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EVALUATION OF HYDRO-ALCOHOLIC EXTRACT OF *PORTULACA OLERACEA* SEEDS AGAINST D-GALACTOSE INDUCED ATTENTION DEFICIT HYPERACTIVITY DISORDER MODEL ON SWISS ALBINO MALE MICE

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ABSTRACT
D-galactose accumulation in brain is one of the major cause for neuronal cell death. Thus the objective of the present study is to evaluate the hyperactivity reducing and cognitive improvement effect of Portulaca oleracea seed extract against D-galactose induced Attention deficit hyperactivity disorder in Swiss albino male mice. Portulaca oleracea seed extract was a highly efficient for treating ADHD and also less toxic.

KEYWORDS: Attention deficit hyperactive disorder, D-galactose, Atomoxetine, Portulaca oleracea.

INTRODUCTION

ADHD is one of the most common neurodevelopmental disorders of childhood. It is usually first diagnosed in childhood and often lasts into adulthood. Children with ADHD may have trouble paying attention, controlling impulsive behaviors (may act without thinking about what the result will be), or be overly active.

The exact pathophysiology of Attention Deficit Hyperactivity Disorder (ADHD) is not clear. With this said, several mechanisms have been proposed as factors associated with the condition. These include abnormalities in the functioning of neurotransmitters, brain structure and cognitive function.

The frontal and prefrontal regions of the brain, as well as possibly the parietal lobe and cerebellum, are thought to be associated with ADHD. These structural areas have been identified with magnetic resonance imaging (MRI), as some research has shown that children with ADHD tended to have altered activation of the brain when performing certain tasks.

ADHD is associated with **abnormally low levels of the neurotransmitters transmitting between the prefrontal cortical area and the basal ganglia i.e., dopamine and noradrenaline**. Dopamine is closely associated with reward centers in the brain, and also interacts with other potent neurotransmitters to regulate mood.

The present study is to prove the memory enhancement and cognitive effect of Portulaca olearacea in ADHD induced by D-galactose in swiss albino male mice using various memory retention experiments such as Elevated Zero Maze, Morris water maze, Pole climbing apparatus, Marble burying behaviour, Beam balance, etc.[1]

METHODOLOGY

COLLECTION ANDAUTHENTICATIONOFPLANTS

Seeds of *PORTULACA OLERACEA* were collected from lake area of Villupuram district. They were duly Authenticated by Dr.K.N.Sunil Kumar from Siddha Central Research Institute, Arumbakkam, Chennai-600106.

PREPARATION OF PLANT EXTRACT

Seeds of *PORTULACA OLERACEA* will be removed from impurities, shade dried, ground into coarse powder individually. This Coarse powder (1 kg) of each plant material were extracted with mixture of ethanol: water (70:30) in Soxhlet extractor. The solvent was completely removed by rotary vacuum evaporator. A dark greenish extract was obtained The extract will be freeze dried stored in a refrigerator at 0-4^{0}C for further use. The yield of the extract was calculated as 75.5%w/w.[2]

PRELIMINARYPHYTOCHEMICAL ANALYSIS

The hydroalcoholic extracts of portulaca oleracea seed was subjected to preliminary Phytochemical screening

for the presence or absence of phytoconstituents by the following methods.

TEST FOR ALKALOIDS[3]

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d) Wagner's test (Iodine-Potassium Iodide Solution)

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

TEST FOR CARBOHYDRATES[4]

a) Molisch's test

The extract was treated with 3ml of alpha-naphthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test (CuSO4.7H2O+KOH+Potassium Tartarate)

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non reducing sugars.

TEST FOR STEROIDS[5]

a) Libermann Burchard test

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids.

TEST FOR PROTEINS

a) Biuret's test

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

TEST FOR TANNIN'S[6]

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

TEST FOR PHENOLS[7]

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

TEST FOR FLAVONOID'S[8]

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and 1 ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta color indicates the presence of flavonoids.

TEST FOR GUMS AND MUCILAGE[9]

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

TEST FOR GLYCOSIDES[10]

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation

of red ring at the junction of two liquids indicates the presence of glycosides.

TEST FOR SAPONINS[11]

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

TEST FOR TERPENES[12]

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

TEST FOR STEROLS[13]

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

EXPERIMENTAL ANIMAL USED FOR INVESTIGATION

The **Swiss albino male mice** weighing 20-30gm were used for this study. The Swiss albino male mice were procured from Mass Biotech Pvt Ltd and caged in the animal house of C.L.Baid Metha college of pharmacy, Thoraipakkam, Chennai- 97. They were housed six per cage under standard laboratory conditions at a temperature 17-23°C with 12:12 hrs light and dark circle. The animals were provided with standard animal feed, water and *ad libitum*. The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This study was approved by Institutional Animal Ethical Committee (IAEC)

Approvalno:03/321/PO/Re/S/01/CPSCEA-dated29/11/2022validupto30/11/2023.

HUSBANDRY CONDITIONS

Animals were housed under standard laboratory conditions, air conditionedwith12-15 filtered fresh air changes/hour. Environment: temperature 17-23°C, relative humidity30-70%, with12 hours fluorescent light (6.00 am to 6.00 pm) and 12 hours dark cycle.

HOUSING

Mice were housed individually in standard polypropylene cages (size: approximately L 410 x B 280xH 140mm), with stainless steel top grill having facilities for pellet food and drinking water in glass bottle.

DIETAND WATER

The control and negative control animals were provided with standard animal feed and water *ad libitium.* Animals were fed with hygienic feed and pure water. The animals were adapted to laboratory conditions one week prior to initiations of experiments.

EXPERIMENTAL PROCEDURE

Animals–30Numbers
Species–Swiss albino male mice
Weight- 20-30 gm
Gender–male
Groups – 5Groupsof6 mice.

PROCEDURE[14]

Thirty swiss albino male mice was used for the study. They were maintained in a well-ventilated room under standard conditions of temperature 27 ± 2°C, relative humidity of 60 ± 5% with 12:12 hour light:dark cycle in polypropylene cages. Standard pellet feed and water ad libitum was provided to the animals. Mice were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethics committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). This study was approved by Institutional Animal Ethical Committee (IAEC).

IAEC Approval no:03/321/PO/Re/S/01/CPSCEA-dated29/11/2022validupto30/11/2023.

TREATMENTPROTOCOL

Swiss albino male mice will be randomly divided into five groups with 6 mice in each group. Group I will serve as normal control and will be given 1 ml/kg saline orally for 21 days. Group II will serve as disease model and will receive D-galactose only for 21 days. Group III will serve as Standard D-galactose and atomoxetine (1 mg/kg) for 21 days. Groups IV and V will serve as treatment groups and receive D-galactose and portulaca oleracea seed extract in two different doses (200mg and 400mg/kg) 21 days orally.

Animals are divided into five groups with 6 mice in each group, n=6.

GROUPING

SNO	GROUPING	TREATMENT
1	GROUP I	Control (untreated)
2	GROUP II	D- Galactose (50mg/kg) SC
3	GROUP III	D-Galactose + Atomoxetine HCL (1mg/ml/kg) PO
4	GROUP IV	D-Galactose + portulaca oleracea seed (200mg/kg) PO
5	GROUP V	D-Galactose + Portulaca Oleracea seed (400mg/kg) PO

EVALUATION PARAMETERS
IN VIVO PARAMETERS
All the parameters were assessed on day 7, day 14 and day 21.

a) ASSESSMENT OF IMPULSIVE BEHAVIOR & HYPERACTIVITY
1. Locomotor Activity Test[15]
Locomotor activity (horizontal activity) was measured using actimeter. Actophotometer, which operates on photoelectric cells, connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each mice pup was placed individually in the activity cage floor for 10 min and the locomotion count was directly read from the digital reading displayed in the actimeter. Actimeter is the combination of hole board and actimeter in which both rats and mice can be placed.Impulsive behaviour was assessed on day 7, 14, 21.

2. Elevated Zero Maze[16]
Elevated zero maze is a modification of the elevated plus maze model of anxiety in rodents, which incorporates both traditional and novel ethological measures in the analysis of drug effects. The elevated zero-maze constitutes a modification in both design and procedure which aims to improve upon the traditional model by increasing the sensitivity to, and facilitating interpretation of, drug action. The novel design comprises an elevated annular platform with two opposite, enclosed quadrants and two open quadrants, and addresses one potentially problematic issue inherent in the plus-maze design, i.e., the unavoidable ambiguity associated with time spent on the centralsquare, removing any ambiguity in the interpretation and allowing uninterrupted exploration. Transfer latency was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. Impulsive behaviour was assessed on day 7, 14, 21.

b) ASSESSMENT OF COGNITIVE BEHAVIOR
1. Morris water maze (MWM)[17]
Behavioural tests were conducted using a Morris water maze (117 cm in diameter, and 50 cm high) in an experimental room. The pool was filled with water to a depth of 40 cm and a temperature of 23 ± 2°c. The water was made opaque with any suitable non-toxic ingredient to prevent the Platform from being visible. The position and orientation of the pool in the testing room remained unchanged throughout the study. Four quadrants on the rim of the pool were designated as north (N), south (S), east (E) and west (W), thus dividing the pool into four quadrants (NW, NE, SE and SW). A removable 8 cm escape platform was placed 2 cm below the water's surface in the centre of any one of the quadrants. Cognitive function was assessed on day 7, 14, 21.

2. Pole Climbing Test[18]
Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. The apparatus has an experimental chamber (25 × 25 × 25 cm) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The study mice pup was placed in the chamber and allowed to explore the chamber for 60 seconds. Conditioned stimulus (CS) i.e. buzzer signal was turned on and unconditioned stimulus (US) i.e. electric shock delivered through grid floor for 60 Sec. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response was defined as climbing reaction time <10 Sec only; and escape response was climbing after applying reaction time >10 Sec. Every rat was subjected to relearning trials (2nd day 3 trials and on third day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response. Animals were screened by using this model and those who demonstrated at least one escape response either at least one escape response either on day one or two were included in the study. Cognitive function is assessed on day 7, 14, 21.

d) ASSESSMENT OF SKELETAL MUSCLE ACTIVITY
1. Rota rod[19,20]
Animals are placed individually in separate lanes on rod rotating at 5rpm such that animals may walk forward to keep balance. After 60s on rod, animals are returned to home cage and apparatus is cleaned with virkon between trials. Procedure is repeated for three trials separated by 10 min inter trial intervals. Trial three may be repeated once if animal falls off rod before 60s cut-off, but no more than four trials should be run per animal. Subjects must be able to stay on rod rotating at 5rpm for 60s before proceeding to testing. After providing the training to the animals, they were used for experimental procedure. The rotating rod is allowed to rotate at 25 rpm and animals from each group are placed individually on the rod and the fall off time was recorded for each animal. Total eight groups were involved and their average fall off time was concluded using MEAN+SEM. Skeletal muscle activity is assessed on day 7, 14, 21.

e) OTHER PARAMETERS
1. Dark-Light Compartment[21]
The apparatus used for the light/dark transition test consisted of a cage (21x42x25 cm) divided into two sections of equal size by a partition with door. Mice are housed six per cage in a room with a 12 hr light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water. All the cages containing mice are transferred to the behaviour testing room 30 min before the first trial

begins. One chamber is brightly illuminated by white diodes (390 lux), whereas the other chamber is dark (2 lux). Mice are placed into the dark side and the door is opened manually 3 seconds. The door is used so that the rat do not enter the light chamber immediately after the releasewith their motivation to escape from experimenter, since the latency to enter the light chambermay serve as an index of anxiety-like behaviour. Mice are allowed to move freely between the two chambers with door open for 10 min. The total number of transitions, the time spent in the each chamber, no of rearing's and the latency to enter the light chamber is recorded manually. After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues. The activity was assessed on day 7, 14, 21.

2. Marble Burying Behaviour[22]

Place one mice pup into a corner of the cage containing marbles, being careful to place the rat on bedding as far from marbles as possible, and place the filter-top cover on the cage. Withhold food and water during the test. Allow mice pup to remain in the cage undisturbed for 30 min. Remove the mice and return it to its home cage after test completion, taking extreme care not to move or dislodge the marbles in the process of removing the subject from the cage. Score a marble as buried if two-thirds of its surface area is covered by bedding. Average scores for the number of marbles buried for each rat pup was recorded manually. Retrieve all 20 marbles from the bedding. Dispose of bedding. The activity was assessed on day 7, 14, 21.

NEUROTRANSMITTERS ESTIMATION[23]

PREPARATION OF TISSUE EXTRACTS

Reagents

Hcl-butanol

Heptanes

0.1M Hcl: (0.85ml conc. Hcl upto 100ml of water)

Procedure

On the day of experiment swiss albino male mice were sacrificed, whole brain was dissected out and the subcortical region (including the striatum) was separated. Tissue was weighed and homogenized in 5ml HCl-butanol for about 1 min followed by the sample was then centrifuged for about 10 min at 2000rpm. An aliquot supernatant phase (1ml) was removed and added to Eppendorf tube containing 2.5ml heptane and 0.31 ml of 0.1M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken for the dopamine, serotonin and norepinephrine assay. All the steps were carried out at 0°C. it was taken in between 50-75 mg of the tissue for homogenate with 5ml of HCl-butanol in correlation of same tissue concentration of 0.1ml of HCl-butanol in Schlumpf method.

1. ESTIMATION OF DOPAMINE[24]

Reagents

0.4M Hcl:3.4ml conc. Hcl upto 100ml water

Sodium acetate buffer pH(6.9)

5M NaOH

0.1M Iodine solution (in ethanol)

Sodium thiosulphate

10M acetic acid: 57ml of glacial acetic acid dissolved in distilled water upto100ml

Procedure

To the 0.2ml of aqueous phase, 0.5ml 0.4M HCl and 0.1ml of EDTA/sodium acetate buffer (pH6.9) were added, followed by 0.1ml iodine solution (0.1M in ethanol) in oxidation. The reaction was stopped after 2 min by addition of 0.1ml Sodium thiosulphate solution. 0.1ml acetic acid is added after 1.5 min. the solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-370nm.)

Test concentration = intensity of test x concentration of standard

Intensity of standard

2. ESTIMATION OF SEROTONIN[25]

The serotonin content was estimated by the method of Schlumpf.

Reagents

1. O-phthalaldialdehyde (OPT) reagent.

Procedure

To 0.2ml of aqueous extract 0.25ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. after the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectrofluroimeter. For serotonin tissue blank, 0.25ml conc. HCl without OPT was added. Internal standard: 500µg ml each of noradrenaline, dopamine and serotonin are prepared in distilled water. HC1 -butanol in 1:2 ratios.

Test concentration = intensity of test x concentration of standard

Intensity of standard

3. ESTIMATION OF NOREPINEPHRINE[26]

Reagents

0.4M HCl: 3.4ml conc. HCl upto 100ml water

Sodium acetate buffer pH (6.9)

5M NaOH:

0.1M Iodine solution (in ethanol)

Sodiumthiosulphate

10M acetic acid: 57ml of glacial acetic acid dissolved in distilled water upto 100ml.

Procedure

To the 0.2ml of aqueous phase, 0.5ml 0.4M HCl and 0.1ml of EDTA/sodium acetate buffer (pH6.9) were

added, followed by 0.1ml iodine solution (0.1M in ethanol) in oxidation. The reaction was stopped after 2 min by addition of 0.1ml Sodium thiosulphate solution. 0.1ml acetic acid is added after 1.5 min. the solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-370nm.

4. ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)[27]

Reagents

Carbonate buffer (100mM, pH 10.2)
Epinephrine (3mm)

Procedure

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480nm in spectrophotometer for 2min at an interval of 15sec. Parallel blank and standard were run for determination of SOD activity.

One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	Uninhibited (Standard)	Inhibited (sample)	Blank
Carbonate buffer	0.900ml	0.800ml	1.0ml
Supernatant	-	0.1ml	-
Epinephrine	0.1ml	0.1ml	-

The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer.

5. INVITRO NITRIC OXIDE RADICAL (NO) SCAVENGING ASSAY[28]

Reagents

Sodium nitroprusside (SNP)
Phosphate buffered saline (pH 7.3)
Griess reagent

Procedure

NO generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al. (1994). Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the plant extract at different concentrations,was incubated at 25°C for 180 min in front of a visible polychromatic light source (25 W tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride).

The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

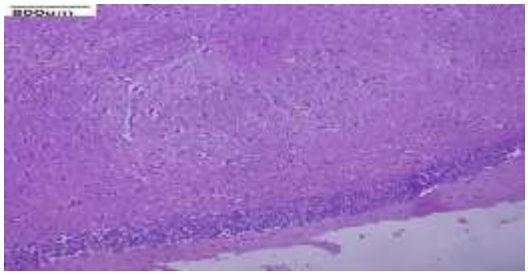
Reagents	Uninhibited (Standard)	Inhibited (sample)	Blank
Carbonate buffer	0.900ml	0.800ml	1.0ml
Supernatant	-	0.1ml	-
Epinephrine	0.1ml	0.1ml	-

HISTOPATHOLOGY OF BRAIN[29,30,31]

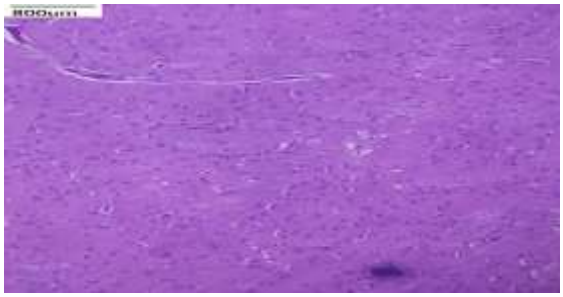
The mice from each group were anaesthetized using intraperitoneal injection of thiopentone sodium. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice cold normal saline and fixed in 10% formalin. Paraffin embedded sections were taken 100µm thickness and processed in alcohol-xylene series and stained with haemoxylin-eosin dye. The sections were examined microscopically for histopathological changes in the hippocampal zone.

HISTOPATHOLOGICAL REPORT OF BRAIN

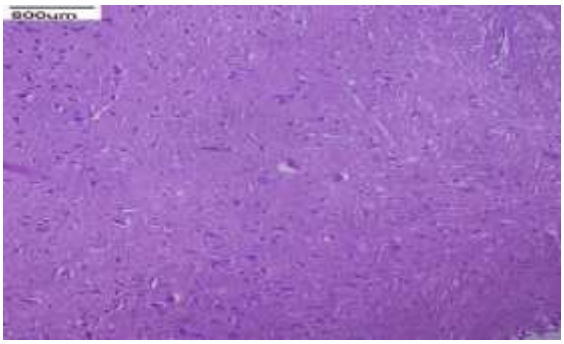
It was observed that there was decrease in the density of neuronal cells and disrupted in the normal distribution of neuronal cells in group II animals with the respect to group I animals. Treatment groups (groups III, IV,V) exhibited improved neuronal configuration and density than groupII.



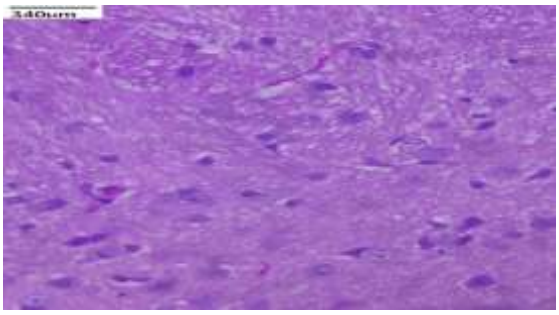
GROUP 1- CONTROL



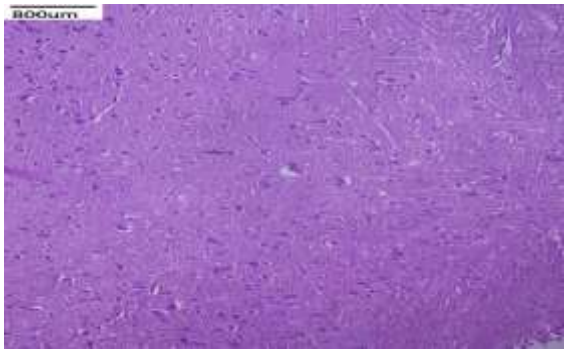
GROUP 2- D-GALACTOSE TREATED.



GROUP 3- STANDARD DRUG(ATOMOXETINE).



GROUP 4-LOW DOSE OF PORTULACA OLERACEA EXTRACT.



GROUP-5 HIGH DOSE OF PORTULACA OLERACEA EXTRACT.

STATISTICAL ANALYSIS[32]

The statistical analysis was carried by one way ANOVA followed by Dunnet's t test. P values <0.05 (95% confidence limit) was considered statistically significant, using Software Graph pad Prism 9.

RESULTS

EFFECT OF PORTULACA OLERACEA EXTRACT IN ELEVATED PLUS MAZE

S.NO	TREATMENT GROUPS	TIME SPEND IN OPEN ARM (SEC)		
		DAY 7	DAY 14	DAY 21
1	Control	25.30 ± 0.84^{ns}	28.30 ± 0.845^{ns}	29.30 ± 0.845^{ns}
2	D-galactose Treated	47.07 ± 1.35^{ns}	45.07 ± 1.35^{ns}	44.27 ± 1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	45.28 ± 0.79^{ns}	33.071 ± 0.5^{ns}	14.11 ± 1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	33.54 ± 1.69^{ns}	23.03 ± 1.35^{ns}	19.05 ± 1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	23.15 ± 0.40^{ns}	17.07 ± 1.44^{ns}	8.09 ± 1.85^{ns}

Values are represented in Mean ± SEM, n=6

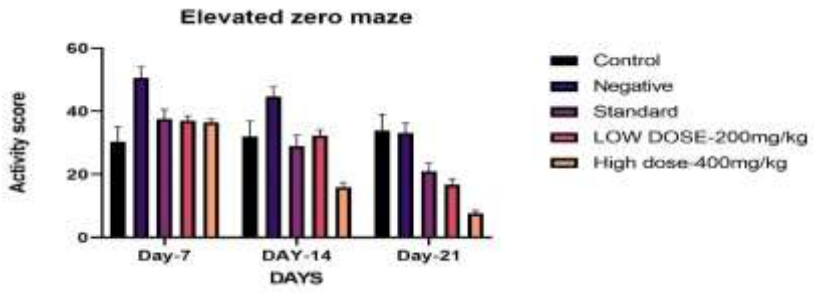
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001

Comparison:

a - Group I vs Group II, Group III, Group IV, Group V

b - Group II vs Group III, Group IV, Group V

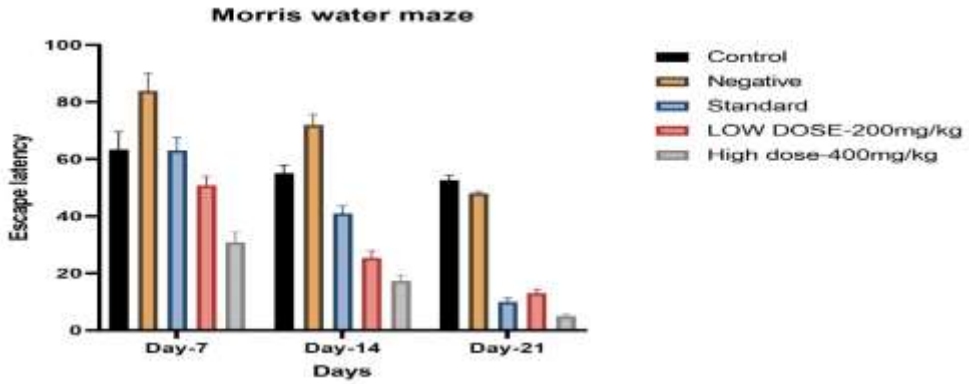
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN MORRIS WATER MAZE

S.NO	TREATMENT GROUPS	ESCAPE LATENCY (seconds)		
		DAY 7	DAY 14	DAY 21
1	Control	62.30±0.84^{ns}	55.30±0.845^{ns}	51.30±0.845^{ns}
2	D-galactose Treated	63.07±1.35^{ns}	56.07±1.35^{ns}	49.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	57.28±0.79^{ns}	31.071±0.5^{ns}	10.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	51.54±1.69^{ns}	32.03±1.35^{ns}	12.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	40.15±0.40^{ns}	12.07±1.44^{ns}	6.09±1.85^{ns}

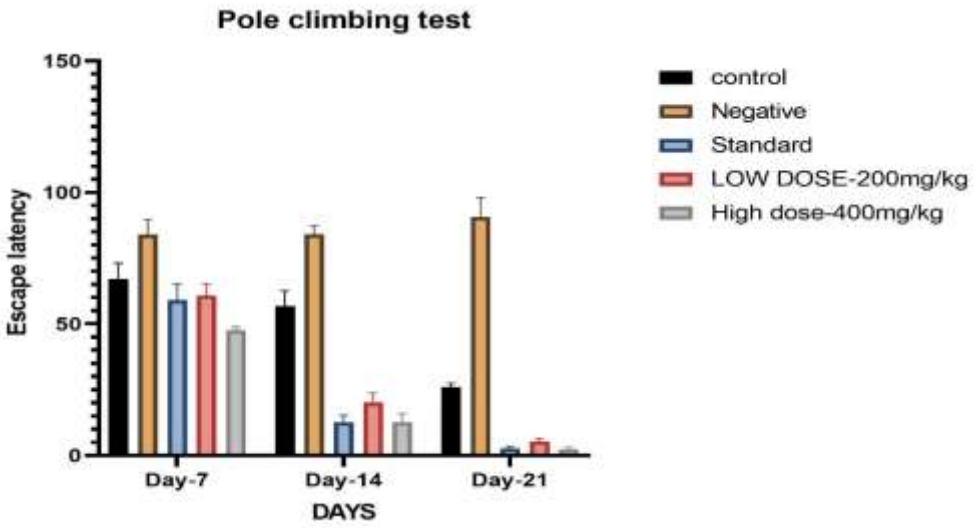
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN POLE CLIMBING TEST

S.NO	TREATMENT GROUPS	ESCAPE LATENCY (SEC)		
		DAY 7	DAY 14	DAY 21
1	Control	62.30±0.84^{ns}	53.30±0.845^{ns}	24.30±0.845^{ns}
2	D-galactose Treated	64.07±1.35^{ns}	57.07±1.35^{ns}	32.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	59.28±0.79^{ns}	8.071±0.5^{ns}	3.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	52.54±1.69^{ns}	13.03±1.35^{ns}	4.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	45.15±0.40^{ns}	5.07±1.44^{ns}	3.09±1.85^{ns}

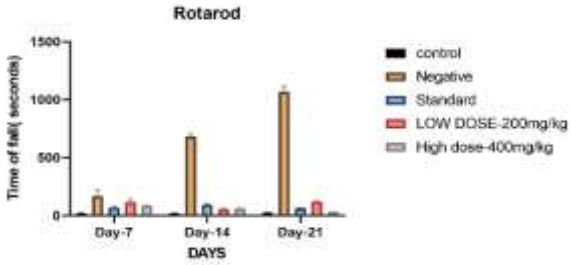
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN ROTA ROD TEST

S.NO	TREATMENT GROUPS	TIME OF FALL (SEC)		
		DAY 7	DAY 14	DAY 21
1	Control	12.30±0.84^{ns}	13.30±0.845^{ns}	23.30±0.845^{ns}
2	D-galactose Treated	575.07±1.35^{ns}	871.07±1.35^{ns}	1010.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	520.28±0.79^{ns}	106.071±0.5^{ns}	64.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	270.54±1.69^{ns}	193.03±1.35^{ns}	122.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	180.15±0.40^{ns}	82.07±1.44^{ns}	33.09±1.85^{ns}

Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).

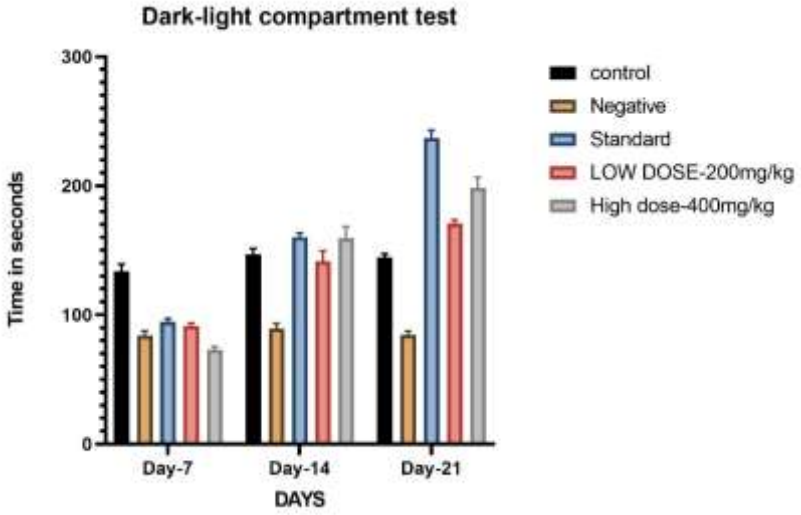


EFFECT OF PORTULACA OLERACEA EXTRACT IN DARK-LIGHT COMPARTMENT TEST

S.NO	TREATMENT GROUPS	TIME SPEND IN LIGHT COMPARTMENT(sec)		
		DAY 7	DAY 14	DAY 21
1	Control	136.30±0.84^{ns}	145.30±0.845^{ns}	148.30±0.845^{ns}
2	D-galactose Treated	85.07±1.35^{ns}	88.07±1.35^{ns}	89.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	90.28±0.79^{ns}	161.071±0.5^{ns}	242.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	106.54±1.69^{ns}	140.03±1.35^{ns}	170.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	91.15±0.40^{ns}	145.07±1.44^{ns}	197.09±1.85^{ns}

Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V

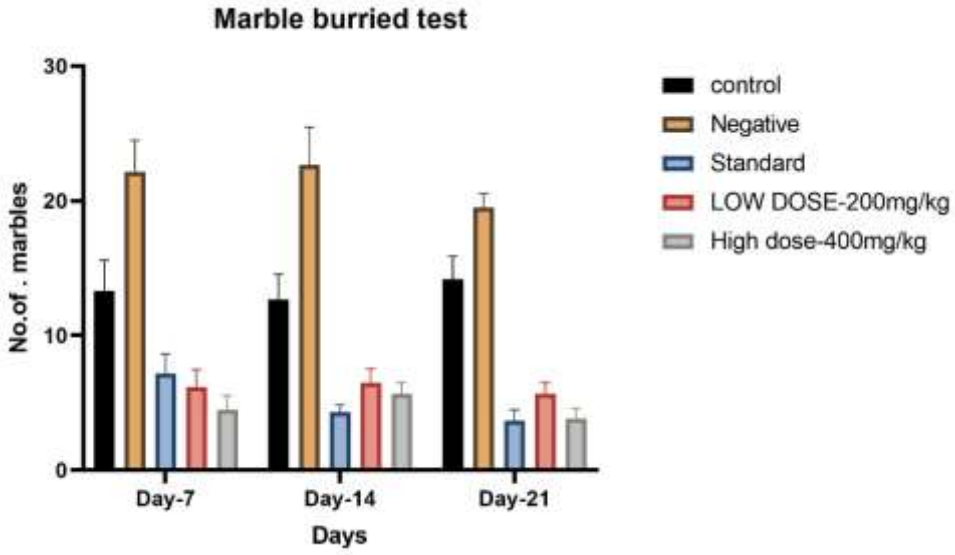
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN MARBLE BURIED TEST

S.NO	TREATMENT GROUPS	NO.OF MARBLES		
		DAY 7	DAY 14	DAY 21
1	Control	12.30±0.84^{ns}	12.30±0.845^{ns}	12.30±0.845^{ns}
2	D-galactose Treated	18.07±1.35^{ns}	17.07±1.35^{ns}	17.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	7.28±0.79^{ns}	5.071±0.5^{ns}	3.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	8.54±1.69^{ns}	5.03±1.35^{ns}	4.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	9.15±0.40^{ns}	4.07±1.44^{ns}	3.99±1.85^{ns}

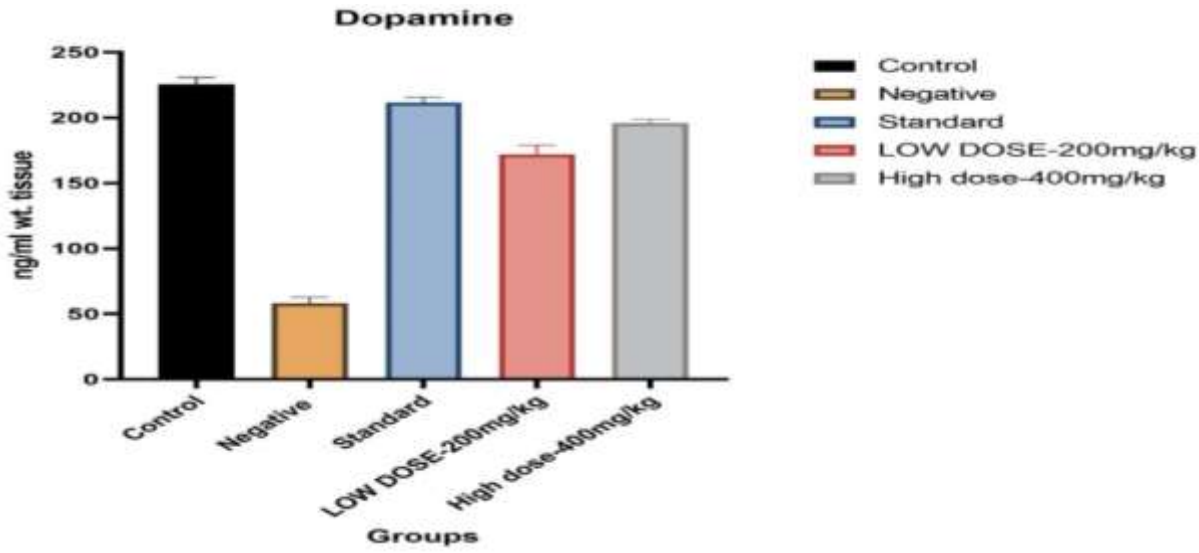
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN DOPAMINE

S.NO	Treatment groups	ng/mg weight of tissue
1	Control	220 ±0.845^{ns}
2	D-galactose Treated	58.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	213.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	160.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	193.99±1.85^{ns}

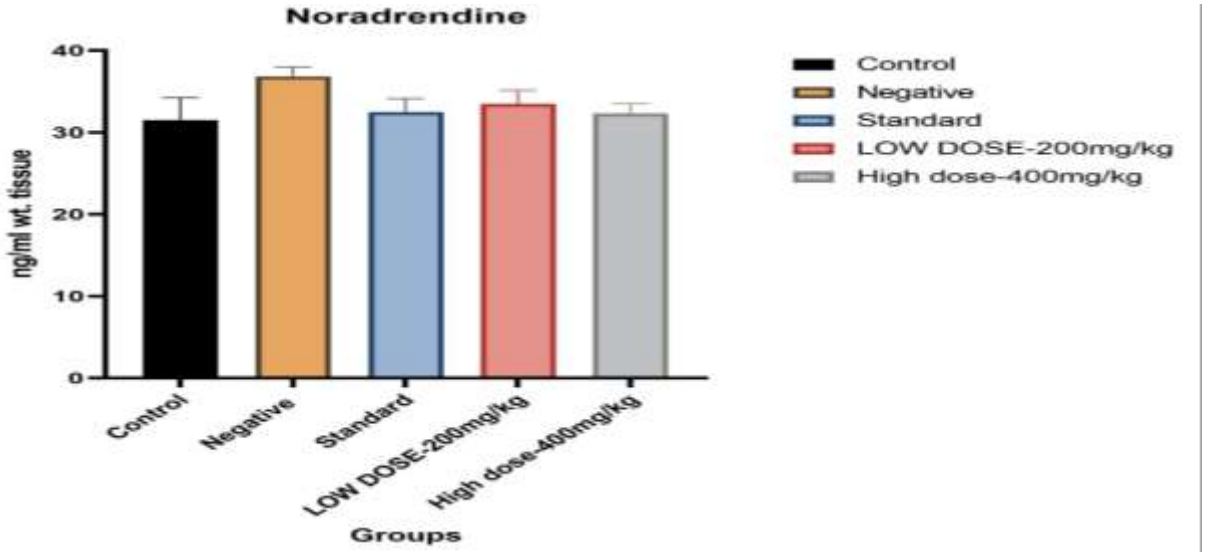
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN NOREPINEPHRINE

S.NO	Treatment groups	ng/mg weight of tissue
1	Control	32 ±0.845^{ns}
2	D-galactose Treated	14.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	33.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	29.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	32.99±1.85^{ns}

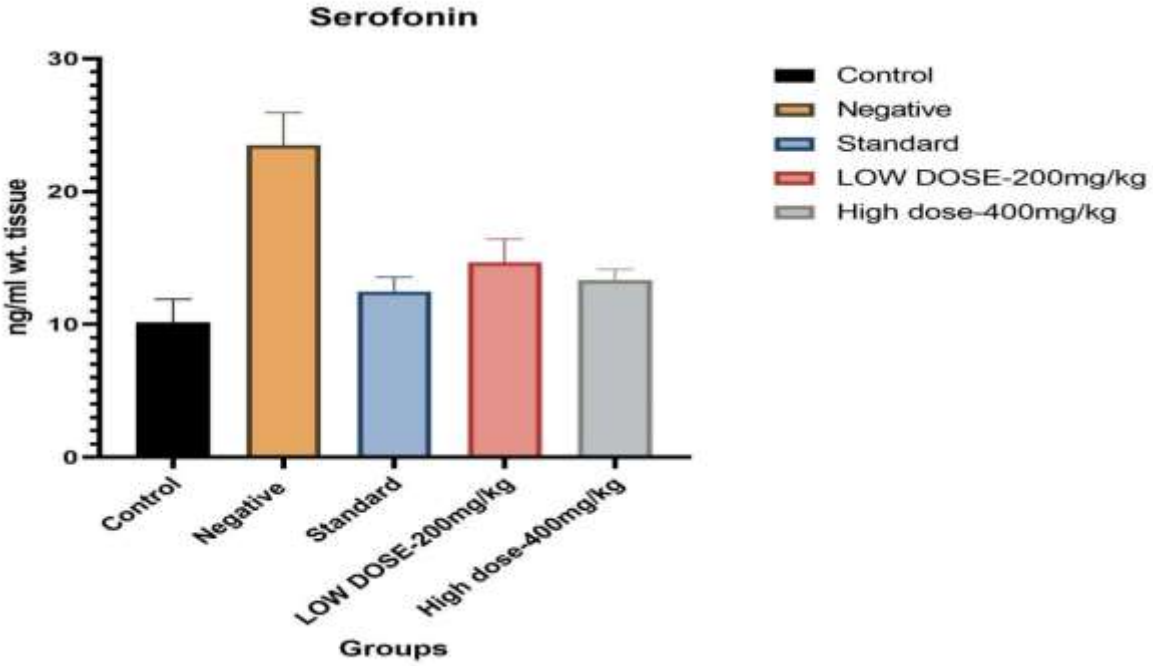
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN SEROTONIN

S.NO	Treatment groups	ng/mg weight of tissue
1	Control	19 ± 0.845^{ns}
2	D-galactose Treated	7.27 ± 1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	15.11 ± 1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	16.05 ± 1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	18.99 ± 1.85^{ns}

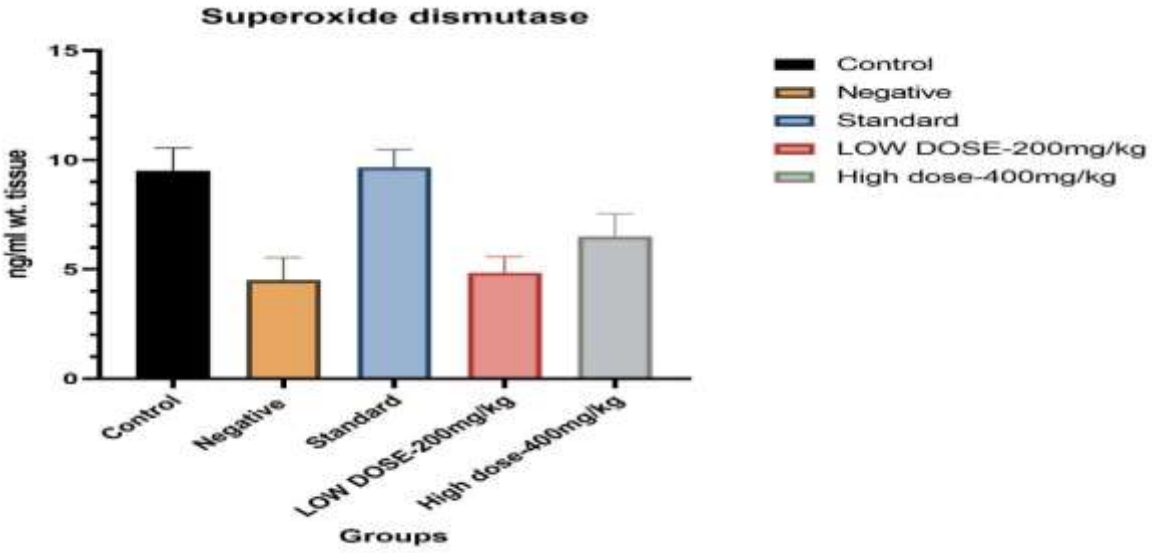
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN SUPEROXIDE DISMUTASE

S.NO	Treatment groups	ng/mg protein
1	Control	9 ±0.845^{ns}
2	D-galactose Treated	4.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	8.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	7.5±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	9.99±1.85^{ns}

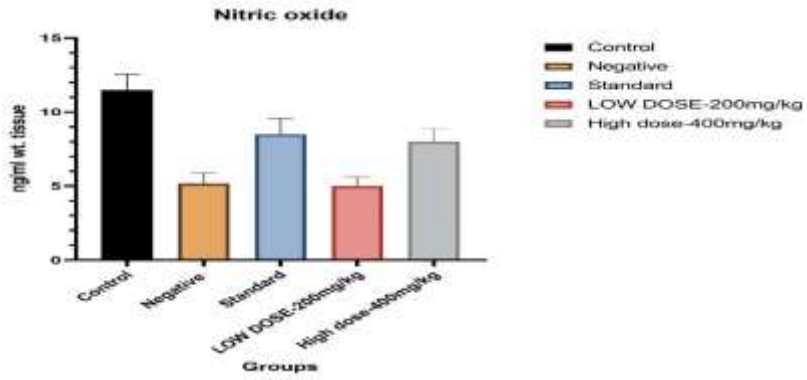
Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



EFFECT OF PORTULACA OLERACEA EXTRACT IN NITRIC OXIDE SCAVANGING ASSAY

S.NO	Treatment groups	ng/mg protein
1	Control	20 ±0.845^{ns}
2	D-galactose Treated	9.27±1.35^{ns}
3	Atomoxetine (1mg/kg/BW)	18.11±1.6^{ns}
4	Portulaca oleracea (200mg/kg/BW)	16.05±1.15^{ns}
5	Portulaca oleracea (400mg/kg/BW)	19.99±1.85^{ns}

Values are represented in Mean ± SEM, n=6
ns- Non significant,*p<0.05, **p<0.01, ***p<0.001
Comparison:
a - Group I vs Group II, Group III, Group IV, Group V
b - Group II vs Group III, Group IV, Group V
c - Group III vs Group IV, Group V (one way ANOVA followed by Dunnett's t' test).



RESULTS

EFFECT OF PORTULACA OLERACEA EXTRACT IN ACTOPHOTOMETER

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in locomotor activity than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the locomotor activity of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing locomotor activity than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing locomotor activity & Group IV also significantly decrease locomotor activity but not as effective as group III & group V.

02) EFFECT OF PORTULACA OLERACEA EXTRACT IN ELEVATED PLUS MAZE

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increases the time spend in open arm than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the of the animal groups showed decreased time spend in open arm in the following manner Group III, V.

The Group III & V showed more effect in time spend in open arm than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing time spend in open arm & Group IV also significantly decrease time spend in open arm but not as effective as group III & group V.

03) EFFECT OF PORTULACA OLERACEA EXTRACT IN MORRIS WATER MAZE

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in escape latency time than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the escape latency time of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing escape latency time than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing escape latency time & Group IV also significantly decrease escape latency time but not as effective as group III & group V.

04) EFFECT OF PORTULACA OLERACEA EXTRACT IN POLE CLIMBING TEST

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in escape latency time than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the escape latency time of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing escape latency time than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing escape latency time & Group IV also significantly decrease escape latency time but not as effective as group III & group V.

05) EFFECT OF PORTULACA OLERACEA EXTRACT IN ROTA ROD TEST

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in the falling time than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the falling time of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing falling time than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing falling time & Group IV also significantly decrease falling time but not as effective as group III & group V.

06) EFFECT OF PORTULACA OLERACEA EXTRACT IN DARK-LIGHT COMPARTMENT

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in time spend in the dark compartment than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the time spend in the dark compartment of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing time spend in the dark compartment than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing time spend in the dark compartment & Group IV also significantly decrease time spend in the dark compartment but not as effective as group III & group V.

07) EFFECT OF PORTULACA OLERACEA EXTRACT IN MARBLE BURRIED TEST

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant increase in no.of marbles buried than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the no.of marbles buried of the animal groups decreased in the following manner Group III, V.

The Group III & V showed more effect in decreasing no.of marbles buried than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in decreasing no. of marbles buried & Group IV also significantly decrease no. of marbles buried but not as effective as group III & group V.

08) EFFECT OF PORTULACA OLERACEA EXTRACT IN DOPAMINE

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant decrease in dopamine level than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the dopamine level of the animal groups increased in the following manner Group III, V.

The Group III & V showed more effect in increasing dopamine level than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in increasing dopamine level & Group IV also significantly increase dopamine level but not as effective as group III & group V.

9) EFFECT OF PORTULACA OLERACEA EXTRACT IN NOREPINEPHRINE

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant decrease in norepinephrine level than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the norepinephrine level of the animal groups increased in the following manner Group III, V.

The Group III & V showed more effect in increasing norepinephrine level than group IV.

Group III compared with Group IV and Group V is considered as c.

When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in increasing norepinephrine level & Group IV also significantly increase norepinephrine level but not as effective as group III & group V.

10) EFFECT OF PORTULACA OLERACEA EXTRACT IN SEROTONIN

Group I Compared with Group II considered as a.

When group I compared with Group II(P<0.001), Group II animal showed significant decrease in serotonin level than group I.

Group II Compared with Group III, Group IV and Group V is considered as b.

When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the serotonin level of the animal groups increased in the following manner Group III, V.
The Group III & V showed more effect in increasing serotonin level than group IV.
Group III compared with Group IV and Group V is considered as c.
When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in increasing serotonin level & Group IV also significantly increase serotonin level but not as effective as group III & group V.

11) EFFECT OF PORTULACA OLERACEA EXTRACT IN SUPEROXIDE DISMUTASE

Group I Compared with Group II considered as a.
When group I compared with Group II(P<0.001), Group II animal showed significant decrease in superoxide dismutase level than group I.
Group II Compared with Group III, Group IV and Group V is considered as b.
When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the superoxide dismutase level of the animal groups increased in the following manner Group III, V.
The Group III & V showed more effect in increasing superoxide dismutase level than group IV.
Group III compared with Group IV and Group V is considered as c.
When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in increasing superoxide dismutase level & Group IV also significantly increase superoxide dismutase level but not as effective as group III & group V.

12) EFFECT OF PORTULACA OLERACEA EXTRACT IN NITRIC OXIDE

Group I Compared with Group II considered as a.
When group I compared with Group II(P<0.001), Group II animal showed significant decrease in nitric oxide level than group I.
Group II Compared with Group III, Group IV and Group V is considered as b.
When group II compared with Group III(P<0.001), Group IV(P<0.001), Group V(P<0.001) the nitric oxide level of the animal groups increased in the following manner Group III, V.
The Group III & V showed more effect in increasing nitric oxide level than group IV.
Group III compared with Group IV and Group V is considered as c.
When Group III compared with Group IV(P<0.001), Group V(P<0.001). Group V almost showed equal effect in increasing nitric oxide level & Group IV also significantly increase nitric oxide level but not as effective as group III & group V.

DISCUSSION

ADHD is now the most common disorder in children and teens. The incidence of ADHD increases with age. Impairment of attention, impulsivity and hyperactivity is the prime and first clinical feature. When the condition progress, additional cognitive abilities are impaired as the ability to calculate and use common objects and tools. Although some studies and people with ADHD suggest that they have high learning capacity. Stimulants and Nonstimulants are the only agents approved by the Food and Drug Administration (FDA) for the treatment of ADHD. All other agents prescribed for the treatment of ADHD are used on an off-label basis.

Due to severe side effects of Drugs available to treat ADHD it becomes complicated to treat the symptoms. The patients may feel insomnia or may feel sedative with these stimulants and non-stimulants. Not all the patients with ADHD become comfortable with the prescribed drugs. Hence alternative treatments are suggested to decrease the symptoms of ADHD. Because the trio symptoms inattentive, impulsivity, hyperactivity cannot be cured with one drug. So herbal therapy like Ayurveda, Siddha and other plant-based medicines are suggested to provide symptomatic relief to these patients.

Based upon literature review many plant-based medicines are used to treat symptoms of ADHD. In literature review it has also been suggested that combination of plant extracts is greatly useful in symptomatic treatment of ADHD. As per previous studies Portulaca oleracea seed extract has excellent antioxidant property, hence it is believed to have actions on CNS disorders and neurodevelopmental disorders.

The present study has revealed the ameliorative effect of Portulaca oleracea seed extract on D-galactose induced attention deficit hyperactivity disorder in Swiss albino male mice. D-galactose induced impairment of memory was assessed by using various behavioral parameters like Pole climbing apparatus and Morris water maze test. It was found that treatment with Portulaca oleracea Seed extract protect cognitive deficits in D-galactose induced induced ADHD.

Spatial learning in the open field habituation was approached to access learning and memory. The decrease in response to a normal environment after repeated exposures to the familiar environment is referred to spatial habitual learning. Recurrent exposure produces a decrease in the exploratory initiatives, which is implicative of memory pertaining to a specific feature of that environment. Exploratory activities may be reduced on subsequent contact with closed field. In the result of this study reduced by the group of animals treated with Portulaca oleracea seed extract indicated increased spatial habitual learning and sleep deprivation decreased spatial habitual learning.

Impulsive and hyperactive behavior was assessed using Actimeter and Elevated zero maze based on locomotion in closed field and novel maze environment and treatment with Portulaca oleracea seed extract reported that the reduction in the locomotor activity and increased time spent duration in open environment when compared to D-galactose induced animals which exhibited high locomotor count and time spent duration in closed field.

Anxiolytic behavior which has some linkage in ADHD was assessed by dark-light compartment and marble burying behavior. D-galactose induced ADHD animals showed treated with Portulaca oleracea seed extract which showed potent reduction in anxiolytic behavior. It was concluded by that the deficit animals buried more no. of marbles when compared with Portulaca oleracea seed extract group and also deficit animals spend more time in dark compartment when compared with treatment group.

Skeletal muscle activity was assessed by rota rod apparatus-galactose induced animals showed high fall off time in rota rod apparatus. Treatment with Portulaca oleracea seed extract in deficit groups greatly reduced fall off time. This indicated that Portulaca oleracea seed extract has muscle relaxant property.

Morris water maze task represents more specific for spatial memory. The essential feature of this technique is that rats are placed into large circular pool of water and can escape into a hidden platform. Thus, the platform offers no local cues to guide escape behavior and the rat can escape from swimming by climbing on to the platform apparently learns the spatial location of the platform any starting position at the circumference of the pool. The only spatial cues are those outside water tank are primarily visual cues. Thus, the versatility of the task makes it a widely acceptable experimental model for the assessment of cognitive tests. Typically, D-galactose induced animals exhibited an increase time for escape latency indicating loss of visual cues to escape to the platform. Such a diminished cognition was reversed by the administration of the Portulaca oleracea seed extract at the specified dosage levels and exhibited escape latency (EL), indicating the well-developed spatial memory in spite of D-galactose induced ADHD.

Both adrenergic and dopaminergic receptors are involved in attention deficit hyperactivity disorder and several studies have suggested their roles in inattention, hyperactivity and impulsivity. Marked dopaminergic deficit is a hallmark of the pathogenesis of ADHD and various drugs including stimulants and non-stimulants have designed to target this dopaminergic receptor. There was a significant reduction in the level of dopamine in the animals and it was treated with Portulaca oleracea seed extract and increased dopamine reduced the hyperactivity and improved impulsivity and cognitive behavior which was impaired by D-galactose induction. Dopamine is the critical neurotransmitter modulating long term hyperactivity along with this serotonin was also involved.

The formation of new memories is thought to require the hippocampus and adjacent medial temporal lobe, but the final storage of memories is widely distributed by neocortical network. Lesion studies have suggested that there is a wide distribution of neocortical memory traces encoded in the strength of synaptic connections among neurons across large areas of the neocortex. Although, $alpha_2$ and D receptors play a major role in learning and memory, hyperactive and impulsive effects have also been detected and on the region implicated in memory storage are richly innervated by the dopaminergic system.

It has been suggested that antioxidant might contribute to the prevention of ADHD. The superoxide dismutase (SOD) constitutes a mutually supportive team of defense against reactive oxygen species. The most remarkable effect of Portulaca oleracea seed extract is increased activity of SOD in brain. Treatment with Portulaca oleracea seed extract preserved the reduced SOD to that of the normal control. Usually increased level of nitric oxide causes oxidative stress. The Portulaca oleracea seed extract has better action on nitric oxide, since the levels of nitric oxide is reduced in the treatment groups.

CONCLUSION

CNS stimulant drugs like atomoxetine, methylphenidate, amphetamine and tricyclic antidepressants like imipramine are used for the treatment of ADHD. But these drugs are more expensive and have addiction problem also more side effects than natural medicines.

The developed method using pretreatment with imipramine instead of desipramine showed good result which was comparable with the standard method available in literature review. So, the Mechanism of action of desipramine is to protect noradrenergic neuron which was used as a standard and pretreatment with imipramine produced similar levels.

Hence, I conclude that desipramine which is difficult to procure in many countries instead of that imipramine can be used.

The combination of Atomoxetine and Quercetin significantly benefited in impulsive, cognitive behavior and stabilized the skeletal muscle activity.

The selected dose 200mg/kg and 400mg/kg of Portulaca oleracea showed significant action in impulsive, cognitive behavior and skeletal muscle activity but higher dose 400mg/kg showed better action than lower dose 200mg/kg.

The present study, relieved the action of Portulaca oleracea on D-galactose induced attention deficit hyperactivity disorder in Swiss albino mice. From the

results it can be concluded that Portulaca oleracea has remarkable effect in memory enhancement and oxidative stress. Further studies are required for the identification of molecular level action and individual phytoconstituent that may responsible for CNS action.

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