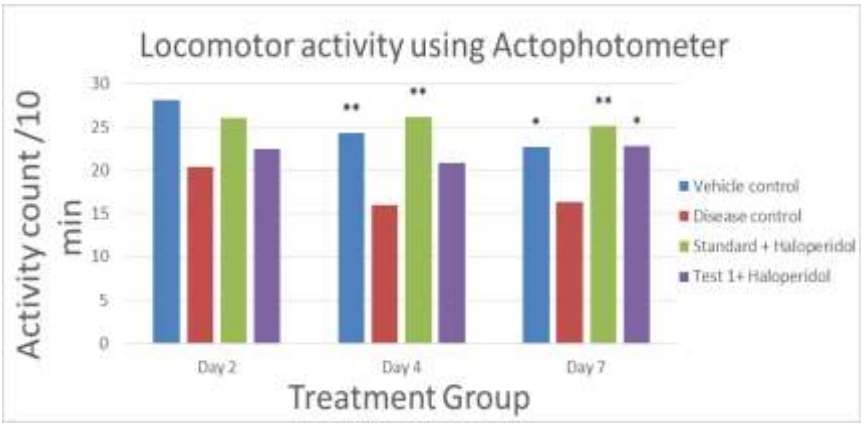
- Behavioural change was caused with reserpine will be administered i.pto mice daily for a period of 5 consecutive days to generate acute DA depletion and tested for behavioural parameter for 7 days as follows:

3.5.2.1 Locomotor activity using actophotometer

Table 3.10: Locomotor activity using actophotometer.

Days	Vehicle control	Disease control	Standard + Haloperidol	Test 1+ Haloperidol
Day 2	28.17±2.88	20.33±1.58	26.00±2.71	22.5±1.26
Day 4	24.33±1.61**	16.00±1.21	26.17±1.62**	20.83±1.49
Day 7	22.67±2.17*	16.33±1.31	25.17±1.25**	22.83±1.56*

- Value were expressed as Mean±SEM for 6 mice in each group. Value were expressed as Mean±SEM for 6 mice in each group. Significance was determined by One-Way ANNOVA followed by Dunnett's multiple comparison tests. *: $p<0.05$ when compared with vehicle control group, ** : $p<0.01$ when compared with Disease control group.



Graph 3.5: Graphical representation of locomotor activity using Rota Rod apparatus.

In case of behavioural change studies in experiments drug group, Standard drug group and vehicle control group were found to be significantly different ($p<0.05$) from disease control group.

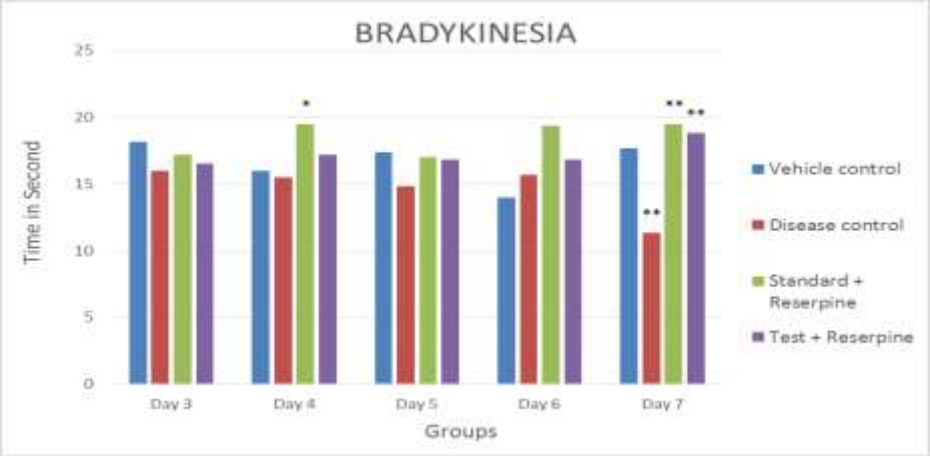
3.5.2.2 Bradykinesia

Table 3.11: Bradykinesia.

Days	Vehicle control	Disease control	Standard + Reserpine	Test + Reserpine
Day 3	18.17±0.70	16.00±1.15	17.17±1.53	16.50±1.43
Day 4	16.00±1.29	15.50±0.76	19.50±1.02*	17.17±1.14
Day 5	17.33±1.28	14.83±1.35	17.00±1.06	16.83±1.01
Day 6	14.00±1.03	15.67±0.99	19.33±0.76	16.83±0.65
Day 7	17.67±1.56	11.33±1.12**	19.50±0.76**	18.83±0.60**

- Value were expressed as Mean±SEM for 6 mice in each group. Value were expressed as Mean±SEM for 6 mice in each group. Significance was determined by One-Way

ANNOVA followed by Dunnett's multiple comparison tests. *: p<0.01 when compared with vehicle control group, ** : p<0.01 when compared with Positive control group.



Graph 3.6: Graphical representation of bradykinesia.

There was a significant decrease in bradykinesia in disease control group as compared to vehicle group. There was a significant increase in bradykinesia in standard group and test group as compared to disease control group. This show that IGA treated group (Test Group) withstand the hold and has a grip on bar for longer duration than other group. Thus it indicate Test drug ha ability to treat muscles weakness or rigidity like Parkinson symptoms.

4. DISCUSSION

Parkinson's is seen as a neurodegenerative disease with a sequence of progression.

It first affects the dorsal motor nucleus of the vagus nerveand the olfactory bulbs and nucleus, t hen the locus coeruleus, and eventually the substantia nigra.

In this study we assayed to evaluate the predictive validity of RES regimen in mice submitted to a set of procedure as a model of motor PD association. Haloperidol induced catalepsy model was used to study behavioural change in mice and accompanied by assessment of biological parameter such as super oxide scavenging activity, Lipid peroxidation and Glutathione activity exhibiting the protective and antioxidant ction of test drug.

Acute toxicity of IGA did not show any toxic or deleterious effect upto 2000 mg/kg oral dose indicating low toxicity of IGA at high dose. As the mice were administered upto maximal possible dose, the thereby suggesting non-toxic nature of IGA fill the end of experimental procedure.

In the present study, the invitro antioxidant havinh potential of Isolated Gallic Acid was evaluated with the help of invitro Reduction power determination of a compound is related to its transferability of electron and may serve as a significant indicator of its potential antioxidant activity. In metal chelation assay, the antioxidant activity present in plant extract forms a coordinate complex with metal ion (chelating activity) and stop the transfer of electron. Thus, Oxygen reaction is arrested and no free radicles are produced.

In the present study, we have focused upon exploring the potential of Isolated Gallic Acid (IGA-100mg/kg & 500mg/kg) for the anti-Parkinsonian activity. Parkinson like effect was induced in mice Haloperidol 2mg/kg in Haloperidol induced catalepsy and Reserpine 5mg/kg in Reserpine induced behavioural change in mice. Trihexyphenidyl Hydrochloride, the establish anti-cholinergic agent was used as a standard in the present study.

In the present study involving mice, in haloperidol treatment group decrease in the level of Dopamine then vehicle control was observed. This decrease in the level of dopamine is responsible for catalepsy and other motor defects. IGA treated group restored the dopamine level dose dependently in haloperidol and reserpine treated groups. Dopamine deficiency in the brain is the major biochemical deficit in PD. Chronic administration of haloperidol for a period of 14 days in mice resulted in decrease in Rotarod task also there are decrease counts in Actophotometer activity cage in Haloperidol treated group as compared to IGA (500mg/kg) treated group. IGA dose dependent increased Rotarod (time for grip on rod at 20) task.

In the present study involving mice, in Reserpine treatment group decrease in the level of dopamine then vehicle control was observed. This decrease in the level of dopamine is responsible for behavioural change in mice. IGA (500mg/kg) treated group restored the dopamine level dose dependent in reserpine treated group. Mice were treated intraperitoneally with reserpine for five consecutive days at a concentration of 5mg/kg to then apply several motor tests. Several PD animal models have tried to resemble particular neuroanatomical, neurochemical, or neurobehavioral abnormalities found in humans; however, only a few have addressed the whole constellation of symptoms. Since its first total synthesis in 1953 by Woodward, reserpine (RES) has become an invaluable agent to reproduce Parkinsonism experimentally. The ubiquitous action of the drug on several amines (both centrally and peripherally) does not emulate the underlying pathology of PD, although it is a useful model to study behavioral and motor symptoms generated by DA depletion. The change in neurochemical balance generated by RES are associated with behavioral deficits

predominantly in motor activity. Studying the behavioral deficits in animal models of PD is of particular interest in order to study the connection between DA depletion and the corresponding behavioral alteration; to then be able to test new therapeutics treatment based on the construct and face validity comprehension of the model. It was found that the administration of RES (Reserpine) to mice induces a reduction in the spontaneous locomotor activity. RES acts at a level of intraneuronal storage vesicles of monoamines (DA, noradrenaline and serotonin) through magnesium and ATP dependent mechanism with the consequent depletion of these monoamines in nerve terminals. The doses administration in the present study were effective in inducing a significant reduction in monoamines level in the CNS of mice. The appearance of bradykinesia, tremor and muscular rigidity induced by RES administration provides a useful animal model of Parkinsonism. Also in Haloperidol induced catalepsy in mice IGA (500mg/kg) has showed a significant increase in Antioxidant enzyme like Superoxide scavenging activity (SOD), Glutathione estimation (GSH) were found to increases significantly in experimental drug group, Standard drug group and vehicle control group as compared to Disease control group and Lipid peroxidation (TBARS) were found to be decreased significantly in experimental drug group, Standard drug group and vehicle control group as compared to Disease control group.

5. CONCLUSION

Neurodegeneration disorder are a heterogeneous group of disease of the nervous system including brain, spinal cord and peripheral nerves. Due to the prevalence, morbidity and mortality of the neurodegenerative disease. They represent significant medical, social and financial binder on the society. These are learnings of basics neuroscience:

1. Understanding fundamental concepts of neurodegenerative disorders.
2. Recognition of specific morphological features of each major disease and their general correlation to disease manifestation.
3. Developing and understanding of genetics, treatment option and clinical feature of each individual disease.

The present study thus, provides sufficients evidence that IGA; an antioxidant of natural origin medicinal plants can be used as an effective anti-PD drug due to its neuroprotective activity. Treatment with IGA exhibited a protective effect may serve as a significant indicator of its potential antioxidant activity. The antioxidant present in plant extract forms a coordinate complex with metal ion (chelating activity) and inhibit the transfer of electron. Thus, Oxygen

reaction is arrested and no free radicles are produced and also showed marked anti-cataleptic effect in haloperidol induced catalepsy in mice. IGA was effective in normalizing haloperidol and reserpine induced behavioral alterations (catalepsy decreased locomotor activity and increased on rota rod activity of muscles rigidity) and brain level of antioxidant enzymes Superoxide scavenging activity (SOD), Glutathione estimation (GSH) were found to increases significantly and in Lipid peroxidation (TBARS) were found to be decreased significantly. IGA exhibited anti-PD activity in *in vitro and in* vivo studies involving mice as a model. IGA can be employed as an effective anti-PD drug as it shows improvement in dopamine neurotransmission and also prevent neurodegeneration. From the above observation it was concluded that the isolated polyphenolic tannin compound (Gallic acid) may possess the anti-Parkinsonian activity.

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