INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Preliminary Ethanopharmacological Survey Of Medicinal Plants From Raigad District

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ARTICLE INFO

Received: 02 Aug 2024
Accepted: 22 Aug 2024
Published: 12 Sep 2024

Keywords:
Ethnopharmacology, Raigad, Field visit, Parkinson Disease, NMDA, Rotenone

DOI:
10.5281/zenodo.13753233

ABSTRACT

Ethnopharmacology is the interdisciplinary science of medicinal plants utilized by humans. These people-plants (typically) relationships have historically and imminently have produced important medicines integral to modern medicine this research is to document the herbs used by traditional healers from Raigad district for treatment of various aliment. There is need to document this data as numbers of traditional healers are declining. Further it is required to establish the correlation between the reported pharmacological activities of the herbs with its actual traditional use. Through this survey various medicinal plants being used by traditional healers will be documented. Further Through systematic review of this data a plant which has been mentioned to possess Neuroprotective activity will be taken up for preclinical evaluation.. From the systematic evaluation of data obtained through Ethanopharmacological survey it was observed that herbalist are using Ruta graveolens for the treatment of Parkinson's.

In Methodology,Acute toxicity study was performed on Zebrafish and parameters were observed. Rotenone induced Parkinson's disease for 3days & MSG induced neurotoxicity in zebratish for 3 days From the systematic evaluation of data obtained through Ethanopharmacological survey it was observed that herbalist claimed the use Ruta graveolense in Parkinson disease. Plant Rata graveolense have a variety of beneficial effects on nervous disorders and can be a potential candidate to be used for the treatment of PD. it can be concluded that Ruta graveolense possess good neuroprotective activity against neurotoxic model of Parkinson's disease via NMDA antagonistic and antioxidant property.

INTRODUCTION

Ethnopharmacology is the interdisciplinary science of medicinal plants utilized by humans. These people-plant relationships have historically and imminently have produced important medicines integral to modern medicine.[1] Ethnopharmacology integrates aspects of botany, natural products chemistry, pharmacology,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

pharmacognosy, anthropology, medicine, psychology and comparative religious study. The discipline researches human interactions with biologically active plants (and other living things) as medicines, poisons, and intoxicants with a primary focus on indigenous and non- Western cultures. Ethnopharmacology seeks to document plants and animals used by various cultures, and describe their use and preparation. These plants and their preparations are then studied to identify, isolate, and characterize the active compounds responsible for the plant's actions on people. Therefore, the ethnopharmacological approach toward the understanding and appraisal of traditional and herbal medicines is characterized by the inclusions of the social as well as the natural sciences. Since time immemorial, traditional medicinal resources, especially plants, have been collected, prepared and administered within indigenous societies, where this knowledge of plant-based medicines has been handed down verbally from generation to generation. In many countries around the world, plant-based medicines contribute significantly to the primary health care of the population. [2] Recent trend shows a decline in the number of traditional herbal healers in the tribal areas since the younger generation is not interested to continue this tradition. Hence, there is an need to record and preserve all information on plants used by different tribal communities for various purposes before it is completely lost. Tribal herbal healers should also be encouraged by some means so that their knowledge is sustained for future generations. Raigad is a district in the Indian state of Maharashtra. It is located in the Konkan region. The district is bounded by Mumbai Harbour to the northwest, Thane District to the north, Pune District to the east, Ratnagiri district to the south, and the Arabian Sea to the west. Raigad has diverse ethanomedicinal data ,Local population has a rich indigenous knowledge, but is s not adequately documented.[3]The data obtained thus will be analyzed by systematical classification and then applying critical statistical analysis such as Fidelity level, Frequency of citations, Use value, Choice Value. [4] Based on the database created from literature survey and through statistical analysis plants with unique characteristics and the plants whose activity is not yet explored by preclinical studies will be segregated and further preclinical studies will be performed. Willing to contribute to the safeguarding of herbal traditional remedies knowledge and to make it easy to find, to use, and to be more familiarized with PD treatment, the present work will be conducted to highlight the medicinal plants used in the traditional preparation for PD treatment. Used parts, methods of preparation, and route of administration will be investigated. Young-onset parkinson's disease is a condition where an individual under 40 years of age may develop pd. The incidence and prevalence of pd increases with advancing age, being present in 1% of people over the age of 65 years.[5] A progressive neurological disorder associated with a loss of dopamine-generating cells in the brain that results in a complex array of symptoms is called as Parkinson's disease (PD), it is primarily associated with progressive loss of motor control. Parkinson's disease is more common in males as compare to female in most populations. There is no homogenous and large epidemiological data on PD from India. [6] Parkinson's disease is defined clinically by the presence of bradykinesia in combination with at least one more manifestation: muscular rigidity, rest tremor or postural instability. Motor symptoms starts unilaterally, and asymmetry persists throughout the course of the disease. Non-motor symptoms are seen in a large proportion of patients. [7] These non-motor symptoms include sleep disorders [for example, frequent waking, rapid eye movement sleep behavior disorder , and day time somnolence],

hyposmia, disturbance in autonomic function [orthostatic hypotension, urogenital dysfunction, and constipation], cognitive impairment, mood disorders and pain.[8,9] Oxidative stress contributes to the cascade leading to dopamine cell degeneration in Parkinson's disease (PD). However, oxidative stress is intimately linked to other components of the degenerative process, such as mitochondrial dysfunction, excitotoxicity, nitric oxide toxicity and inflammation. Furthermore, impairment of proteasomal function leads to free radical generation and oxidative stress.[10]. the current medications available are associated with some major therapeutic problems such as 1. Decreased control of parkinsonian symptoms 2. Increased involuntary movements 3. Alterations in mentation 4. Increased diurnal fluctuations; "on-off' periods 5. Episodes of a kinetic freezing and "crisis" 6. Increased fatigue and neurasthenia. Current medication is associated with several ADRs and are also unable to reverse the disease hence herbal plants with lesser side effects can be potential candidates to treat various chronic diseases. Though there is advancement in recent modalities of treatment of various disease, many allopathic drugs are yet associated with potential side effects which limits their use. In recent years herbal drug research has gained tremendous popularity due to their favorable safety profile as compared to allopathic drugs. There are examples of potential drug candidates being first identified to possess pharmacological activity which were isolated from herbal extracts and later being introduced in treatment of allopathy. In India herbs are being used for treatment of various ailments for time immemorial. The knowledge of local communities regarding use of plants can be used to find out specific constituent of medicinal plants, which can further be evaluated for their potential efficacy for treatment of various diseases. Ruta graveolense is a rich source of flavanoids, glycosides, alkaloids, terpenes and steroids, phenols, phenolic compound, tannins, saponins, and essential volatile oils. It was used for its antioxidant activity, anticonvulsant, anti-inflammatory, hepatoprotective analgesic, Stimulating, antispasmodic, stomachic, irritant, abortifacient arthritis, anti-epileptic, emmenogogue, rubefacient property ; used externally for sciatica, headache, muscular chest pain, bronchitis.[11]From the systematic evaluation of data obtained through Ethanopharmacological survey it was observed that herbalist are using Ruta graveolens Linn for the treatment of Parkinson's. Plant Ruta graveolens Linn have a variety of beneficial effects on nervous disorders and can be a potential candidate to be used for the treatment of PD.

MATERIALS AND METHODS

Study Area –Raigad District

Raigad district in the state of Maharashtra lies between 17°51′ - 19°80′ N latitude and72°51′ - 73°40′ E longitude. It covers an area of 7162 sq. km.The district is bounded at the west with the aid of arabian sea, thane district lies to the north, pune district to the east, ratnagiri district to the south while satara district shares a boundary in south-east. Raigad district bureaucracy an crucial element of the traditional konkan vicinity. [12] There are several hill levels stretching out from the foremost sahyadri range which runs nearly parallel to the west coast. On the north-east boundary of the district, the sahyadri variety is crossed by means of numerous passes or ghats. Interesting wood land plants is reflected due to various physiological, geological, edaphic and climatic conditions.

Method of study and data collection

Information on the use of medicinal plants was gathered through field surveys and data is recorded using following methods.

field visits: Personal visits to the area selected for the study.

Personal Interviews: Personal interviews to be conducted with the knowledgeable persons, viz. Traditional healers, herbalist, Vaidya's, Sirahas, Guniyas, Birth attendants etc. Information's were collected by asking questions in interview session in their own dialect. The information was collected through conducting interviews, discussion and field observation of the study area using semi-structured questionnaire.

Field Visit: Raigad has diverse ethanomedicinal data, Local population has a rich indigenous knowledge, but is s not adequately documented. So Ethanopharmacological survey has been conducted in Karnal, Phansad and Bhimashankar area of Raigad district. No in bracket signifies number of traditional healers we interacted. [13,14,15,16,17,18]

Figure 1: Evaluation of activity

Ethnopharmacological Survey

Raigad has diverse ethanomedicinal data. Local population has a rich indigenous knowledge, but is s not adequately documented. So Ethanopharmacological survey has been conducted in Karnal, Phansad and Bhimashankar area of Raigad district.To perform this data collection the help of traditional medical practitioners was needed. They are faith healers with a great competence to practice traditional medicine. They were identified after inquiries taken next to the elders of the tribe but equally on the base of their personal notoriety.The information was collected through conducting interviews, discussion and field observation of the study area using semi-structured questionnaire. Traditional medicinal practitioners were questioned according to the parameters and data sheet was filled.[19,20]

Figure 2. Field data sheet

Selection Of Drug

Through statistical analysis and through literature review of the obtained ethnopharmacological data a plant was selected for further pharmacological evaluation. The data collected was divided into three main class of drugs through statistical analysis and literature review. The plant whose activity against a particular disease has not been previously explored and will show maximum potential against the particular disease will be further used for its pharmacological evaluation. Statistical analysis of the obtained data was done using two statistical tools.[21,22]

Figure 3: Two Statistical Tools

Collection And Authentication:

Figure. 4: Authentication of study plant

Dried Leaves powder of Ruta graveolens was procured from D G. Sangrah, Andheri. The Dried Leaves powder of Ruta graveolens was authenticated by Dr. Harshad Pandit, HOD of Botany, Andheri. The authentication number of the plant is ssc p 12193690.(Specimen number ssc p 12193690.) The dried powder was brought to Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar-03. for further research work.

Physico-Chemical Analysis Of Plant Powder:

Physicochemical investigation [23]

Total Ash value-

Weigh and ignite flat, thin, porcelain dish or a tarred silica crucible. Weigh about 2 gm of the powdered drug into the crucible. Heat with a burner, using a flame about 2 cm high and supporting the crucible. About 7 cm above the flame heat till vapors almost cease to be evolved, then lower the dish and heat more strongly until all the carbon is burnt off. Cool in desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air-dried sample of the crude drug. (Divide this ash into 2 approximately equal parts)

Calculation Weight of the empty dish = X, Weight of the drug taken = Y

Weight of dish + ash (after complete incineration) = Z Weight of ash = (Z-X) gm

Y gm of crude drug gives (Z-X) gm of ash.

Therefore 100 gm of the crude drug gives 100/Y (Z-X) gm of the ash. Total ash value of sample= 100(Z-X) /Y %.

Acid insoluble ash value-

Take first part with 25ml of dilute HCl, wash the ash from the dish used for total ash into 100 ml of beaker. Place wire gauze over a Bunsen burner and boil 5 mins. Filter through an ashless filter paper wash the residue Keep it containing residue in a nickel crucible. Get the ash (insoluble acid ash value).

Calculation Weight of the residue = X gm

Y gm of the air-dried drug gives = X gm of acid insoluble ash.

Therefore 100 gm of the air-dried drug give = 100 × X/ Y gm of acid insoluble ash. Acid insoluble ash value of the sample = 100×X / Y %.

Note: Acid insoluble ash value of a crude drug is always less than total ash value of the same drug.

Water soluble ash value-

Take second part with 25ml of D/W. wash the ash from the dish used for total ash into 100 ml of beaker. Place wire gauze over a Bunsen burner and boil 5 mins. Filter through an ashless filter paper wash the residue Keep it containing residue in a nickel crucible. Get the ash (insoluble acid ash value).

Calculation Weight of the residue = X gm

Y gm of the air-dried drug gives = X gm of water-soluble ash.

Therefore 100 gm of the air-dried drug give = 100 × X/ Y gm of water-soluble ash.

Water soluble ash value of the sample = 100×X / Y

Loss Of Drying

Weigh 2gm of powder drug into porcelain dish. Dry it in 100ºCelsius into hot air oven. Cool it. The loss in weight is usually recorded as moisture.

Extraction Of Plant:

Materials:

Ruta graveolense - leaves powder

Ethanol (PubChem CID: 702)

Soxhlet extraction apparatus

Rotary evaporator with vaccum pump

Hot air oven

Procedure:.[24]

Figure 5: Extraction Of Plant

Preliminary Phytochemical Analysis:[25]

Preliminary phytochemical test for aqueous extract of Ruta graveolense was carried out to identify phytoconstituents present in AERG.

Test for glycosides: 0.2 ml of Fehling's solutions A and B was mixed with 5 ml of the filtrate until it became alkaline (tested with litmus paper). A brick-red colorations on heating showed a positive result.

Test for steroids:

Salkowski reaction: - To 2ml aqueous extract, add 2ml chloroform (Pub chem CID: 6212) and 2ml conc. H2SO4 (Pub chem CID: 1118), shake well, the chloroform layer will show red and acid layer will show yellow fluorescence

Test for alkaloids:

Dragendroff's test: To 2-3ml filtrate, add few drops of dragendroff's reagent, orange brown colour will be formed.

Test for Triterpenoid:

Salkowski reaction: -To 2ml aqueous extract, add 2ml chloroform and 2ml conc. H2SO4, shake well, the chloroform layer will show red and acid layer will show greenish yellow fluorescence.

Test for Saponin:

Liberman buchard test: -To the extract solution a mixture of acetic anhydride and concentrate sulphuric acid (19:1) was dissolved in suitable anhydride solvent; development of violet purple color, indicting the presence of steroidal-saponins.

Test for flavonoids:

Alkali reagent test-To the test solution add few drops of sodium hydroxide (Pub chem CID: 14798) solution, formation of an intense yellow colour which turns to colourless by the addition of few drops of dilute acetic acid indicate the presence of flavonoids.

Test for carbohydrates:

Molisch's test: A small quantity of AERG was dissolved in distilled water. In 2-3ml aqueous extract, add Molisch's reagent and conc. H2SO4 from the sides of the test tube, violet ring will be obtained.

Test for volatile oil:

Solubility test: To 2ml aqueous extract, add 90% alcohol, volatile oils are soluble in 90% alcohol.

Test for tannins: A small quantity of extract was diluted with water and tested with following reagent.

Lead acetate solution (Pub chem CID-9317) (10%) - Gives white colored precipitate for tannins compounds.

Dilute ferric chloride (FeCl3) solution (5%)-. An appearance of violet colour indicates the presence of tannins.

Test for phenolic compound

Lead acetate solution (Pub chem CID-9317) (10%)- Gives buff colored precipitate for phenolic compounds.

Confirmatory test for phenolic acids

Dilute ferric chloride (FeCl3) solution (5%)- Intense blue, green, red or purple colour indicates the presence of phenolic compounds.

pharmacological evaluation:

In- Vivo Study (Chemicals And Animal Details):

Standard Drug: Selligiline (Pub chem CID: 26757)

Disease inducing drug: Monosodium glutamate (Pub chem CID: 23672308) - (Himedia Laboratories) Rotenone -(Pub chem CID: 6758) (Sigma Aldrich)

Animal details: Zebra fish

Species: Danio rerio (Adult wild type)

Age/Weight/Size: 4-6 months old; 0.5-1g

Gender: Both sex

Number to be used Zebra fish: 116

Number of Days each animal: 3 months approximately will be housed

Proposed source of animals: Vikrant Aqua Culture, Bandra.

Animal House Conditions:

The adult wild type zebrafish, 0.5-1g were procured from Vikrant aqua culture, Bandra. The rats were brought to animal house. and zebrafish were brought to zebrafish facility, M. Pharm Pharmacology laboratory of Dr. L.H. Hiranandani college of pharmacy, opposite to Ulhasnagar railway station, CHM campus, Ulhasnagar-03. Zebrafish were acclimatized in Zebrafish facility located in M. Pharm Pharmacology laboratory under standard husbandry conditions, i.e., temperature of 28 ± 5ºC, optimum pH of 7-8, conductivity of 0.25ppt-0.75ppt and 14:10 hr. light/dark cycle. 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. PZEB/IAEC/2019/17 (for zebrafish).

Acute Toxicity Study [26]:

Limit test: Limit test will be performed on test compound according to OECD 203

The Fishes will be exposed to the test compound at a maximum dose of 100mg/l for 30mins daily for an over period of 96 hours. Mortalities are recorded at 24, 48, 72 & 96 hours. If all the fishes survive, 1/10th and 1/20th dose is been selected for actual study.

Limit test Parameters

1.Number and duration of freezing episodes
2. Complete cataleptic time
3.Time spent near the bottom of tank
4. Latency to travel from one point to another
5.Total distance moved and swimming velocity

Table 1: Acute Toxicity Study

In-Vivo Model

Rotenone induced Parkinson's disease in Zebrafish: [27]

Purpose and Rationale:

Rotenone: Rotenone generates an experimental animal model of Parkinson's disease (PD) that mimics and elicits PD-like symptoms, such as muscular rigidity (catalepsy), bradykinesia, postural instability, unsteady gait and sleep disturbances. Due to its high lipophilicity, rotenone can readily cross the blood–brain barrier and enter all cells without being dependent on a specific transporter. Application of low doses of rotenone in vitro and in vivo have been shown to affect many of the mechanisms involved in the pathogenesis of PD, such as altered calcium signaling, induction of oxidative stress and apoptosis, loss of tyrosine hydroxlase, proteasomal dysfunction, nigral iron accumulation and the formation of fibrillar cytoplasmic inclusions that contain ubiquitin and a-synuclein.

Selegiline (Standard): Overactivation of NMDA receptors exerts excitotoxicity. Over activation of NMDA along with other glutamate/glycine receptors disturb the calcium homeostasis, which is the key mediator of glutamate-induced excitotoxic neuronal damage. Selegiline, diminishes potentiation of the NMDA receptor by the polyamine binding site. Hence in the present study, we will use selegiline against Rotenone-induced neurotoxicity.

Objective:

The main objective of this study was to evaluate the neuroprotective effect of EERG in Rotenone induced Parkinson's disease in Zebrafish.

Parameters studied are as follows: Behavioral parameter evaluation:

1.Number and duration of freezing episodes
2. Complete cataleptic time
3.Time spent near the bottom of tank
4. Latency to travel from one point to another
5.Total distance moved and swimming velocity

Study design:

Table.2: Study design- Rotenone induced Parkinson's disease in Zebrafish

Preparation of drug:

The dose of EERG i.e., 5µg/ml and 10µg/ml were prepared by suspending EERG in distilled water. Dose of Selegiline 0.03µg/ml was prepared by suspending it in distilled water. Vehicle containing 10% DMSO in water was used as control. All the solutions were freshly prepared every day prior to the exposure.

Induction of Parkinson's disease: Induction of Parkinson's disease was done by the exposure of fishes to Rotenone at a dose of 3pg/ml in water once daily for 72 hours for 30 min.

Dosing schedule: All fishes were exposed to Rotenone at a dose of 3pg/ml for 30 min. After inducing Parkinson's disease fishes were transferred to fresh aerated water for 15 min. Later fishes were exposed to EERG or Standard drug according to the groups for 30min and then in fresh aerated water for 15min. This schedule was followed for 3 days and on the 3rd day, fishes were placed in experimentation tank.

Experimentation tank was filled up to 5L fresh aerated tank water. The tank was marked with horizontal and vertical lines for easy evaluation of behavioural parameters. All behavioural evaluation was done using camera.

Behavioral parameters:

Figure 6. Behavioral Parameters

Observation:

Total distance moved and swimming velocity: There is decreased in total distance moved and swimming velocity in Parkinson's disease.

Time spent near bottom of the tank

When they are transferred to a new environment they initially spend more time near the bottom of the tank and after sometime they come towards surface, this is attributed towards their exploratory behavior and most often due to their anxiety. Thus, the time spent near the bottom of the tank gives idea about the extent of anxiety of fish.

Latency to travel from one fixed point to another

The time taken by the fish to travel from first vertical end to last will be calculated at different time. This gives an idea about the speed of fish under examination. Catalepsy diminishes the speed of fishes due to rigidity of muscular movements.

Complete cataleptic time

Time for which the fish doesn't move at all i.e. the time for which fish remains completely cataleptic during examination period at various time intervals will be measured.

Number and duration of freezing episodes

Total absence of movement will be measured.

Monosodium glutamate (MSG) induced Neurotoxicity in zebrafish [28]

Purpose and Rationale:

Monosodium glutamate (Inducing agent): Monosodium glutamate (MSG) is the sodium salt of the glutamic acid, a nonessential amino acid. MSG is known to affect dopaminergic neurons causing neurotoxicity. It acts through the activation of both ionotropic and metabotropic glutamate receptor (iGluR and mGluR) found in the central nervous system (CNS). Hyperactivation of these receptors has been reported to produce excitotoxicity and neuronal death. It is also known that MSG or sodium salt of glutamate exerts excitotoxicity by over activation of glutamate receptors namely N- methyl-D-aspartate (NMDA) receptor.

Selegiline (Standard): Overactivation of NMDA receptors by the MSG or sodium salt of glutamate exerts excitotoxicity. Over activation of NMDA along with other glutamate/glycine receptors disturb the calcium homeostasis, which is the key mediator of glutamate-induced excitotoxic neuronal damage. Selegiline, diminishes potentiation of the NMDA receptor by the polyamine binding site. Hence in the present study, we will use selegiline against MSG-induced neurotoxicity.

Objective:

The main objective of this study was to evaluate the neuroprotective effect of EERG in MSG induced neurotoxicity in Zebrafish.

Parameters studied are as follows:

Behavioral parameter evaluation:

1.Number and duration of freezing episodes
2. Complete cataleptic time
3.Time spent near the bottom of tank
4. Latency to travel from one point to another
5 Total distance moved and swimming velocity

Study design:

Table 3: Study design: Monosodium glutamate (MSG) induced Neurotoxicity in zebrafish Preparation of drug The dose of EERG i.e., 5µg/ml and 10µg/ml were prepared by suspending EERG in distilled water. Dose of Selegiline 0.03µg/ml was prepared by suspending it in distilled water. Vehicle containing 10% DMSO in water was used as control. All the solutions were freshly prepared every day prior to the exposure.

Induction of neurotoxicity:

Induction of neurotoxicity was done by the exposure of fishes to MSG at a dose of 30ug/ml in water once daily 30 mins for 96 hours.

Dosing schedule:

All fishes were exposed to MSG at a dose of 30ug/ml for 30 min. [29] After inducing neurotoxicity fishes were transferred to fresh aerated water for 15 min. Later fishes were exposed to EERG or Standard drug according to the groups for 30min and then in fresh aerated water for 15min. This schedule was followed for 4 days and on the 4th day, fishes were placed in experimentation tank. Experimentation tank was filled up to 5L fresh aerated tank water. The tank was marked with horizontal and vertical lines for easy evaluation of behavioural parameters. All behavioural evaluation was done using camera.

Behavioral parameters:

Observation:

Total distance moved and swimming velocity: There is decreased in total distance moved and swimming velocity in Parkinson's disease

Time spent near bottom of the tank

When they are transferred to a new environment they initially spend more time near the bottom of the tank and after sometime they come towards surface, this is attributed towards their exploratory behavior and most often due to their anxiety. Thus, the time spent near the bottom of the tank gives idea about the extent of anxiety of fish.

Latency to travel from one fixed point to another: The time taken by the fish to travel from first vertical end to last will be calculated at different time. This gives an idea about the speed of fish under examination. Catalepsy diminishes the speed of fishes due to rigidity of muscular movements.

Complete cataleptic time

Time for which the fish doesn't move at all i.e. the time for which fish remains completely cataleptic during examination period at various time intervals will be measured.

Number and duration of freezing episodes: Total absence of movement will be measured.

Statistical analysis:

The results of Neuroprotective activity were expressed as Mean ± SEM from 8 zebrafish in each group. Results were statistically analyzed using one–way ANOVA followed by Tukey's multiple comparison tests; all groups were compared with disease control group and $P<0.05$, $P<0.01$, $P<0.001$ was considered significant.

RESULTS AND DISCUSSION:

Ethnopharmacological Survey:

The present study was performed to carry out the phytochemical and pharmacological evaluation of aqueous extract of Ruta graveolense . The results are presented in tables and graph format. The result displayed include physicochemical, phytochemical evaluation and confirmatory test for AERG, acute toxicity study for AERG, in vitro antioxidant activity and neuroprotective activity of AERG and pharmacological effect of AERG on MSG induced excitotoxicity and rotenone induced Parkinson's disease.

Data collected through ethnopharmacological survey included plant species with their vernacular names, uses and mode of preparation In the present study, 150 plant species were reported after undertaking the survey and having discussion with local traditional healers of different age groups. It was found that dominated medicinal plants of this area are major source of primary health care. Traditional healers are using these plants to cure diseases related to stomach pain, fever, jaundice, dysentery, skin diseases, snake bites, neurological disorders, wounds, cold & cough diabetes, cancer, asthma and worms. The plant material is employed in the form of decoctions, extracts, pastes, fermented decoctions, juice & Powder some times in combination with other parts of same or different plants other substances, such as sugar candy, curd, honey, hair oil, milk and turmeric powder, are also used in various preparations.

Through this survey, the availability and presence of many medicinal plants have been investigated and verified. We suggested that these plants can be used as drugs by pharmacologically unexplored areas of India, which may be utilized for the better human health. As a result of the present study, we recommend giving priority for further phytochemical investigation to plants that scored highest Use value and choice value, as such values could be considered as good indicator of prospective plants for discovering new drugs.

Ruta graveolens was found to be of interest for additional research since it has not been mentioned before in any folk or evidence-based medicines for the treatment of PD and its pharmacological effects were not reported.

Selection Of Drug

The data collected was divided into three main class of drugs ie. Plants cited for Antitumor and Antimicrobial activity and plants acting on CNS related disorder through statistical analysis. The plant whose activity against a particular disease has not been previously explored and will show maximum potential against the particular disease will be further used for its pharmacological evaluation.

Plants used in antimicrobial disorder

Table no 4 : Plants used in Antimicrobial disorder

From statistical analysis Amorpophalus commutatus and Cassia tora showed maximum potential for further preclinical studies antimicrobial disorder . But through literature review it was found that antimicrobial activity of Amorpophalus commutatus and Cassia tora has been explored preclinically .

Plants used in Anticancer

Table no 5: - Plants used in Anticancer

From statistical analysis Moringa pterdospcrma showed maximum potential for further preclinical studies against anticancer disorder. But through literature review it was found that anticancer activity of Moringa pterdospcrma has been explored preclinically.

Plants used in CNS related disorder

Table no 6 : - Plants used in CNS related disorder

Selection of Plant:

Through Statistical analysis a herb with high frequency of citation and yet have not been explored by preclinical testing has been chosen for evaluation of activity. Preferably an herb with mentioned antimicrobial, anticancer, CNS related disorder have been chosen.

Figure 1: Evaluation of activity

From statistical analysis Ruta graveolense and Alpinia galanga showed maximum potential for further preclinical studies against CNS disorder. On the basis of literature review, Ruta graveolense was further selected to determine its efficacy preclinically. The data obtained thus will be analyzed by systematical classification and then applying critical statistical analysis such as Fidelity level, Frequency of citations, Use value, Choice Value. Based on the database created from literature survey and through statistical analysis plants with unique characteristics and the plants whose activity is not yet explored by preclinical studies will be Segregated and further preclinical studies will be performed. Willing to contribute to the safeguarding of herbal traditional remedies knowledge and to make it easy to find, to use, and to be more familiarized with PD treatment, the present work will be conducted to highlight the medicinal plants used in the traditional preparation for PD treatment. Used parts, methods of preparation, and route of administration was investigated.

Physicochemical Evaluation:

The leaves powder of Ruta graveolense (crude powder) was subjected to physicochemical parameter evaluation such as total ash value, acid insoluble ash value , water soluble &, loss on drying was performed. Result was shown in table no.7.

Table No. 7: Physicochemical Evaluation

Plant Extraction:

The plant powder of Ruta graveolense was subjected to soxhlet extraction method. The % yield, colour, solubility and consistency of EERG was shown in table no. 8.

Table no 8 : % yield, color, solubility and consistency of EERG

Figure 8. Herbal extract

Phytochemical Analysis:

Preliminary phytochemical analysis of AESD

Table no. 9: Preliminary phytochemical analysis of AESD

As per above phytochemical analysis, EERG extract showed presence of Glycosides, Alkaloids, Steroids, Triterpenoid, Volatile oil,carbohydrates ,Saponin ,Flavanoids ,Tannins,Phenolic compound.

Confirmatory Test For Phenolic Compound (Plant Origin Compound):

Table no. 10 Confirmatory test of Phenolic compound

Figure. 9: Phytochemical analysis and Confirmatory test of AERG

Acute Toxicity Study:

The following condition were maintained during acute toxicity study.

In Zebrafish:

0 = Mortality Standard Values:

pH= 7-8(Optimum)

Temperature= 28±5ºC

Conductivity= 0.25-0.75 ppt

Table no. 11: Observation table for acute toxicity study of EERG in zebrafish

Number of freezing episodes

Table no 12. Number of freezing episodes

Duration of freezing episode

Table no 13. Duration of freezing episode

Complete Cataleptic time

Table no.14 Complete Cataleptic time

Time spent near the bottom of the tank

Table no. 15. Time spent near the bottom of the tank

Latency to travel from one point to another

Table no. 16. Latency to travel from one point to another

Total distance moved

Table no.17 Total distance moved

Swimming velocity

Table no. 18. Swimming velocity

From the above observation table, it was concluded that EERG showed no sign of toxicity at the dose 100mg/L in adult zebrafish. Hence, 1/10th (10µg/ml) and 1/20th (5 µg/ml) dose was selected for main study.

In-Vivo Study

Rotenone induced Parkinson's disease in zebrafish

Behavioural Parameters Observed

Latency to travel from one point to another

Table no 19: Latency to travel from one point to another

Figure 10: Latency to travel from one point to another

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's ** p≤0.01, *** p≤0.001, when compared with disease group.

Latency to travel from one point to another decreased in group administered with EERG as compared to disease control. Hence it can be concluded that muscle rigidity –a symptom of PD was improved by the EERG.

Complete Cataleptic Time

Table no 20: Complete cataleptic time

Figure 11: Complete cataleptic time

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was, ** p≤0.01, when compared with disease group. Complete cataleptic time was absent in group administered with EERG as compared to disease control. Hence it can be concluded that Complete immobility –a symptom of PD was improved by the EERG. EERG* -Ethanolic extract of Ruta graveolens

Time Spent Near The Bottom Of The Tank

Table no.21: Time spent near the bottom of the tank

Figure:12 Time spent near the bottom of the tank.

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.05, *** p≤0.05, when compared with disease group. Disease control group was observed to spent more time near the bottom of the tank than the group administered with EERG. Hence it can be concluded that anxiety –a symptom of PD was improved by the EERG. EERG* -Ethanolic extract of Ruta graveolens.

3.8.1.1.4 Number of freezing episodes

Table no .22: Number of freezing episodes

Figure .13: Number of freezing episodes

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was ** p≤0.01, when compared with disease group. EERG* -Ethanolic extract of Ruta graveolens Number of freezing episodes were absent in group administered with EERG as compared to disease control. Hence it can be concluded that Dyskinesia –a symptom of PD was improved by the EERG.

Duration of freezing episodes

Table no 23 - Duration of freezing episode

Figure .14: Duration of freezing episode

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was *** p≤0.05, when compared with disease group.

Duration of freezing episodes were absent in group administered with EERG as compared to disease control. Hence it can be concluded that Dyskinesia –a symptom of PD was improved by the EERG.

Total distance moved

Table no 24: Total distance moved

Figure .15: Total distance moved

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.05, *** p≤0.05, when compared with disease group. Total distance moved by zebrafish was increased in group administered with EERG as compared to disease control. Hence it can be concluded that bradykinesia –a symptom of PD was improved by the EERG.

Total swimming velocity

Table no 25 : Total swimming velocity

Figure .16: Total swimming velocity

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with disease group.Swimming velocity of zebrafish was increased in group administered with EERG as compared to disease control. Hence it can be concluded that bradykinesia –a symptom of PD was improved by the EERG.

Model 2: MSG Induced Neurotoxicity In Zebrafish

Table no.26. MSG induced neurotoxicity in zebrafish

Fate of animals: Sacrification

Latency to travel from one point to another

Table no 27: Latency to travel from one point to another

Figure 17: Latency to travel from one point to another values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's ** p≤0.01, *** p≤0.001, when compared with disease group. Latency to travel from one point to another decreased in group administered with EERG as compared to disease control. Hence it can be concluded that muscle rigidity –a symptom of PD was improved by the EERG.

Complete Cataleptic Time

Table no 28: Complete Cataleptic Time

Figure 18: Complete cataleptic time

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was, ** p≤0.01, when compared with disease group Complete cataleptic time was absent in group administered with EERG as compared to disease control. Hence it can be concluded that Complete immobility –a symptom of PD was improved by the EERG. EERG* -Ethanolic extract of Ruta graveolens.

Time spent near the bottom of the tank

Table no 29: Time spent near the bottom of the tank Figure 19: Time spent near the bottom of the tank Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was

determined by One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.05, *** p≤0.05, when compared with disease group. Disease control group was observed to spent more time near the bottom of the tank than the group administered with EERG. Hence it can be concluded that anxiety –a symptom of PD was improved by the EERG.

Duration of freezing episodes

Table no 30: Duration of freezing episodes

Figure 20: Duration of freezing episodes

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was *** p≤0.05, when compared with disease group. Duration of freezing episodes were absent in group administered with EERG as compared to disease control. Hence it can be concluded that Dyskinesia –a symptom of PD was improved by the EERG. EERG* -Ethanolic extract of Ruta graveolens.

Number of freezing episodes

Table no 31: Number of freezing episodes

Figure 21: Number of freezing episodes

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance determined was ** p≤0.01, when compared with disease group. Number of freezing episodes were absent in group administered with EERG as compared to disease control. Hence it can be concluded that Dyskinesia –a symptom of PD was improved by the EERG. EERG* -Ethanolic extract of Ruta graveolens

Total distance moved

Table no 32: Total distance moved

Figure 22: Total distance moved

Values were expressed as Mean ± SEM for 8 zebrafish in each group.Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.05, *** p≤0.05, when compared with disease group. Total distance moved by zebrafish was increased in group administered with EERG as compared to disease control. Hence it can be concluded that bradykinesia –a symptom of PD was improved by the EERG.

Total swimming velocity

Table no 33: Total swimming velocity

Figure 23: Total swimming velocity

Values were expressed as Mean ± SEM for 8 zebrafish in each group. Significance was determined by One way ANOVA followed by Tukey's multiple comparison test's *p≤0.05, ** p≤0.01, *** p≤0.001, when compared with disease group. Swimming velocity of zebrafish was increased in group administered with EERG as compared to disease control. Hence it can be concluded that bradykinesia –a symptom of PD was improved by the EERG.

DISCUSSION:

Ethnopharmacology is the interdisciplinary science of medicinal plants utilized by humans. These people-plants (typically) relationships have historically and imminently have produced important medicines integral to modern medicine this research is to document the herbs used by traditional healers from Raigad district for treatment of various aliment. There is need to document this data as numbers of traditional healers are declining. Further it is required to establish the correlation between the reported pharmacological activities of the herbs with its actual traditional use. Through this survey various medicinal plants being used by traditional healers will be demerited. Further Through systematic review of this data a plant which has been mentioned to possess Neuroprotective activity will be taken up for preclinical evaluation. Ruta graveolense is a rich source of flavonoids glycosides alkaloids, terpenes and steroids, phenols. Phenolic compound tannins, saponins, and essential volatile oil.it was used for its antioxidant activity, anticonvulsant, anti-inflammatory, analgesic Stimulating From the systematic evaluation of data obtained through

Ethanopharmacological survey in was observed that herbalist are beneficial effects on nervous disorders and can be a potential candidate to be used for the treatment of PD. Acute toxicity study was performed on Zebrafish and parameters were observed. Rotenone induced Parkinson's disease for 3days & MSG induced neurotoxicity in zebrafish for 3 days was performed which resulted in alterations in behavioral pattern. The present study provides evidence that ethanolic extract of Ruta graveolens has an important potential for improving the symptoms of Parkinson's disease induced by neurotoxicity in zebra fish caused by rotenone and monosodium glutamate. From the systematic evaluation of data obtained through Ethanopharmacological survey in was observed that herbalist claimed the use Ruta graveolens in Parkinson disease. Plant Ruta graveolens have a variety of beneficial effects on nervous disorders and can be a potential candidate to be used for the treatment of PD, it can be concluded that Ruta graveolens, possess good neuroprotective activity against Neurotoxic model of Parkinson's disease via NMDA antagonistic and antioxidant.

CONCLUSION:

In the screening models of PD in Zebra fish, the administration of the ethanolic extract of Ruta graveolens showed a significant relief in symptoms of PD. The above results suggest an improvement in controlling the progression of PD when compared with disease control group; hence it could be of great benefit in symptomatic treatment of PD. The activity may be due its ability to influence the receptor and its antioxidant activity. [58, 59] Also, presence of Phenolic compound must have contributed in its activity. The ethanolic extract of Ruta graveolens showed significant neuroprotective activity against Rotenone induced PD and Monosodium glutamate induced neurotoxicity in zebrafish model and its efficacy can be further determined in higher animal species such as rodents which will further help to statistical compare its neuroprotective activity. Furthermore, the efficacy of plant extracts and their active ingredients in PD models should be further investigated for its molecular mechanism. The medicinal plants obtained through this ethnopharmacological survey are future crucial sources of novel natural products with therapeutic potential.

FUTURE PERSPECTIVES

The ethanolic extract of Ruta graveolense showed significant neuroprotective activity against rotenone induced pd and monosodium glutamate induced neurotoxicity in zebrafish model and its efficacy can be further studied in higher animal species such as rodents, which can further suggest the statistical comparison regarding its neuroprotective activity. Further kinetics studies would help us understand the mechanism of action of the ethanolic extract of leaves of Ruta graveolense. Studies are required to explore the molecular mechanism of neuroprotection of ethanolic extract of leaves of Ruta graveolense.

ACKNOWLEDGEMENT

At this moment of accomplishment, I express my gratitude to my guide, Dr. Priti P. Patel (M.Pharm, Ph.D) Department of pharmacology. Under her guidance I successfully overcame many difficulties and learned a lot. I am immensely pleased to record my profound gratitude and heartful thanks to my guide for her constant support throughout the course of my work. It’s my privilege to work under her guidance. I would like to thank Dr. Paraag Gide, principal, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar-03, for his help, support and for the arrangement of all the necessary facilities made available for the current dissertation work. I am thankfully indebted to HSNCB board and Dr. L.H. Hiranandani College of Pharmacy for providing excellent facilities and working conditions for the research work.

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HOW TO CITE: Sonali Gopale*, Priti Patel, Preliminary Ethanopharmacological Survey Of Medicinal Plants From Raigad District, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 9, 615-645. https://doi.org/10.5281/zenodo.13753233

Group	Dose	No of animals
Test compound	100mg/L	16
Total		16

Table 1: Acute Toxicity Study

Group	Dose	No. of zebrafish
Vehicle	10% DMSO (Pub chem CID: 679)	8
Toxic (Rotenone)	3pg/ml	8
Standard (Selegiline)	0.03µg/ml (Selegiline) + 3pg/ml (Rotenone)	8
Test1(EERG)	5µg/ml (EERG)+3pg/ml (Rotenone)	8
Test2(EERG)	10µg/ml (EERG)+3pg/ml (Rotenone)	8
Total		40

Table.2: Study design- Rotenone induced Parkinson's disease in Zebrafish

Group	Dose	No. of zebrafish
Vehicle	10% DMSO (Pub chem CID: 679)	8
Toxic (MSG)	30µg/ml	8
Standard (Selegiline)	0.03µg/ml (Selegiline)+ 30µg/ml (MSG)	8
Test1(EERG)	5µg/ml (EERG)+3pg/ml (MSG)	8
Test2(EERG)	10µg/ml (EERG)+3pg/ml (MSG)	8
Total		40

Table 3: Study design: Monosodium glutamate (MSG) induced Neurotoxicity in zebrafish

Preparation of drug

Sr. No	Plants	No of citation	Use value	Choice value	Literature review
1	*Bombax ceiba*	4/8	0.5	0.66%	Antibacterial, Antimicrobial
2	*Amorpophalus commutatus*	5/8	0.625	0.83%	Antibacterial, Antimicrobial
3	*Drypetes roxburghi*	2/8	0.25	0.33%	Antimicrobial Anthelmintic
4	*Cassia tora*	5/8	0.625	0.83%	Antibacterial, Antimicrobial
5	*Ziziphus xylopyra*	3/8	0.375	0.5%	Antibacterial
6	*Eranthemum nervosum*	3/8	0.375	0.5%	Antimicrobial

Table no 4: Plants used in Antimicrobial disorder

Sr. No	Plants	No of citation	Use Value	Choice value	Literature review
1	*Macaranga peltate*	4/8	0.5	0.13%	Cytotoxic, anticancer
2	*Cassia absauss*	3/8	0.375	1%	Anticancer
3	*Moringa pterdospcrma*	5/8	0.625	1.6%	Immunomodulatory Antitumor Action

Table no 5: - Plants used in Anticancer

Sr. No	Plants	No of citation	Use value	Choice value	Literature review
1	*Terminalia chebula*	4/8	0.5	0.44%	Neuroprotective
2	*Cuscuta reflexa*	3/8	0.375	0.33%	Analgesic
3	*Mimosa pudica*	4/8	0.5	0.44%	Epilepsy
4	*Costus specious*	2/8	0.25	0.22%	Neuroprotective
5	*Commelina benghalensis*	3/8	0.375	0.33%	Anxiolytic effects, Neuroprotective
6	*Celastrus Paniculatus*	4/8	0.5	0.44	Neuroprotective , antioxidant
7	*Ruta graveolens*	6/8	0.75	0.66 %	Anti convulsant, antioxidant, Analgesic
8	*Mucuna preriens*	3/8	0.375	0.33%	Antiparkinson
9	*Alpinia galanga*	6/8	0.75	0.66 %	Neuroprotective , antioxidant CNS stimulant

Table no 6: - Plants used in CNS related disorder

	Sr. No	Parameter	Observation
PHYSICOCHEMICAL EVALUATION	1	Total ash	30%
	2	Acid insoluble ash	2.0%
	3	Water soluble ash	1.02%
	4	Loss on drying	3.42%

Table no. 7: Physicochemical Evaluation

	Sr. No	Parameter	Observation
PLANT EXTRAC	1	Plant part used	Leaves
	2	Solvent	Ethanol
	3	% yield	13.46%
	4	Color of isolated compound	Green
	5	Solubility	Distilled water
	6	Consistency	Semisolid

Table no 8: % yield, color, solubility and consistency of EERG

Sr. No	Test	Observation	Inference
1	Legal test (Glycosides)	Pink colour	Glycosides may be present
2	Dragendroff's test (Alkaloids)	Orange colour	Alkaloids may be present
2	Salkowski reaction (Steroid)	No red & greenish colour	Steroids may be Absent
4	Salkowski test (Triterpenoid)	Yellowish colour turns red	Triterpenoid may be present
5	Solubility test (Volatile oils)	Soluble	Volatile oil may be present
6	Molisch test (Carbohydrates)	Violet ring is formed at the junction of two liquids	Carbohydrates may be present
7	Liberman Buchard test (Saponin)	Violet Purple colour	Saponin may be present
8	Alkali reagent Test (Flavonoid)	Yellow colour to colourless	Flavonoids may be present
9	Lead acetate Test (Tannins)	White ppt	Tannins may be present
10	Lead acetate Test (Phenolic compound)	buff colored precipitate	Phenolic compound may be present

Table no. 9: Preliminary phytochemical analysis of AESD

Sr.no	Test	Observation	Inference
1	Ferric Chloride test	Deep Blue black colour	Phenolic compound are present

Table no. 10 Confirmatory test of Phenolic compound

PARAMETER / TIME PERIOD	Mortality	PH	TEMPERATURE	CONDUCTIVITY
Day 1-30 min	0	7.2	28±5	0.312 ppt
60 min	0	7.2	28±5	0.326ppt
120 min	0	7.2	28±5	0.341 ppt
Day 2	0	7.2	28±5	0.350 ppt
Day 3	0	7.2	28±5	0.376 ppt
Day 4	0	7.2	28±5	0.392 ppt

Table no. 11: Observation table for acute toxicity study of EERG in zebrafish

Time interval (min) / Groups	0	15	30	45	60
	Number of freezing episodes				
Vehicle 10% DMSO	0	0	0	0	0
Test compound EERG100mg/L	0	0	0	0	0

Table no 12. Number of freezing episodes

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	0	0	0	0	0
Test compound EERG 100mg/L	0	0	0	0	0

Table no 13. Duration of freezing episode

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	0	0	0	0	0
Test compound EERG 100mg/L	0	0	0	0	0

Table no.14 Complete Cataleptic time

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	7.37 ±1.72	18.5 ± 3.52	20.5 ± 2.44	19.5 ± 2.30	14.87 ± 4.74
Test compound EERG 100mg/L	6.25 ± 0.73	7 ± 0.91	6.25 ± 1.25	6.88 ± 0.64	7.63 ± 0.60

Table no. 15. Time spent near the bottom of the tank

Time interval (min) / Groups	0	15	30	45	60
	Time in seconds				
Vehicle 10% DMSO	6.12 ±1.20	4.25± 0.3	4.37 ± 1.49	4.62 ± 0.92	3.25 ± 1.12
Test compound EERG 100mg/L	4.13 ± 0.48	3.50 ± 0.3	3.75 ± 0.25	3.13 ± 0.35	2.88 ± 0.33

Table no. 16 Latency to travel from one point to another

Time interval (min) / Groups	0	15	30	45	60
	Distance moved in cm				
Vehicle 10% DMSO	549.75 ± 35.89	592.5 ± 70.29	618.75 ± 58.26	622.5 ± 26.15	616.5 ± 23.53
Test compound EERG 100mg/L	553.50 ± 36.10	572.25 ± 32.41	626.57 ± 22.52	640.50 ± 58.71	639.75 ± 59.68

Table no.17 Total distance moved

Time interval (min) / Groups	0	15	30	45	60
	Velocity (cm/s)				
Vehicle 10% DMSO	4.58 ± 0.29	4.93 ± 0.58	5.15 ± 0.48	5.19 ± 0.21	5.13 ± 0.19
Test compound EERG 100mg/L	4.71 ± 0.28	4.76 ± 0.27	5.08 ± 0.21	5.33 ± 0.48	5.43 ± 0.46

Table no. 18. Swimming velocity

Groups Time interval	**0 min**	**15min**	**30min**	**45min**	**60 min**
Vehicle	3.25± 0.366	3.125±0.3981	3.25± 0.491	3.285± 0.5489	3.375±0.1499
Rotenone (3pg/ml)	21± 0.47702**	21.125±0.48357**	20.25±0.914112**	18.375±1.482475**	18.125±1.36318**
Selegiline (0.03µg/ml)	6.125±0.35714***	4.625± 0.40161***	5.375± 0.48277***	3.75±0.461447***	6.25±0.64052***
EERG (5 µg/ml)	4.25±0.20631**	4.375± 0.21031**	3.5± 0.25613**	3.25±0.36111**	3.125± 0.4868**
EERG (10µg/ml)	4± 0.21981**	4.25± 0.24994**	3.75±0.29822**	3.125±0.38297**	3±0.5 **

Table no 19: Latency to travel from one point to another

Time interval / Groups	0 min	15 min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
Rotenone (3pg/ml)	10± 0.722531**	11.625±0.68649**	10.375±0.79281**	9.5± 0.89742**	7± 0.69165**
Selegiline (0.03 µg/ml)	0	0	0	0	0
EERG (5 µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0	0	0	0

Table no 20: Complete cataleptic time

Time interval / Groups	0 min	15 min	30min	45 min	60min
Vehicle	7.5± 0.77919	7.625± 1.05115	7.1429± 1.12995	7.875± 1.28782	6.625± 0.8851
Rotenone (3pg/ml)	23.875± 0.8378*	25± 0.94719*	21.375± 1.36685*	19.5± 1.70482*	15.125±1.2886*
Selegiline (0.03 µg/ml)	11.125±0.49612**	10.375±0.53647**	9.25± 0.57747**	6.875±0.66888**	6± 0.5931**
EERG (5 µg/ml)	7.75± 0.34934**	7± 0.35987*	5.875± 0.37211**	5.5± 0.50071*	4.5± 0.4414**
EERG (10µg/ml)	5.44± 0.41708**	7.444±0.46663**	8.6667± 0.49962**	10.1111± 0.54964**	10± 0.4047**

Table no.21: Time spent near the bottom of the tank

Time interval / Group	0 min	15 min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
Rotenone(3pg/ml)	0.1577±0.14741**	0.182±0.17015**	0.2191±0.21264*	0.3377±0.2452**	0.3212±0.125**
Selegiline (0.03µg/ml)	0	0	0	0	0
EERG (5µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0	0	0	0

Table no .22: Number of freezing episodes

Groups Time interval	0 min	15min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
Rotenone (3pg/ml)	3.625±21.83**	3±9.649***	3.875 ±6.825***	2.875 ±8.099***	3±0.6471***
Selegiline (0.03 µg/ml)	0	0	0	0	0
EERG (5 µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0		0	0

Table no 23 - Duration of freezing episode

Groups Time interval	0 min	15min	30 min	45 min	60 min
Vehicle	233.68125± 29.4448	246.47± 33.5121	257.125±38.193	260.97± 40.6767	268± 36.9985
Rotenone (3pg/ml)	120.11771±17.5007*	133.98± 20.3723*	143.12± 23.508*	163.16± 25.422*	165± 23.2674*
Selegiline (0.03 µg/ml)	215.45513± 27.75955***	254.6± 31.6259***	235.878± 35.6681***	274.63±41.77722***	269.5± 42.63332***
EERG (5 µg/ml)	230.84902± 30.9127***	234.16± 35.2514***	248.866± 40.715*	260.6± 44.1697***	288± 42.0931***
EERG (10µg/ml)	244.31324± 30.946***	230.19±34.944***	247.561± 41.163***	265.58± 47.1599***	280.6±45.00066***

Table no 24: Total distance moved

Groups Time interval	0	15	30	45	60
Vehicle	3.8625±29.1837	4.07396± 33.1537	4.25±37.9666	4.313542±40.940205	4.424±38.170453
Rotenone (3pg/ml)	1.98542±0.08902*	2.21458± 0.10055*	2.3656±0.1102*	2.696875± 0.1108037*	2.7354± 0.1852628*
Selegiline (0.03µg/ml)	3.79375±0.089283***	4.15729±0.100457***	3.9458± 0.119734***	4.516667± 0.14302802***	4.52188±0.21550 707***

EERG (5 µg/ml)	4.05417±0.0885***	4.09688±0.096108***	4.3396± 0.111768*	4.530208± 0.12513758***	5.0021± 0.15438169***
EERG (10µg/ml)	4.29063± 0.079608***	4.02708±0.092468***	4.3167± 0.090365***	4.617708± 0.08909742***	4.86667± 0.11367333***

Table no 25: Total swimming velocity

Groups	Dose	No. of Animals
Vehicle	10% DMSO	8
Disease control	30µg/ml	8
Standard (Selegiline)	0.03 µg/ml	8
Test group 1 EERG	5µg/ml	8
Test group 2 EERG	10µg/ml	8
Total		40

Table no.26. MSG induced neurotoxicity in zebrafish

Groups Time interval	0 min	15 min	30min	45 min	60min
Vehicle	4±0.2853	5±0.32529	4±0.37912	3.625±0.40825	3.75±0.5224
MSG (30µg/ml)	9.25±0.46047**	9.375±0.52625**	9.25±0.67548**	7.5±0.63161**	6.25±0.5508**
Selegiline (0.03 µg/ml)	3.625±0.257577***	4.75±0.29395***	4.625±0.352704***	4.5±0.411987***	3.75±0.50951***
EERG (5 µg/ml)	2.75±0.136285**	3.125±0.15865**	2.875±0.21848**	.360555**	3.125±0.50951**
EERG (10µg/ml)	3.125±0.18519**	3.5±0.21172**	3±0.28237**	3.75±0.39192*	2.875±0.4963**

Table no 27: Latency to travel from one point to another

Groups Time interval	0 min	15 min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
MSG (30µg/ml)	3.375 ± 0.152753**	1.75± 0.145389**	0.625 ± 0.155778*	0.875± 0.46188**	0.875± 0.61623**
Selegiline (0.03 µg/ml)	0	0	0	0	0
EERG (5 µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0	0	0	0

Table no 28: Complete Cataleptic Time

Groups / Time interval	0 min	15 min	30 min	45 min	60 min
Vehicle	5.375±0.274767	5.25± 0.303707	4.875± 0.311653	4.25± 0.378682	3.125± 0.32789
MSG (30µg/ml)	6.875± 0.319028*	6.125± 0.32046*	4.5± 0.308836*	4± 0.359528**	3.125± 0.29523*
Selegiline (0.03 µg/ml)	4.125± 0.2353**	3.875±0.2672**	4.125± 0.2924**	4± 0.400748**	3.25± 0.35551**
EERG (5 µg/ml)	3.5± 0.208436**	4± 0.232706**	3.875± 0.2574**	3± 0.341001**	3.5± 0.36472**
EERG (10µg/ml)	2.428± 0.1849**	3± 0.2117**	2.7143±0.2312**	2.28± 0.2987**	2.42857± 0.310**

Table no 29: Time spent near the bottom of the tank

Time interval Groups	0 min	15 min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
MSG (30µg/ml)	1.75±0.14494**	1.875 ± 0.17623**	1.875± 0..18955*	1.5±0.1632**	1.375± 0.15005**
Selegiline (0.03 µg/ml)	0	0	0	0	0
EERG (5 µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0	0	0	0

Table no 30: Duration of freezing episodes

Groups Time interval	0 min	15min	30 min	45 min	60 min
Vehicle	0	0	0	0	0
MSG (30µg/ml)	1.75± 0..15689**	2.125 ± 0.182044***	1.375 ± 0.2290***	2. ± 0.301***	1.75±0.4531***
Selegiline (0.03 µg/ml)	0	0	0	0	0
EERG (5 µg/ml)	0	0	0	0	0
EERG (10µg/ml)	0	0	0	0	0

Table no 31: Number of freezing episodes

Groups Time interval	0 min	15 min	30 min	45 min	60 min
Vehicle	188.243± 24.518	227.883± 28.11432	206.141± 31.1162	214.838±33.33881	220.6± 30.541
MSG (30µg/ml)	124.440± 29.4330*	131.017± 29.08891*	150.938± 34.4274*	139.425± 29.92894*	148.1± 23.63724*
Selegiline (0.03 µg/ml)	226.637± 25.5281***	230.13± 33.4169***	248.035± 38.229***	263.034± 40.4417***	238.8± 34.9117***
EERG (5 µg/ml)	232.450± 30.3715***	233.57± 34.46331***	247.976± 39.481***	253.248± 42.4336***	274.9± 39.98824***
EERG (10µg/ml)	239.034± 30.83101***	248.517± 34.98312***	264.583± 40.4647****	253.188± 44.73756***	262.1± 38.632***

Table no 32: Total distance moved

Groups Time interval	0 min	15 min	30 min	45 min	60 min
Vehicle	3.111458±0.068306	3.766667±0.069307	3.40729±0.08458	3.5510417± 0.09733194	3.64583± 0.12234279
MSG (30µg/ml)	2.185417±0.072668*	2.285417±0.083561*	2.61979±0.098166*	2.4020833± 0.1250601*	2.53854± 0.17861166*
Selegiline (0.03 µg/ml)	3.980208±0.068087***	4.02604± 0.078026***	4.325± 0.092093 ***	4.572917± 0.1183426***	4.1313± 0.15845968***
EERG (5 µg/ml)	201.7688±0.084249***	223.4536±0.092075***	211.793± 0.095816**	247.64865± 0.11280173***	245.981± 0.06770032***
EERG (10µg/ml)	4.197917±0.070036***	4.348958 ±0.081621**	4.61563± 0.092216***	4.4± 0.13096199***	4.54167± 0.14690085***

Table no 33: Total swimming velocity

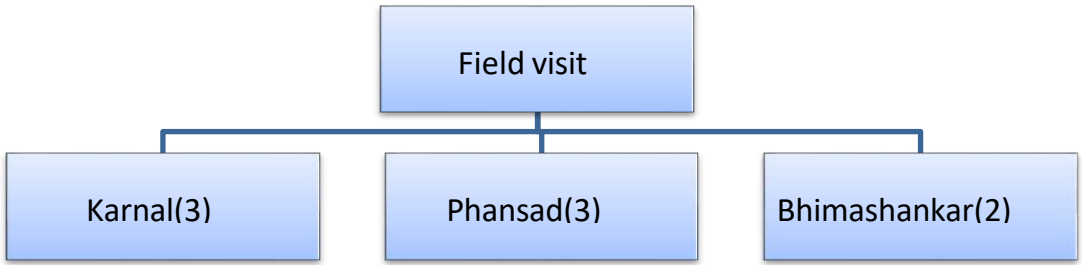


Figure 1: Evaluation of activity

FIELD DATA SHEET	
DATE:	COLLECTION NO:
AREA:	DISTRICT:
COMMON NAME:	
STATUS:	
PART USED:	
USED FOR:	
PREPARATION AND DOSAGE:	
TYPE OF VEGETATION:	
SOIL:	
BOTANICAL SOURCE: FAMILY:	

Figure 2. Field data sheet

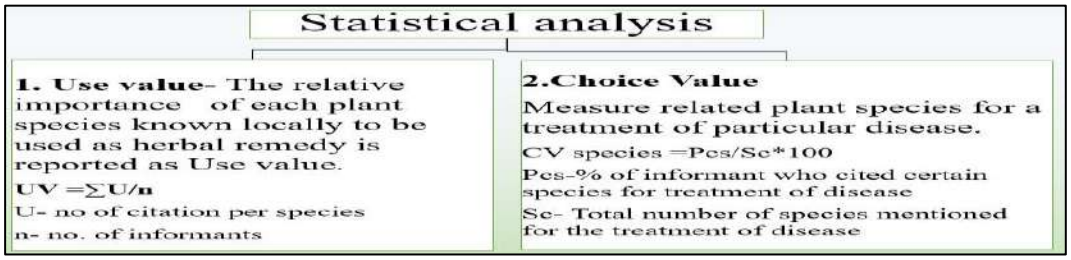
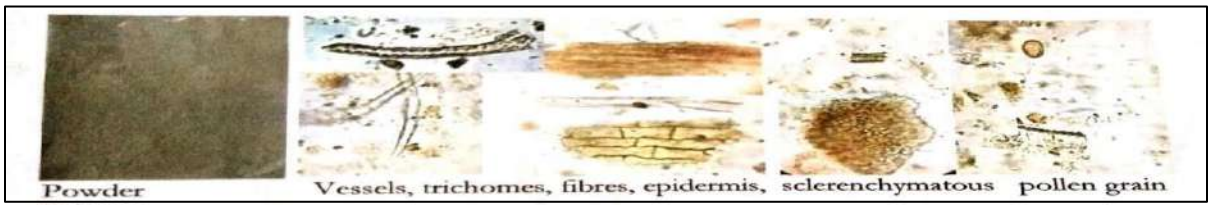


Figure 3: Two Statistical Tools



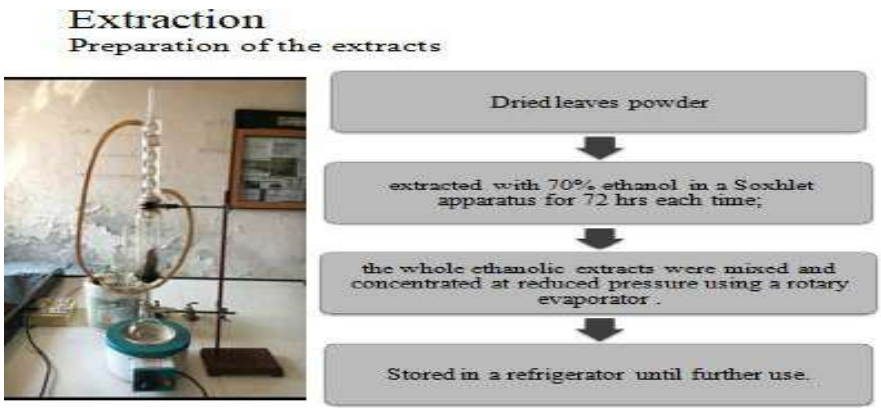


Figure 5: Extraction Of Plant

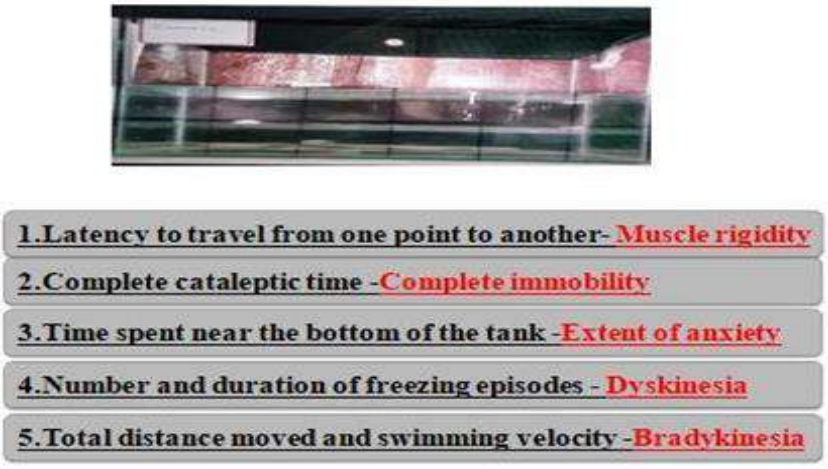


Figure 6. Behavioral Parameters

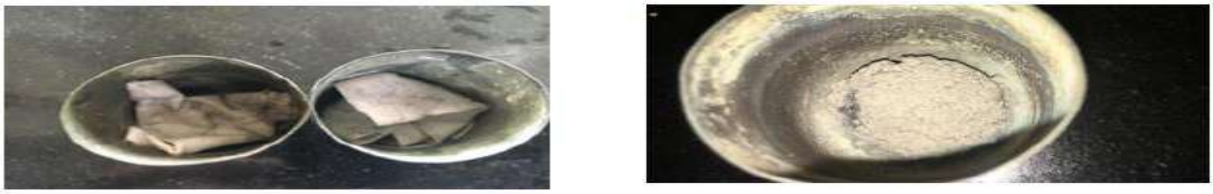


Figure 7. Physicochemical analysis of crude powder

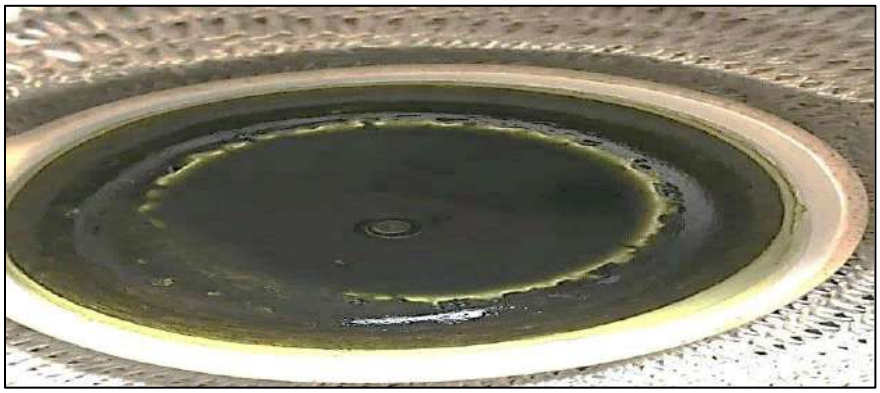


Table no 8 : % yield, color, solubility and consistency of EERG

Figure 8. Herbal extract

Table no 8 : % yield, color, solubility and consistency of EERG

Figure 8. Herbal extract

Figure. 9: Phytochemical analysis and Confirmatory test of AERG

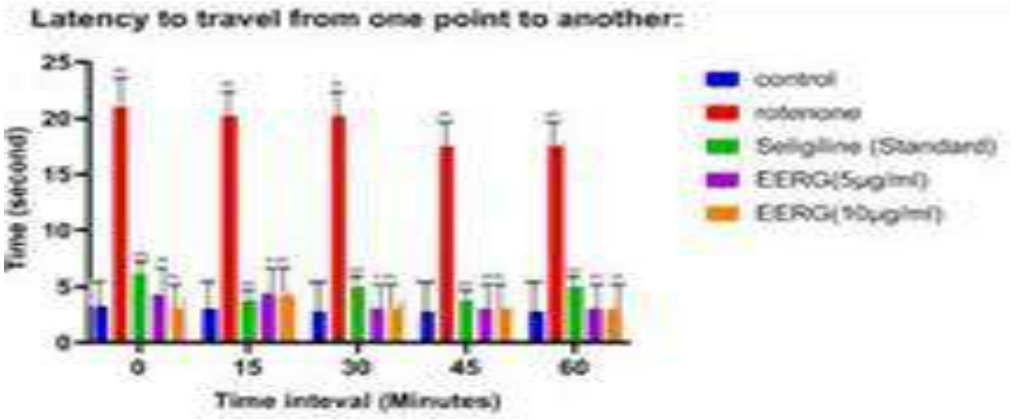


Figure 10: Latency to travel from one point to another

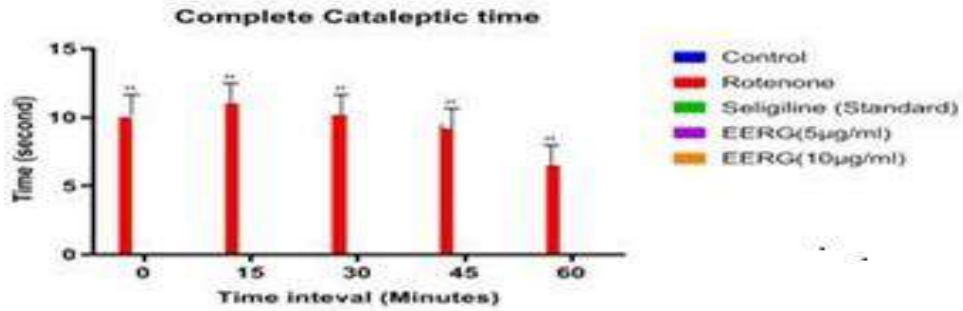


Figure 11: Complete cataleptic time

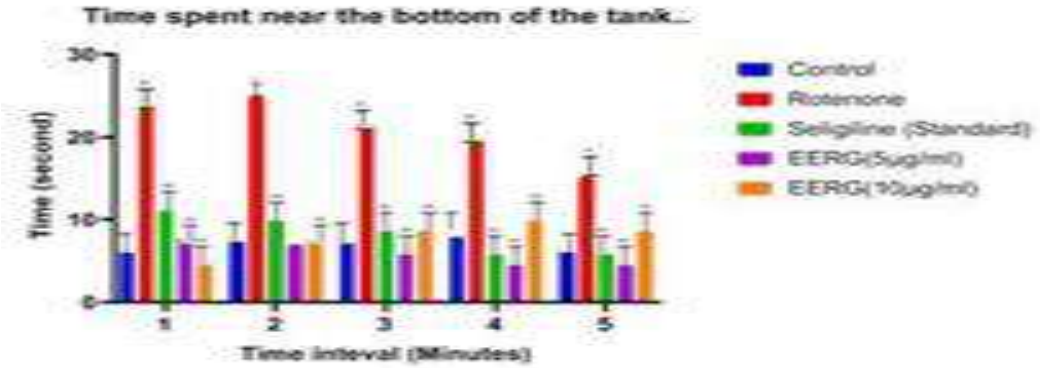


Figure:12 Time spent near the bottom of the tank.

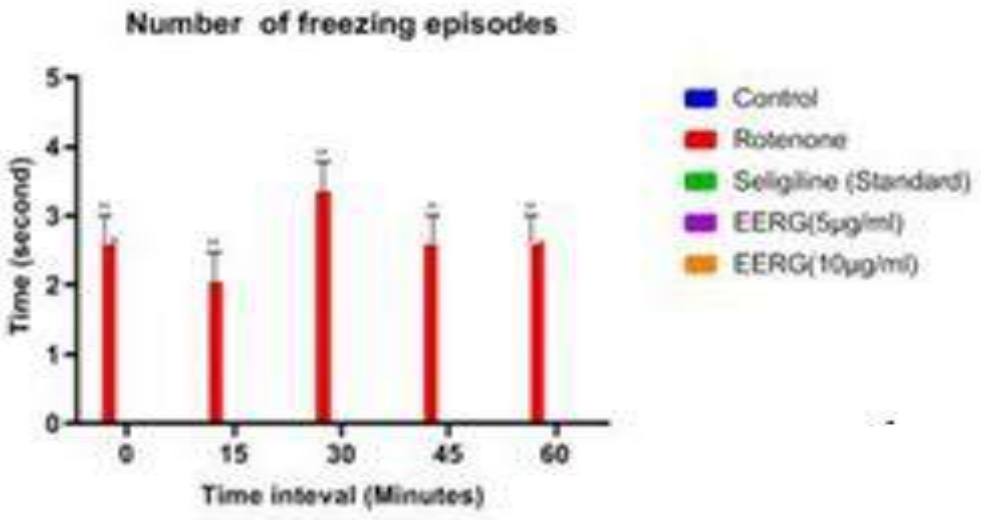


Figure .13: Number of freezing episodes

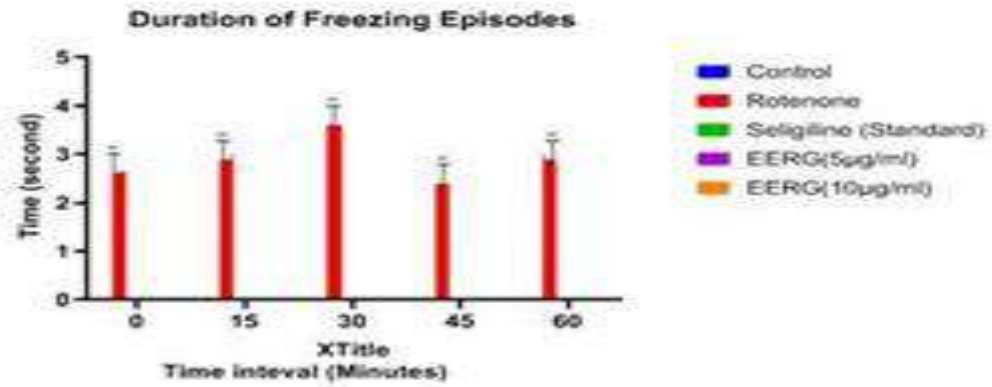


Figure .14: Duration of freezing episode

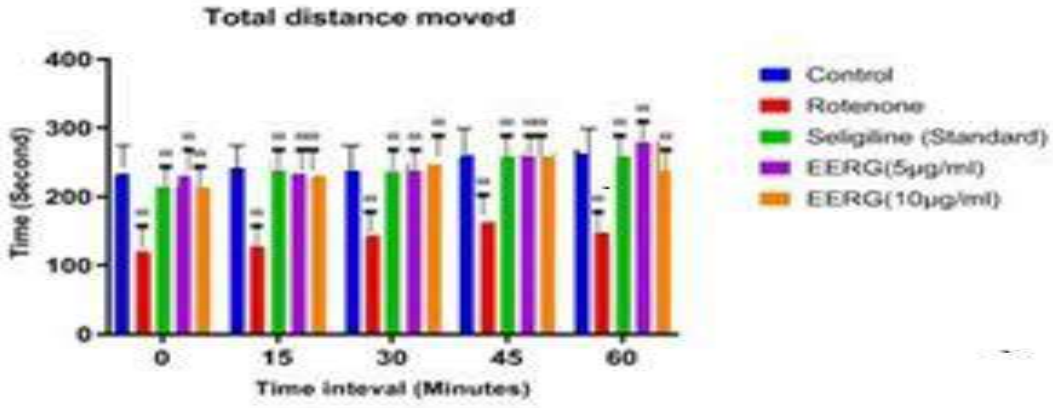


Figure .15: Total distance moved

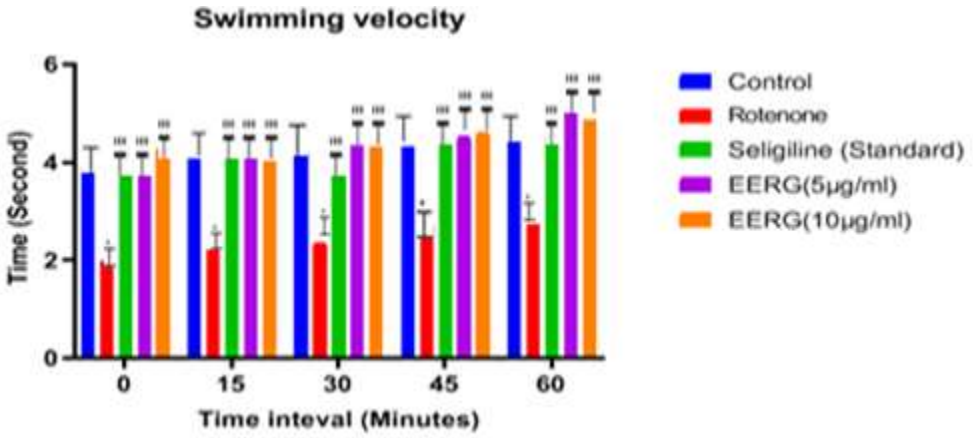


Figure .16: Total swimming velocity

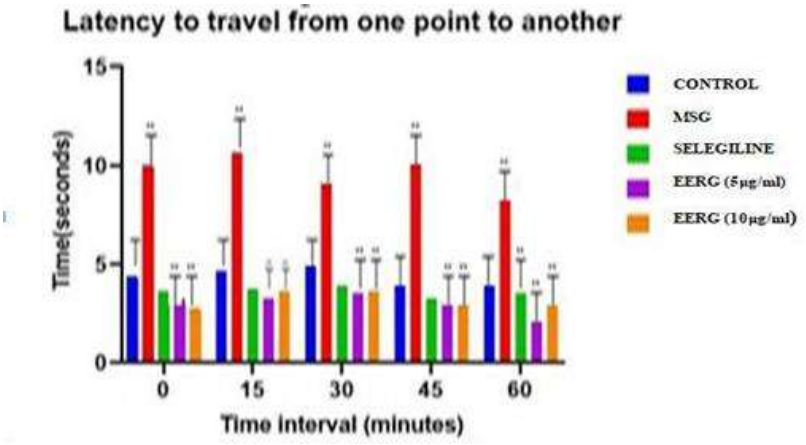


Figure 17: Latency to travel from one point to another

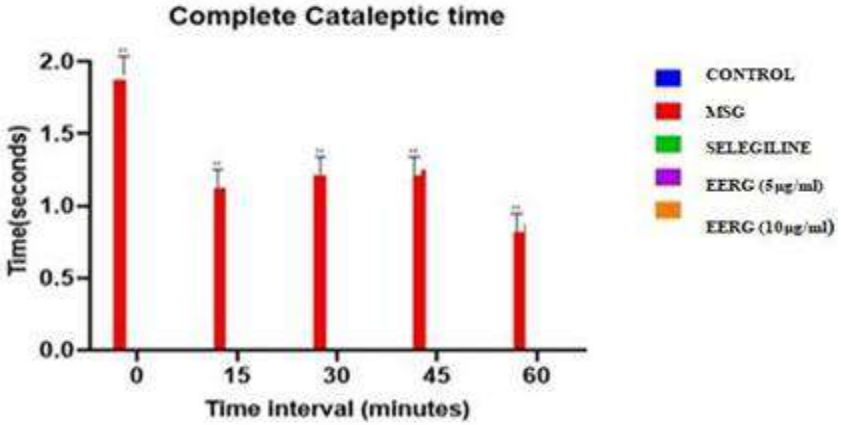


Figure 18: Complete cataleptic time

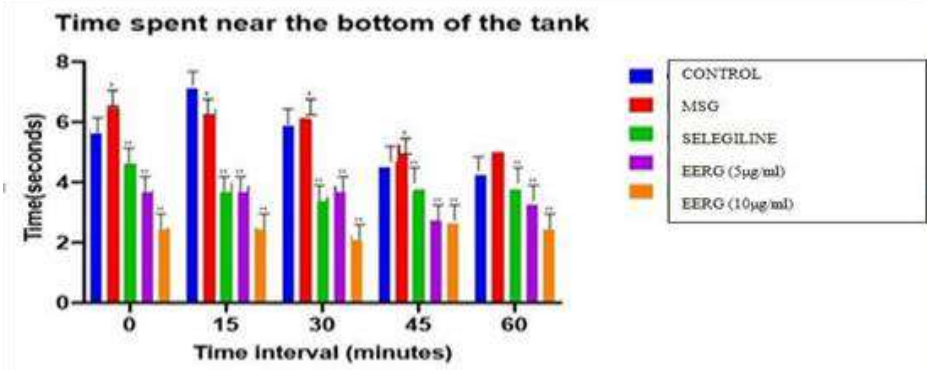


Figure 19: Time spent near the bottom of the tank

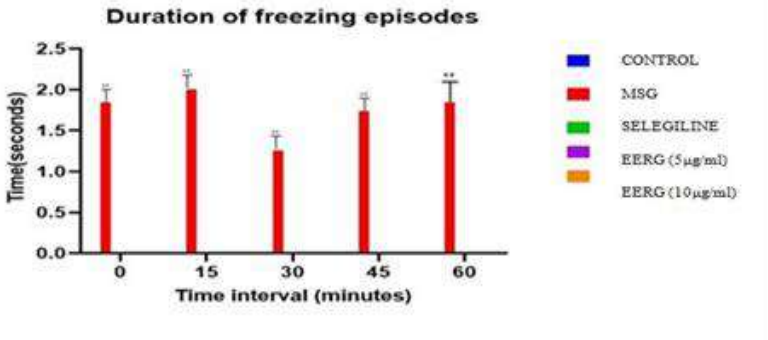


Figure 20: Duration of freezing episodes

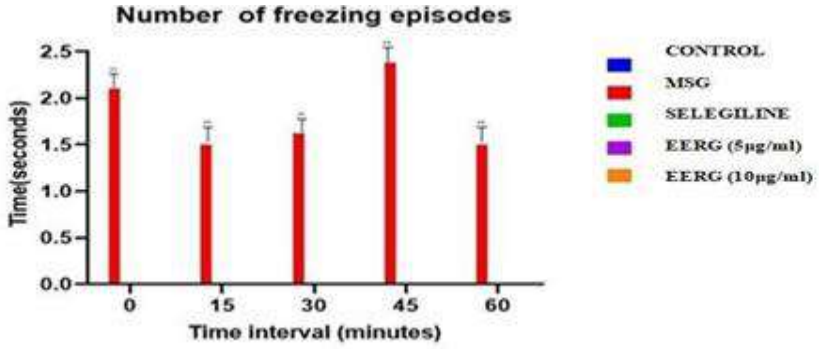


Figure 21: Number of freezing episodes

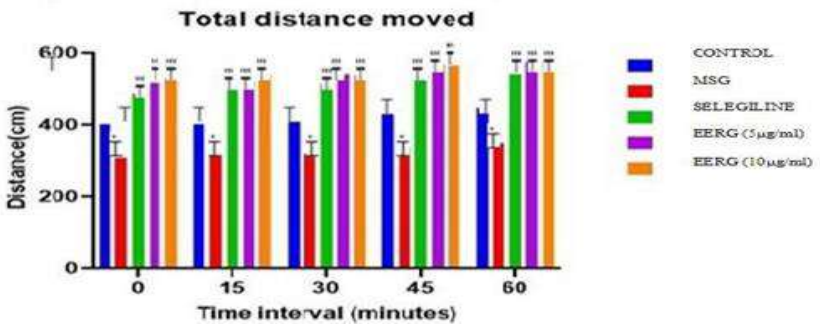


Figure 22: Total distance moved

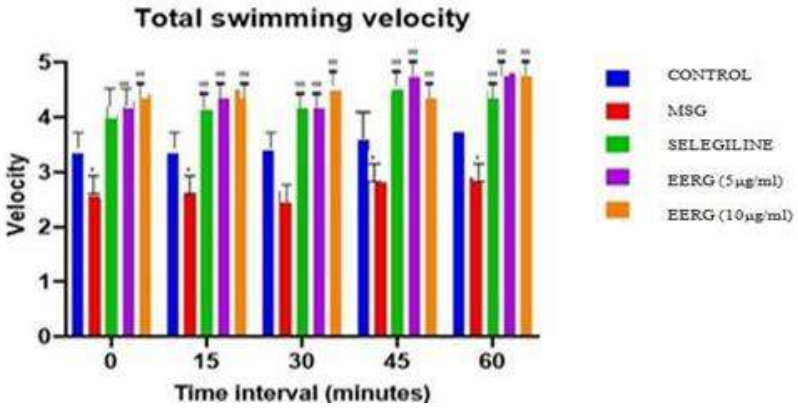


Figure 23: Total swimming velocity