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Integrated Phytochemical Profiling And Biological Assessment Of Coccinia Grandis Fruits In The Management Of Alzheimer's Disease

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Abstract:

***Coccinia grandis* (L.) Voigt fruit extract (FECG) was investigated for its phytochemical composition, antioxidant potential, and neuroprotective effects in experimental models of Parkinson's (PD) and Alzheimer's disease (AD). Phytochemical screening of the ethanolic extract confirmed the presence of alkaloids, phenolics, flavonoids, tannins, steroids, saponins, glycosides, and mucilage. The extract exhibited notably high total phenolic (302.82 mg GAE/g) and flavonoid (264.64 mg CE/g) content, accompanied by strong dose-dependent DPPH, hydroxyl, and superoxide radical scavenging activity. LC-MS profiling identified several bioactive compounds, including trigonelline, kojic acid, coumarin derivatives, DHEA, and quinazolinone analogues, supporting its multifunctional pharmacological potential. Acute toxicity studies indicated that FECG is safe up to 2000 mg/kg. In the 6-OHDA-induced PD model, FECG (200 and 400 mg/kg) significantly improved body weight, food and water intake, motor coordination, and reduced apomorphine-induced rotations while restoring antioxidant enzyme activities and dopaminergic neurotransmitter levels. In the $AlCl_3$-induced AD model, 90-day FECG administration alleviated body weight loss, enhanced behavioral and cognitive performance, and normalized urinary and intake parameters, showing efficacy comparable to rivastigmine. These findings demonstrate that FECG possesses potent antioxidant, anti-Parkinsonian, and anti-Alzheimer's activities, highlighting its potential as a promising phytotherapeutic agent for neurodegenerative disorders.**

Keywords: *Coccinia grandis*, phytochemical profiling, LC–MS analysis, antioxidant activity, neuroprotection, Parkinson's disease, 6-OHDA model, Alzheimer's disease.

Introduction

Neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) impose an escalating global health burden and are characterized by progressive neuronal loss, oxidative stress, and neurochemical disturbances that impair motor and cognitive functions (1). PD primarily involves dopaminergic neuronal degeneration in the nigrostriatal pathway, whereas AD is associated with cholinergic dysfunction, behavioral changes, and progressive memory impairment (2). Increasing evidence highlights oxidative stress as a central contributor to the onset and progression of these disorders, making antioxidant-based therapeutic strategies a promising area of investigation (3). *Coccinia grandis* (L.) Voigt is a traditional medicinal plant recognized for its pharmacological diversity and historical therapeutic use (4). Phytochemical screening performed in the present study revealed that the ethanolic fruit extract (FECG) contains alkaloids, flavonoids, phenols, tannins, steroids, glycosides, saponins, proteins, and mucilage, confirming a rich chemical profile supportive of biological activity (4). The extract also exhibited high levels of total phenolics and flavonoids, constituents known to exert strong antioxidant effects (5,7). Correspondingly, in vitro antioxidant assays demonstrated potent scavenging of DPPH, hydroxyl, and superoxide radicals, indicating strong free-radical neutralizing capacity (6,7). LC-MS profiling further identified several bioactive molecules such as trigonelline, kojic acid, coumarin

derivatives, DHEA, and quinazolinone analogues many of which have documented antioxidant, anti-inflammatory, and neuroprotective properties (8–11). Acute toxicity assessment confirmed the extract's safety up to 2000 mg/kg, demonstrating a favorable toxicological profile (12). To evaluate neuroprotective efficacy, two validated models were employed. In the 6-OHDA model of PD, FECG improved body weight, motor coordination, intake behaviors, and significantly reduced apomorphine-induced rotations, alongside restoration of antioxidant enzyme levels and dopaminergic neurotransmitters (13,14). In the $AlCl_3$-induced AD model, chronic FECG administration improved cognitive and behavioral outcomes, normalized physiological parameters, and exhibited efficacy comparable to rivastigmine (15,16). Collectively, the phytochemical richness, strong antioxidant activity, and robust neuroprotective outcomes support *Coccinia grandis* fruit extract as a promising candidate for the development of phytotherapeutic interventions targeting neurodegenerative diseases.

Materials & Methods:

Collection and Authentication of the plant material:

The fruits of *Cocania grandis* were collected from the nearby regions of Tirupati and was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S V University, Tirupati.

Extraction:

The fruits of *Cocania grandis* were carefully washed with tap water, and the pulps were separated using the glove box technique under a vacuum. Then they were lyophilized at -50 ºC for 72 hours, ground into powder, and stored in a vacuum desiccator. The grind materials were passed through sieve no. 44 and 72, and the powdered materials of identical size between those two sieves were collected in a glove box and stored in a vacuum desiccator for further use. About 100 gm of powdered materials were taken in a 1000 ml round bottom flask and extracted with 500 ml of 70% ethanol for 72 hours using a cold maceration process with continuous stirring. The extract was decanted into pre-weighed glass vials. The solvent present in the extract was removed to obtain a syrupy brownish-yellow mass. The extract was weighed and preserved in a vacuum desiccator for subsequent use in the study.

Preliminary Phytochemical Analysis:

The ethanolic extract of *Cocania grandis* Linn fruit pulp (FECG) was subjected to qualitative tests to identify various plant constituents using standard procedure.

PHARMACOLOGICAL EVALUATION

Housing and feeding conditions

The male Wistar rats weighing above 230 - 250 g were utilized in this current investigation, and the same was acquired from the institutional animal facility. All rats were caged in a clean con- fines beneath the well-organized cabin with 22 ± 28 °C temperature and 12 hours light/dark cycle. All animals were permitted food, water, and *ad libitum* throughout the study. All the experimental procedures were performed on the animals after approval from the Institutional Animal Ethical Committee, IAEC (Ref. no.: 1837/ PO/ RcBiBt/ S/2015/CCSEA) and by the recommendations for the proper care and use of laboratory animals.

Acute toxicity test

An acute oral toxicity study for FECG was carried out per OECD guideline 423. The test procedure minimizes the number of animals required to estimate the acute oral toxicity study. The test allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Experimental Protocol

i) 6-OHDA-induced Parkinson's disease model

All animals were segregated into four groups I-IV. The treatment period comprised of 07 days, and the animals were grouped under the following regimen: i) Sham-operated received saline treatment ii) 6-OHDA lesioned group + saline iii) 6-OHDA lesioned group + Fruit pulp of ethanol extract of *Cocania grandis* 200 mg/kg (6-OHDA + FECG- I) iv) 6-OHDA lesioned group + Fruit pulp of ethanol extract of *Cocania grandis* 400 mg/kg (6-OHDA + FECG-II).

Each group consisted of 6 animals. After surgical procedures for the intra-striatal injection of 6-hydroxydopamine (6-OHDA) with saline, the animals were divided into four groups (n = 6 per group). Group I received saline alone instead of 6-OHDA as a unilateral lesion. Group II received an intra-striatal injection of 6-OHDA as a unilateral lesion. Groups III & IV received ethanol extract of fruit extract of *Cocania grandis* (FECG-I & II) 200 & 400 mg/kg for 07 days, consecutively after 6-OHDA injection. 0.8 µg 6-OHDA, dissolved in 4 µL physiological saline containing 0.1% ascorbic acid, was administrated through a Hamilton syringe over 4.50 min; the syringe was left in place for 5 min after injection before being slowly removed. Sham-operated rats were injected with saline on the seventh, fifteenth, and twenty-ninth days after surgery. After receiving a 6-OHDA treatment, the animals' general behaviour was monitored for four weeks continuously, including body weight, food and water intake, body temperature, and urine output. Then behavioural tests were performed to evaluate the

motor abilities of the animals. The animals were sacrificed on the 29th day following the beginning of the treatment.

ii) AlCl3-induced Alzheimer's disease model

Before the experiment started, 30 rats were meticulously divided into five groups, each comprising six. The dosage administered to them was calculated based on their body weight and administered daily for six consecutive weeks to the following groups: The first group, known as the control group, was given a solution of normal saline orally for the 90-day experiment. The second group was given a solution of AlCl3 in their drinking water from day 01 to 30, with the dose being 17 mg/kg of body weight. The third group received oral rivastigmine (0.3 mg/kg) for 90 days, one hour before exposure to AlCl3 from the 1st to the 30th day. The fourth and fifth groups were given a dosage of FECG I and FECG II (200 mg/kg and 400 mg/g of body weight) for 90 days, respectively, an hour before being exposed to AlCl3 from day 1 to 30.

The general behavioural parameters such as body weight, food and water intake, urinary output and urinary ions were noted for the first and last day of treatment, and then the data were interpreted. All animals underwent baseline measurements of their behaviours in the following behavioural studies. Then the animals were sacrificed on the 90th day following the beginning of the treatment.

BEHAVIORAL STUDY

i) 6-OHDA-induced Parkinson's disease model

Apomorphine-induced rotation test

As previously described for our group, the apomorphine-induced rotation test (also named rotameter) was performed three weeks after the 6-OHDA lesion to evaluate the degree of dopaminergic depletion. 6-OHDA lesion results in nigrostriatal denervation and subsequent hypersensitivity of the DA receptors.[243] Administration of apomorphine, a potent DA agonist, in lesioned-side animals generates an imbalance in the striatal dopaminergic transmission, preferentially stimulating the affected side. This unequal stimulation leads to the contralateral rotation of the animals. For this test, rats were subcutaneously administrated in the neck with 0.05 mg/kg apomorphine hydrochloride dissolved in 0.9% NaCl. Total net contralateral rotations (the difference between the total number of contralateral and ipsilateral rotations) were quantified using an automated metal testing bowl over a 45-min session. Since apomorphine is a potent DA agonist, continuous overstimulation of the dopaminergic system could result in an inadequate interpretation of the other outcomes evaluated in this study.

Narrow beam maze test

Animals were positioned on a narrow beam 1 m long and 80 cm above the ground. The animal wouldn't be hurt if it fell; a 12 cm thick foam was placed exactly beneath the beam. The animal was set at one end of the beam, while the animal's residence cage was set at the other. A stopwatch measured how long the animals travelled from their initial starting positions to the other end. The test was recorded as "timed out" at 2 minutes if animals refused to finish a beam run. The latency to start and the total time to cross the beam were recorded throughout this test.

Rotarod test

Motor performance was also evaluated with Rotarod equipment under the protocol previously described by Monville C. The first three days of testing before the 6- OHDA administration served as training. The animals underwent a four-trial test under an accelerating protocol going from 4 rpm to 40 rpm in 5 minutes, being allowed to rest for at least 20 minutes between trials. Like accelerating protocol, the latency to fall was recorded on the 7th, 15th, 21st, and 29th days.

Grip strength test

The Forelimb strengths of rats were measured using a grip strength meter.[246] The animals were trained for six training sessions on day 1 for a reliable assessment of holding capability, and the grip strength test was carried out on day 29. Rat forelimbs were placed on the tension bar as they were positioned facing the grip strength meter's T bar. The rat was gently and steadily dragged away from the T bar by the root of the tail when it grabbed the bar. The grip strength meter automatically calculated and recorded the force each animal could produce in grams. Each animal's measures were computed. Between measurements, the rats were given the 30s to rest.

Sensorimotor test

The corner test assesses the direction pattern of sensorimotor dysfunction. It was initially applied to rats. It has been an established technique for evaluating sensory- motor function, an excellent way to spot and measure sensory and postural asymmetry.It offers a quick approach for identifying ipsilateral and contralateral steering deviation.

Disengage test

A tactile stimulus was placed towards the vibrissae, and a stopwatch was used to measure how long it took the rat to turn around and touch the probe with a paw. Paw extension was thus measured for the disengage test.

AlCl3-induced Alzheimer's disease model

Morris Water Maze test

Spatial learning and memory were assessed as previously described by Morris.The experiment was based on the ability of the rats to learn how to escape from the pool by locating a transparent, submerged platform, climbing, and staying on it to be returned to their cage. The Morris water maze test was carried out in a large circular pool (160 cm diameter) filled with water (30 cm depth) and included an acquisition trial and a probe trial. Four points around the edge of the tank were designated north (N), south (S), east (E), and west (W), thus providing four alternative start positions and dividing the pool into four quadrants (NW, NE, SE, and SW). The invisible platform was placed 2 cm below the water level of the northeast quadrant. During the acquisition trial, the rats were trained to locate the platform (up to 90 s) thrice a day for five days. All the rats were placed on the platform for 30 s before being placed at a start point. If the rats reached the platform during the 90 s, they were allowed to remain for 30 s in the platform. When the rats failed to reach the platform during the 90 s, they were guided to the platform and then allowed to remain for 30 s. On the sixth day, the rats were individually subjected to a 90-probe trial in which the platform was removed from the pool. The time spent swimming in the target quadrant (within 90 s probe test time) was recorded. Rats with no or less deterioration in memory functions (negative control and treated groups) were expected to remember the platform location and spend more time swimming within the target quadrant, looking for it, compared to the Alzheimer's rats.

Passive Avoidance Test

The apparatus of the Passive Avoidance test consisted of a light compartment (depth 270 mm × width 370 mm × height 360 mm) and a dark compartment (depth 270 mm × width 370 mm × height 360 mm) used for the measurement of emotional memory of rats.The two compartments of this apparatus were connected by a sliding door having 90 mm of diameter in the middle part. The floor of this apparatus consisted of a metal grid spaced 0.9 cm apart and connected to a shock generator, able to generate shock in the range of 0.5 mA. Each test consists of two separate trials: the acquisition and retention trials. For the acquisition trial, each rat was placed in the light compartment, and when the rat entered the dark compartment, an electrical foot shock of 0.5 mA was administered for 3 sec. With the help of a stopwatch, the latency times, once the rat had entered the dark compartment were recorded as escape latency (EL). After that, the rat was returned to its home cage. A retention trial was performed after 24 hrs of the acquisition trial, in which no shock was given when the rat entered the dark compartment and latency times to re-enter the dark chamber were measured as retention latency (RL) up to 600 sec. The apparatus was cleaned after each test with 70% ethanol to remove any olfactory clue.

Biochemical Studies

The animals were deeply anaesthetized after behavioural assessment (i.e., 29 days after surgery in PD and 90th day in AD). The brains were quickly removed and placed in an ice-cold Petri dish. Rat brains will be fixed using a Microwave Fixation System. Striata from both sides are dissected from all the groups of animals. The striatal tissue is homogenized in 250 µl acetonitrile to eliminate debris before being centrifuged at 13,000 g for 30 min. A nitrogen stream evaporates the acetonitrile after the supernatant has been collected and cleaned with heptane. Re-suspending the sample in 75 µl of mobile phase (37.5 mM phosphoric acid, pH 8.5) to estimate total protein and lipid peroxidation. The supernatant was centrifuged at 15000 rpm for 1 hour at 4°C. The supernatant obtained was used for further estimation of biochemicals described below,

TBARS activity

TBARS in the brain tissue was estimated following the method of Ohkawa *et al.* The principle depends on the reaction between thiobarbituric acid and malondialdehyde (MDA), a secondary product of lipid peroxidation at pH 4. A reddish pink colour developed was estimated at 532 nm, indicating the extent of peroxidation. Briefly, 0.2 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) and 1.5 ml of 8% thiobarbituric acid were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95 °C in a water bath for 60 minutes. The tubes were cooled to room temperature under running water, and the final volume was made to 5 ml in each tube. 5 ml of butanol: pyridine (15:1 ratio) mixture was added, and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken, and its absorbance was measured at 532 nm against an appropriate reagent blank without the sample using an extinction coefficient of 1.56 x 105 MLcm^{-1}. The extent of lipid peroxidation was expressed as nmol/gm protein.

Superoxide dismutase activity

Enzymatic activity is measured in a reaction mixture containing nine mM of epinephrine, 0.1 mM EDTA and 0.05 M of sodium carbonate at pH 10.2 and 30°C. The rate of change of absorbance for Adrenochrome formation, SOD's potential to stop adrenaline's auto-oxidation to adrenochrome in an alkaline pH) should be 0.025/min, and the amount of SOD needed to achieve 50% of inhibition is

measured. The ability of SOD to inhibit the autooxidation of epinephrine at alkaline pH. This oxidation can be followed spectrophotometrically at 480 nm.

Catalase activity

Catalase is assayed by the method of Caliborne M. The mixture consists of 1.95 ml of phosphate buffer (0.05 M, pH-7), 1 ml of H_2O_2 (0.019 M) and 0.05 ml sample (10% w/v) in a final volume of 3 ml. control cuvette contains all the components except substrate. The change in absorbance is then recorded at 240 nm, the catalase activity is calculated, and nM H_2O_2 consumed/min/mg protein was used to measure catalase activity.

CAT = (OD_{240} x Volume of Assay) x mg of protein /(0.081x Volume of Enzyme)

Where,

OD is optical density observed at 240nm 0.081 is the extinction coefficient

GRH activity

Glutathione reductase (GRH) activity was assayed by the method of Carlberg and Mannervik (1975), as modified by Mohandas *et al.* (1984). The reaction system consisted of potassium phosphate buffer (100 M, pH 7.4), EDTA 0.5 mM, oxidized glutathione (GSSG) 1 mM, NADPH 0.1 mM and PMS 10 %. Enzyme activity was quantified at 25 ºC by measuring the disappearance of NADPH at 340 nm and expressed as nmol NADPH oxidized/min/mg/protein. The amount of nmol NADPH/min/mg^{-1} protein used to express the enzyme activity.

GPx activity

The GPx reaction was performed in a medium containing 300 µL of a solution composed of 48 mM buffer phosphate, pH 7.7, 0.38 mM EDTA, 0.95 mM azide (to inhibit catalase), 1 mM of glutathione, 0.12mM of nicotinamide adenine dinucleotide phosphate (NADPH), 3.2 U of glutathione reductase, 0.02 mM DL-dithiothreitol and 0.0007% hydrogen peroxide (Wendel, 1981). The decrease in absorbance was recorded for 5 min in a spectrophotometer with a wavelength set at 340 nm. The glutathione peroxidase activity values are expressed as unit NADPH/min/mg−1 protein. One unit catalyzes the oxidation by H_2O_2 of 1 mol. Of reduced glutathione to oxidized glutathione per minute at 25°C, pH 7.0.

Total protein content

Total thiols were assayed on the principle of formation of a relatively stable yellow colour by sulfhydryl groups of DTNB. Protein content in the samples was determined by the method of Lowry *et al.*; This method uses two separate reactions: the reaction of aromatic amino acids with the Folin-Ciocalteau reagent and the reaction of peptide bonds with cupric ions in an alkaline environment (biuret reaction).

Nitric oxide estimation

he level of nitrite in the rat striatal tissue is an indicator of the production of nitric oxide (NO), which was determined according to the method of Kumar *et al.*, Nitric oxide was estimated by indirect measurement of nitrite, nitrate, and total nitrite in rat brain extract supernatants obtained after centrifugation. The absorbance was noted at 620 nm and 550 nm, respectively. The nitrite and total nitrite levels were normalized to the total protein estimated by the Bradford method.

Neurochemical Analysis

6-OHDA-induced Parkinson's disease model

The method described by Patel was used to estimate the levels of brain catecholamines with a little modification. Catecholamines such as dopamine (DA) and their metabolites [3,4-dihydro phenylacetaldehyde (DOPAC), homovanillic acid (HVA)] levels were estimated by HPLC using an electrochemical detector. The study used a water standard system consisting of a high-pressure isocratic pump, a 20 µl manual injector valve, a C18 reverse phase column, and an electrochemical detector. The mobile phase consisted of sodium citrate buffer (pH 4.5), acetonitrile (87:13), and v/v). Sodium citrate buffer consisted of 10 mM citric acid, 25mM NaH_2PO_4, 25 mM ethylenediaminetetraacetic acid (EDTA), and 2 mM of 1-heptane sulfonic acid. Electrochemical conditions for the experiment were +0.75 V, and sensitivity ranged from 5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 µl) were injected manually. On the day of the experiment, frozen brain samples were thawed and homogenized in a solution containing 0.2 M perchloric acid. After that, samples were centrifuged at 12,000 g for 5 min. The supernatant was filtered through 0.22 µm nylon filters before injecting it in the High-performance liquid chromatography (HPLC) sample injector. Data were recorded and analyzed with the help of Breeze software. Neurotransmitters' concentrations and metabolites were calculated from the standard curve generated using a standard in a 10-100 ng/ml concentration range.

AlCl3-induced Alzheimer's disease model

Acetylcholinesterase activity:

The activity of the enzyme acetylcholinesterase (AChE) was measured using a method described by Ellman. The endpoint is the formation of yellow due to the reaction of thiocholine with dithiol-bis-

nitrobenzoate ions. A 0.4 ml aliquot of the prepared homogenate was added to a cuvette containing 2.6 ml sodium phosphate buffer (pH 7.2, 0.1M). 100µl of Ellman's reagent (DTNB) was added and taken into a photocell. The absorbance was set to zero at 412 nm when the fluctuations stopped. Of the substrate (Acetyl thiocholine iodide), 20 µl was added. A change in the absorbance per minute was noted. AChE activity is calculated using the following formula: $R= (\Delta A/1.36 \times 10^4) \times (1/[400/3120]C_0)$

$= 5.74 (10^{-4}) \Delta A/ C_0$

Where,

ΔA = Change in absorbance per minute

C_0 = Original concentration of the tissue (mg/ml)

R = Rate in moles substrate hydrolyzed per minute per gram of tissue

Transmembrane protein activity

The activity of the enzyme Na^+/K^+ ATPase (NKA) was measured using a method suggested by Li *et al.*, The supernatant obtained from the homogenates of brain tissue was incubated in a mixture of 0.2 ml of the buffer, 0.2 ml of calcium chloride, 0.2 ml of ATP, and 0.2 ml of distilled water at 37 °C. The mixture was incubated for 10 min at 37 ºC with 2.0 ml of 20 % TCA. A blue colour developed after adding 1.0 ml of ammonium molybdate and 0.8 ml of 4-aminonaphthol sulphonic acid. The absorbance of the resulting mixture was measured at 650 nm.

Histopathological Examination

6-OHDA-induced Parkinson's disease model

At the end of the tests, to evaluate the histological changes of the forebrain & brainstem in 6-OHDA-induced PD, the rats are given a high dosage of ketamine (150 mg/kg) to induce profound anaesthesia before being given glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and 100 ml of 0.1% PB containing 10% sucrose after the conclusion of behavioural trials. After perfusion, the forebrain and brainstem blocks were produced, immersed in paraffin, and slices were cut to a thickness of 30 m on a microtome and collected in PB (0.1 M). Sections are stained with 0.1% cresyl violet and Nissl stain.

AlCl3-induced Alzheimer's disease model

For 24 hours, the hippocampus region of each brain is fixed in a formalin buffer (10%). After washing with tap water, dehydration is achieved using successive dilutions of alcohol (methyl, ethyl, and 100% ethyl). Specimens are cleaned in xylene and embedded in paraffin for 24 hours at 56 degrees Celsius in a hot air oven. A microtome is used to create paraffin-beeswax tissue blocks for sectioning at a thickness of 4 microns. For histological analysis under a light microscope, the resulting tissue sections are collected on glass slides, deparaffinized, and stained with hematoxylin and eosin stains.

Statistical Analysis

The study presented the findings as mean ± standard error mean (SEM). The researchers analyzed the behaviour data using a statistical method called ANOVA, which compares multiple groups, and a follow-up test called Dunnett's test & Turkey's test to identify specific differences. A p-value of less than 0.05 was used as the threshold for determining statistical significance throughout the analysis.

Results & Discussion:

Table 1: List of Phytochemical Constituents screened in FECG

S. No.	Constituents	Ethanolic Extract
1.	Alkaloids	+
2.	Carbohydrates	-
3.	Protein	+
4.	Steroids	+
5.	Phenols	+
6.	Tannins	+
7.	Flavonoids	+
8.	Gums and Mucilage	+
9.	Glycosides	+
10.	Coumarins	-
11.	Saponins	+
12.	Terpenoids	-

+ = Present

- = Absent

Pharmacological Studies
Biochemical Analysis

Table 2. Effect of FECG post-treatment on Brain Antioxidants of 6-OHDA lesioned rats

Groups	Drugs/extracts administered group	LPO	SOD	CAT	GSH	GPx	NO
I	Sham-operated group	0.2757 ± 0.05061	7.620 ± 0.14470	7.791 ± 0.02671	21.43 ± 0.1236	17.52 ± 0.06331	17.14 ± 0.9766
II	6-OHDA-lesioned group	0.5915 ± 0.121**	3.14 ± 0.02075****	3.231 ± 0.01332****	12.14 ± 0.6100****	9.867 ± 0.05164****	34.43 ± 0.1236****
III	FECG I	0.3717 ± 0.01757*	4.911 ± 0.07461****	5.781 ± 0.06953****	16.77 ± 0.4322****	12.43 ± 0.1423****	26.44 ± 1.2460***
IV	FECG II	0.211 ± 0.05459^{ns}	6.98 ± 0.01356****	7.341 ± 0.01696****	19.89 ± 0.02321****	14.18 ± 0.09453****	22.06 ± 0.4023***

Table 3: Effect of FECG post-treatment on Brain Neurochemicals in 6-OHDA lesioned rats

Groups	Drugs/extracts administered group	Dopamine	DOPAC	HVA
I	Sham-operated group	98.28 ± 0.86	102.2 ± 1.07	101.4 ± 1.01
II	6-OHDA-lesioned group	21.38 ±6.12****	230.8 ±5.23****	178.4 ±17.45****
III	FECG I	48.57 ±0.65****	184.9 ±4.37****	142.7 ±25.68***
IV	FECG II	76.63 ±6.42***	107.9 ± 1.17*	128.6 ± 0.36*

AlCl3-induced Alzheimer's disease Model

Table 4. Grouping of animals in AlCl3-induced Alzheimer's disease Model

Groups	Drugs/extracts	Post- treatment protocol	Route of Administration	Dose
I	Control group (Normal Saline)	90 days	p.o	0.9% w/v
II	Aluminium chloride (AlCl3)	First 30 days	p.o	17mg/kg
III	AlCl3 + rivastigmine	90 days	p.o	0.3mg/kg
IV	AlCl3 + FECG I	90 days	p.o	200mg/kg
V	AlCl3 + FECG II	90 days	p.o	400mg/kg

AD induced using AlCl3, p.o., at a 17 mg/kg b dose. wt., daily for one month.

Table 5: Effect of FECG treatment on change in body weight in AlCl3-induced rats

Groups	Drugs/extracts administered group	Change in body weight (g)
I	Control	121.60 ± 12.48
II	AlCl3	-44.70 ± 3.04****
III	AlCl3 + Rivastigmine	82.30 ± 12.39**
IV	AlCl3 + FECG I	66.75 ± 11.13***
V	AlCl3 + FECG II	78.65 ± 14.47**

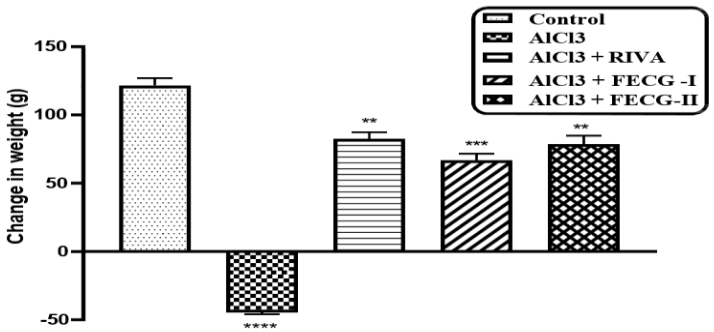


Fig.1: Effect of AlCl3 and FECG treatment on the change in body weight

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 6: Effect of FECG treatment on change in food intake in AlCl3-induced rats

Groups	Drugs/extracts administered group	Change in food intake (g)
I	Control	7.34 ± 0.51
II	AlCl3	-11.00 ± 0.57****
III	AlCl3 + Rivastigmine	0.85 ± 0.77****
IV	AlCl3 + FECG I	-6.66 ± 0.99****
V	AlCl3 + FECG II	-3.94 ± 0.61****

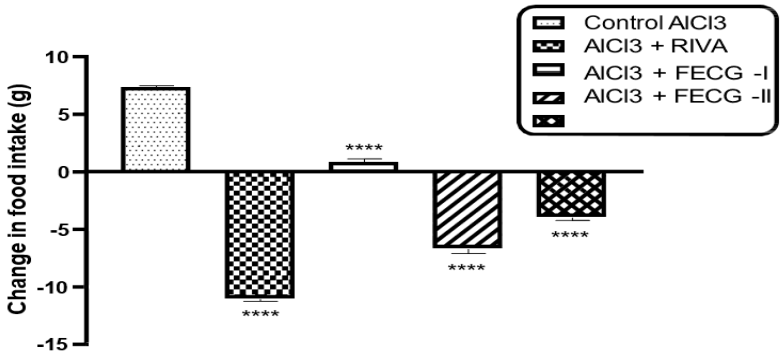


Fig. 2: Effect of AlCl3 and FECG treatment on the change in food intake

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 7: Effect of FECG treatment on change in water intake in AlCl3-induced rats

Groups	Drugs/extracts administered group	Change in water intake (ml/day)	
		Initial intake (ml)	Final intake (ml)
I	Control	7.99 ± 0.13	10.60 ± 0.36
II	AlCl3	12.00 ± 0.42^{ns}	7.93 ± 0.88****
III	AlCl3 + Rivastigmine	7.98 ± 0.12^{ns}	10.14 ± 0.38****
IV	AlCl3 + FECG I	6.50 ± 0.11^{ns}	7.92 ± 0.16****
V	AlCl3 + FECG II	7.88 ± 0.12^{ns}	10.51 ± 0.46****

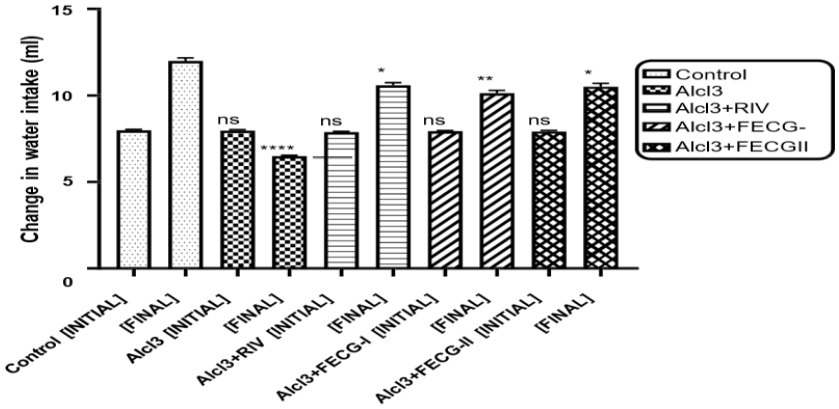


Fig.3: Effect of AlCl3 and FECG treatment on the change in water intake

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by repeated measures, one-way ANOVA, and Turkey's multiple comparison test.

Table 8: Effect of FECG treatment on change in urinary output in AlCl3-induced rats

Groups	Drugs/extracts administered group	Change in urinary output (ml/day)
I	**Control**	4.01 ± 0.41
II	**AlCl3**	-1.47 ± 0.10****
III	**AlCl3 + Rivastigmine**	2.72 ± 0.41**
IV	**AlCl3 + FECG I**	2.20 ± 0.37***
V	**AlCl3 + FECG II**	2.60 ± 0.47**

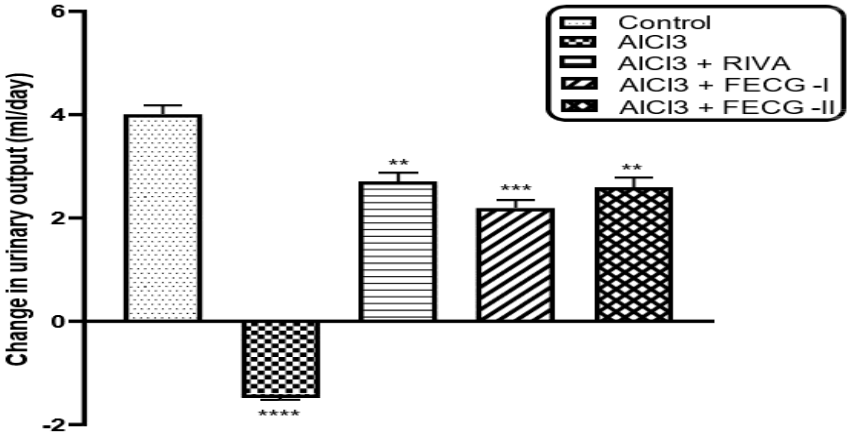


Fig.4: Effect of AlCl3 and FECG treatment on the change in urinary output

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 9: Effect of FECG treatment on change in urinary sodium in AlCl3-induced rats

Groups	Drugs/extracts administered group	Change in urinary sodium (mEq/day)
I	**Control**	0.05 ± 0.58
II	**AlCl3**	-0.37 ± 0.06***
III	**AlCl3 + Rivastigmine**	-0.03 ± 0.02^{ns}
IV	**AlCl3 + FECG I**	-0.24 ± 0.02****
V	**AlCl3 + FECG II**	-0.10 ± 0.04**

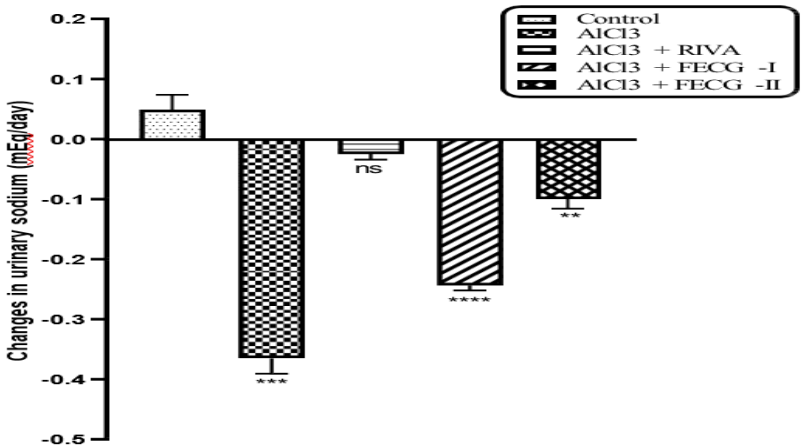


Fig.28 Effect of AlCl3 and FECG treatment on the change in urinary sodium

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by Repeated measures, one-way ANOVA and Dunnett's post hoc comparison test.

Table 10: Effect of FECG treatment on change in urinary potassium in AlCl3- induced rats

Groups	Drugs/extracts administered group	Change in urinary potassium (mEq/day)
I	**Control**	0.04 ± 0.03
II	**AlCl3**	-0.41 ± 0.09***
III	**AlCl3 + Rivastigmine**	0.01 ± 0.03^{ns}
IV	**AlCl3 + FECG I**	-0.21 ± 0.05***
V	**AlCl3 + FECG II**	-0.05 ± 0.06*

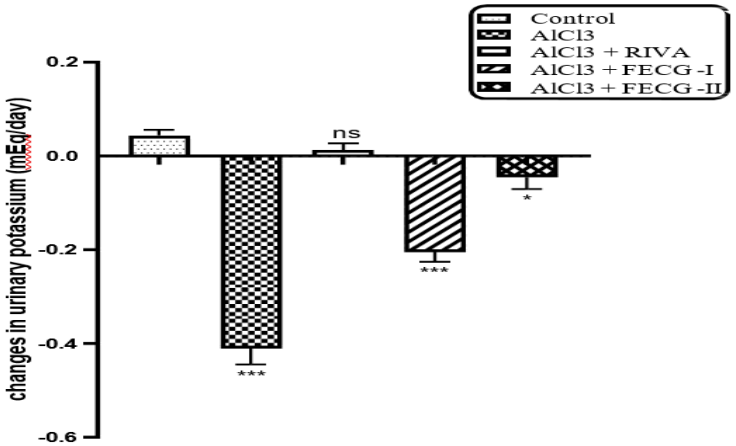


Fig.5: Effect of AlCl3 and FECG treatment on the change in urinary potassium

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 11: Effect of FECG treatment on Morris Water Maze in AlCl3-induced rats

Groups	Drugs/extracts administered group	Duration of mean escape latency (sec)				
		Day 1	Day 10	Day 30	Day 60	Day 90
I	**Control**	45.57	32.11	22.12	16.84	16.54
II	**AlCl3**	47.35	45.38	42.51	46.8	48.12
III	**AlCl3 + Rivastigmine**	46.86	32.14	23.32	18.31	17.56
IV	**AlCl3 + FECG I**	47.32	39.23	27.12	31.13	29.16
V	**AlCl3 + FECG II**	46.12	33.13	23.16	19.42	17.93

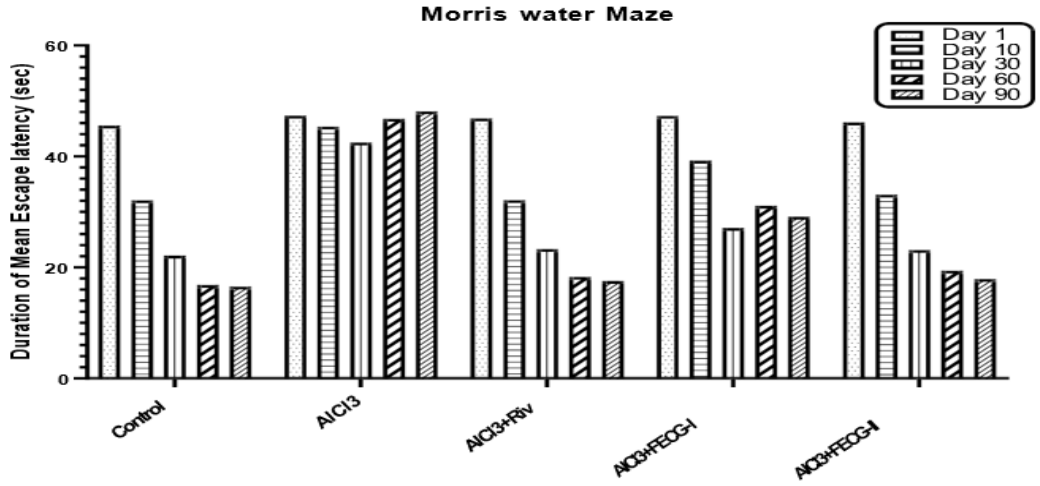


Fig.6: Effect of AlCl3 and FECG post-treatment on the duration of mean escape latency of rats

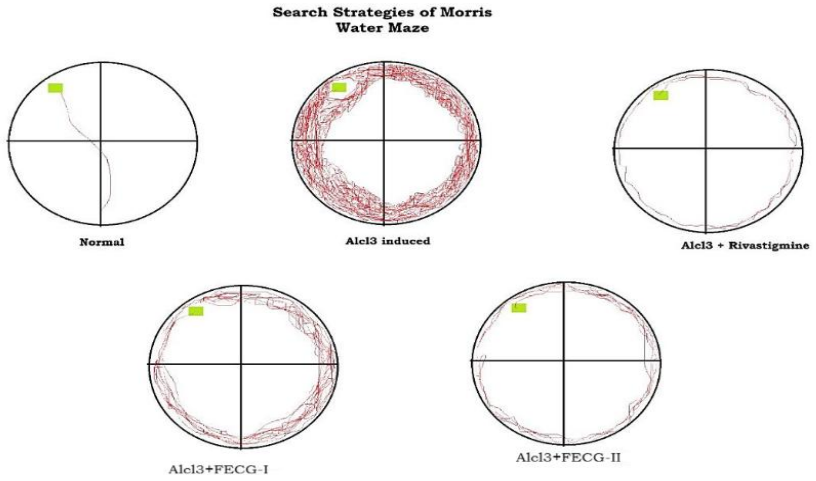


Fig.7: Effect of FECG treatment in the search strategies of Morris water maze test using AlCl3-induced Alzheimer model in rat

Table 12: Effect of FECG treatment on step-through latency of passive avoidance in AlCl3-induced rats

Groups	Drugs/extracts administered group	Step through latency (sec)	
		Acquisition Trial	Retention Trial
I	Control	30.33 ± 4.97	235.8 ± 5.49
II	AlCl3	41.00 ± 2.19**	103.7 ± 2.16****
III	AlCl3 + Rivastigmine	24.83 ± 5.12^{ns}	215.3 ± 2.16**
IV	AlCl3 + FECG I	34.50 ± 11.15^{ns}	155.3 ± 21.56***
V	AlCl3 + FECG II	33.33 ± 5.57*	205.7 ± 1.63***

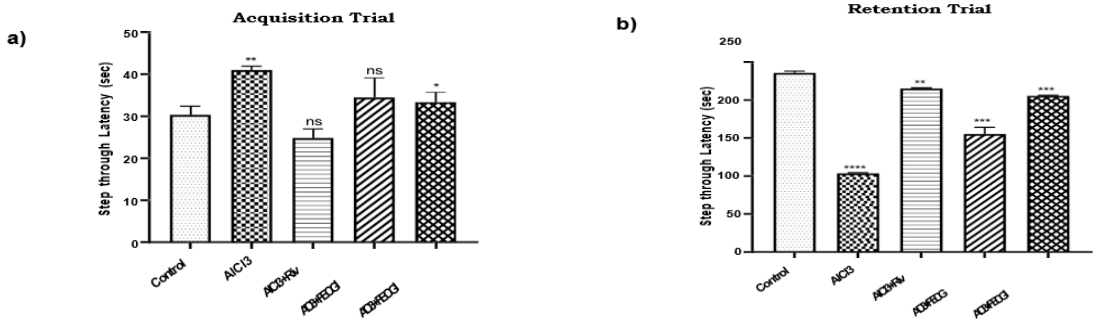


Fig.8: Effect of AlCl3 and FECG treatment on step-through latency in passive avoidance test

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 13: Effect of FECG treatment on no. of crossings of passive avoidance in AlCl3-induced rats

Groups	Drugs/extracts administered group	Step through latency (sec)	
		Acquisition Trial	Retention Trial
I	Control	2.17 ± 0.98	1.17 ± 0.41
II	AlCl3	4.00 ± 0.89***	3.17 ± 0.41**
III	AlCl3 + Rivastigmine	1.50 ± 0.55^{ns}	1.33 ± 0.52^{ns}
IV	AlCl3 + FECG I	2.17 ± 0.41^{ns}	2.00 ± 0.89^{ns}
V	AlCl3 + FECG II	2.17 ± 0.98^{ns}	1.67 ± 0.82^{ns}

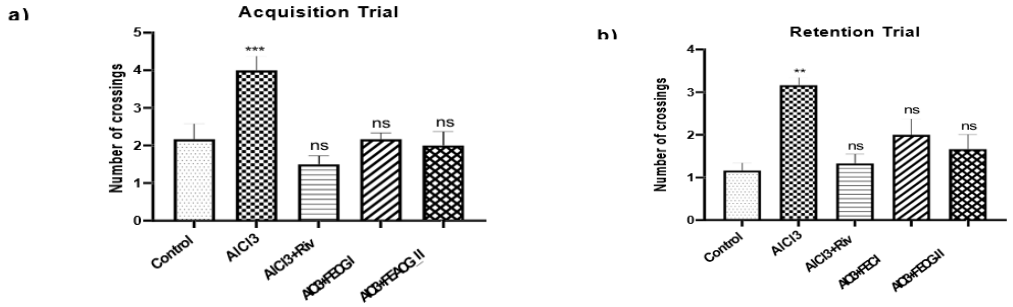


Fig.9: Effect of AlCl3 and FECG treatment on the no. of crossings in passive avoidance test

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 14: Effect of FECG Treatment on Brain Antioxidants in AlCl3-induced Rats

Groups	Drugs/extracts administered group	LPO	SOD	CAT	GSH	TP	NO
I	Control	0.62 ± 0.06	3.13 ± 0.23	1.89 ± 0.15	3.19 ± 0.30	6.11 ± 0.05	1.89 ± 0.27
II	AlCl3	1.42 ± 0.08****	0.87 ± 0.13****	0.71 ± 0.13****	0.87 ± 0.02****	4.41 ± 0.19****	3.73 ± 0.04****
III	AlCl3 + Rivastigmine	0.77 ± 0.15^{ns}	2.15 ± 0.10**	1.59 ± 0.06**	2.18 ± 0.13**	5.98 ± 0.07*	2.67 ± 0.06**
IV	AlCl3 + FECG I	1.26 ± 0.22***	1.98 ± 0.08***	0.92 ± 0.60****	1.94 ± 0.05***	5.11 ± 0.09****	3.05 ± 0.03***
V	AlCl3 + FECG II	0.86 ± 0.10***	2.04 ± 0.06***	1.48 ± 0.05**	2.04 ± 0.03***	5.83 ± 0.11**	2.91 ± 0.04**

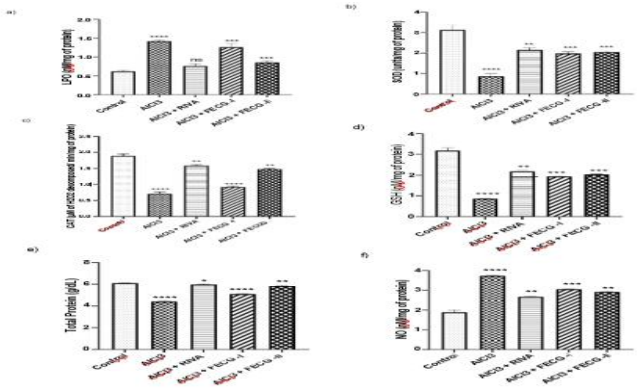


Fig.10: Effect of AlCl3 and FECG treatment on the Brain Antioxidants level

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Table 15: Effect of FECG Treatment on Brain Neurochemicals in AlCl3-induced Rats

Groups	Drugs/extracts administered group	AchE	Na^+K^+ATPase
I	Control	1.55 ± 0.08	4.08 ± 0.07
II	AlCl3	3.75 ± 0.13	2.48 ± 0.02
III	AlCl3 + Rivastigmine	1.78 ± 0.07	4.02 ± 0.02
IV	AlCl3 + FECG I	2.13 ± 0.14	3.44 ± 0.02
V	AlCl3 + FECG II	1.69 ± 0.10	3.80 ± 0.06

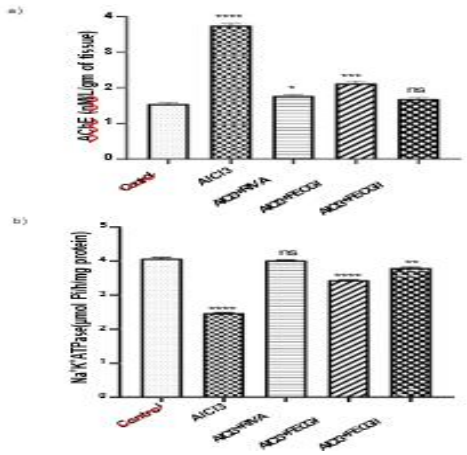


Fig.11: Effect of AlCl3 and FECG treatment on the Brain neurochemical level

Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

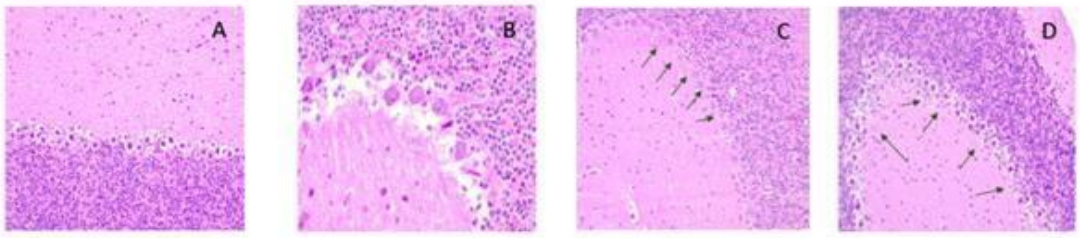


Fig.12: Histopathology of 6-OHDA-induced Parkinson's disease model

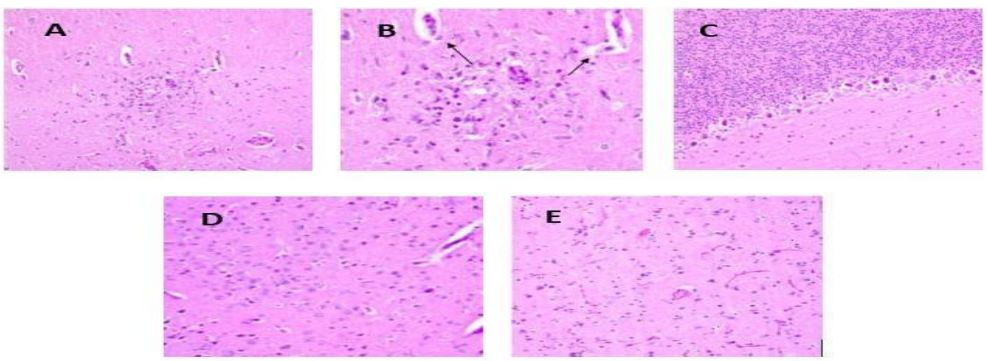
Histopathology

a) Forebrain

The effect of FECG and 6-OHDA on the density of neurons in the forebrain region of 6-OHDA lesioned rat brains. Photomicrographs of cresyl-violet stained forebrain sections. (Panel a) Sham-operated control group showing healthy neurons; (Panel b) 6- OHDA-treated group showing signs of neuronal death with a marked reduction in the glial cells and neuronal density located in the forebrain region; (Panel c) FECG-I (200 mg/kg) little protective effect on 6-OHDA-induced neurodegeneration; (Panel d) FECG-II (400 mg/kg) per treatment showing intact neurons; (magnification:40×).

b) Brain Stem

The effect of FECG and 6-OHDA on the neuronal density in brainstem regions of lesioned rat brains. Photomicrographs of cresyl-violet stained brain stem sections. (Panel a) Sham-operated control group showing healthy neuronal cells with clusters of nerve nuclei surrounded by glial cells; (Panel b) 6-OHDA treated group showing signs of neuron death with condensed nuclei, vacuolation and chromatolysis, necrosis of nerve nuclei with reactive astrogliosis; (Panel c) FECG I (200 mg/kg) showing clusters of nerve nuclei surrounded by less reactive astrogliosis glia cells; (Panel d) FECG II (400 mg/kg.) showing normal cells with clusters of nerve nuclei and also it shows more protection against 6-OHDA-induced parkinsonism by reducing neuron swelling and vacuolation (magnification:40×).



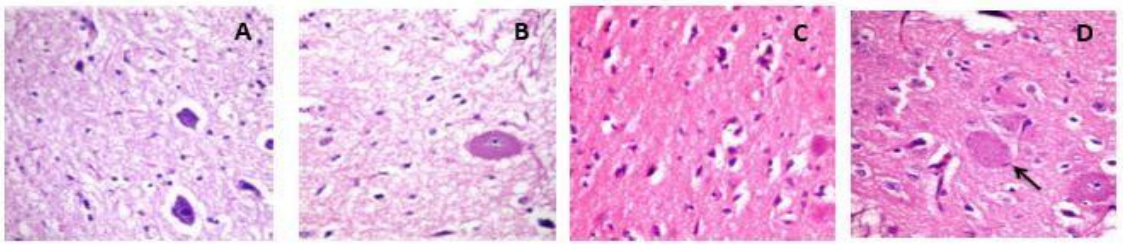


Fig.13 Histopathology of AlCl3-induced Alzheimer's disease model

The effect of FECG and AlCl3 on the neuronal density in the brain regions of rat brains. Photomicrographs of haematoxylin and eosin stained brain sections. (Panel a) Control group showing healthy neuronal cells with clusters of nerve nuclei ; (Panel b) AlCl3 treated group showing signs of neuron death with deteriorated cells with plague formation; (Panel c) AlCl3 + Rivastigmine showing healthy neuronal cells with clusters of nerve nuclei; (Panel d) FECG I (200 mg/kg) showing clusters of nerve nuclei with reduction of deteriorated cells; (Panel e) FECG II (400 mg/kg.) showing normal nerve nuclei with prominent reduction of deteriorated cells (tangles formed cells) and vacuolation (magnification:40×).

Statistical Analysis

Statistical analysis was done using GraphPad Prism software version 9.0. Data are shown as Mean ± SEM; n = 06 rats for each group with p values denoted as *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, which are compared with control groups. Statistical analysis was done using one-way and two-way ANOVA and post hoc comparison tests with Dunnett's and Turkey's tests.

Discussion:

The present study demonstrates that the ethanolic fruit extract of *Coccinia grandis* (FECG) exhibits significant neuroprotective potential in validated animal models of Parkinson's disease (PD) and Alzheimer's disease (AD). The phytochemical analysis revealed a rich presence of alkaloids, phenolics, flavonoids, tannins, steroids, saponins, glycosides, and mucilage, constituents commonly associated with antioxidant and neuroprotective properties. High levels of total phenolic (302.82 mg GAE/g) and flavonoid (264.64 mg CE/g) content correlate with the strong radical scavenging activity demonstrated in vitro, suggesting that the antioxidant capacity of FECG may serve as a key mechanism underlying its neuroprotective actions.

In the 6-OHDA PD model, FECG markedly improved behavioral outcomes, including motor coordination, rotarod performance, grip strength, and narrow-beam traversal times. These behavioral improvements were supported by neurochemical findings, where FECG prevented dopamine depletion and normalized DOPAC and HVA levels, indicating restoration of dopaminergic neurotransmission. Furthermore, FECG reduced lipid peroxidation (LPO) and enhanced endogenous antioxidant enzymes such as SOD, CAT, GSH, and GPx, suggesting that oxidative stress mitigation plays a central role in its anti-Parkinsonian activity. Histopathological examination reinforced these findings by showing reduced neuronal degeneration and restored cellular architecture in both forebrain and brainstem tissues following treatment. Similarly, in the AlCl3-induced AD model, FECG significantly ameliorated body weight loss, food and water intake disturbances, and urinary biochemical alterations, indicating improved systemic physiology. Importantly, FECG enhanced cognitive performance in the Morris Water Maze and passive avoidance tests, demonstrating preserved spatial learning, memory retention, and exploratory behaviors. Biochemically, FECG lowered LPO levels while improving SOD, CAT, GSH, and total protein, indicating robust antioxidant responses in AD conditions. Additionally, reduced acetylcholinesterase activity and improved Na^+/K^+-ATPase function reflect restored cholinergic transmission and membrane integrity, essential for cognitive function. Histopathological analysis further confirmed neuroprotection, showing decreased neuronal loss, reduced vacuolation, and prominent reduction in plaque-like deteriorated cells.

Collectively, these findings suggest that the neuroprotective efficacy of FECG is multifactorial, involving antioxidant defense enhancement, dopaminergic and cholinergic neurotransmission restoration, reduction of neuroinflammation, and structural protection of neuronal tissues. The presence of diverse bioactive molecules (e.g., trigonelline, coumarin derivatives, DHEA, kojic acid, and quinazolinone analogues) likely contributes synergistically to these therapeutic effects. The extract's favorable safety profile supports its potential development as a phytopharmacological agent for managing neurodegenerative diseases.

Summary

This study evaluated the phytochemical composition and neuroprotective effects of *Coccinia grandis* fruit extract in animal models of PD and AD. The extract

contained abundant neuroactive constituents and demonstrated strong antioxidant capacity. In PD rats, FECG improved motor behavior, restored dopamine levels, and reduced oxidative stress. In AD rats, it enhanced cognitive function, normalized physiological parameters, and protected neuronal structure. Biochemical analyses confirmed enhanced antioxidant defenses and improved neurotransmitter-related enzyme activity. Overall, FECG displayed significant therapeutic potential across both models.

Conclusion

The findings from the present investigation establish that *Coccinia grandis* fruit extract possesses potent antioxidant, anti-Parkinsonian, and anti-Alzheimer's activities. Its efficacy is mediated through restoration of neurotransmitter balance, reduction of oxidative stress, improvement of behavioral and cognitive functions, and preservation of neuronal integrity. Given its safety and broad-spectrum neuroprotective effects, FECG holds promise as a natural therapeutic candidate for managing neurodegenerative disorders. Further mechanistic, toxicological, and clinical studies are warranted to advance its development.

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