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INVESTIGATIONS OF HERBAL EXTRACTS USING ANIMAL MODELS FOR ANTIDEPRESSANTS AND ANTI-ANXIETY ACTIVITIES

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Abstract:

The aim of this dissertation was to investigate the potential antidepressant effects of the methanolic extract of Sagittaria Trifolia and Litchi Chinensis in a mouse model. The study focused on assessing behavioral changes and antioxidant properties of the extract in order to provide insights into its therapeutic potential.The primary objectives included evaluating the antidepressant-like effects through behavioral assessments using the Forced Swim Test, Tail Suspension Test, and Open Field Test. Additionally, the study aimed to determine the antioxidant capacity of the extract and investigate its potential mechanism of action.Mice were treated with the methanolic extract of Sagittaria Trifolia and Litchi Chinensis for 14 days, followed by behavioral assessments on days 1, 7, and 14. Four groups were formed, including a control group, a standard group treated with Imipramine, and two test groups treated with different doses of the extract. Biochemical parameters, such as serotonin, norepinephrine, catalase activity, lipid peroxidation, superoxide dismutase (SOD) activity, and reduced glutathione (GSH), were measured to assess the extract's impact on the central nervous system and antioxidant capacity.The results of this study suggest that the methanolic extract of Sagittaria Trifolia and Litchi Chinensis exhibits potential antidepressant-like effects in mice, as indicated by reduced immobility duration in behavioral tests, particularly at the higher dose. Moreover, the extract demonstrated promising antioxidant properties, with increased catalase activity and reduced lipid peroxidation. While these findings are encouraging, further research is needed to elucidate the precise mechanisms of action and to assess the safety and efficacy of Sagittaria Trifolia and Litchi Chinensis extract for potential clinical use. This study contributes to the understanding of natural product-based alternatives for depression treatment, emphasizing the need for continued investigation into the therapeutic potential of this plant extract.

Keywords:*Sagittaria Trifolia,Litchi Chinensis,antidepressant effects, methanolic extract*

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INTRODUCTION:

Depression: It is basically acknowledged as illness with symptoms such as anxiety and sleep disturbances. It can be a persistent, recurring illness that can cause many personal suffering for individuals and their families. At present, disability caused by depression is estimated to be the fourth most important cause of worldwide loss of life years. This has resulted into a requirement of search for effective treatments, including antidepressant drugs, herbal remedies, psychotherapy and electroconvulsive shock therapy. [1]

PLANT PROFILE

Sagittaria Trifolia and Litchi Chinensis

Sagittaria Trifolia

Fig No.1 Sagittaria Trifolia

Common Name	Chinese Arrowroot, Threeleaf arrowhead
Family	Alismataceae
USDA hardiness	10-12
Known Hazards	None known
Habitats	Ponds, lakes, marshes, paddy fields and channels[266].
Range	Southern European Russia, through temperate Asia to tropical southeastern Asia in Malaysia, Indonesia and the Philippines.

Litchi Chinensis

Fig No.2 Litchi Chinensis

Common Name: lychee
Type: Broadleaf evergreen
Family: Sapindaceae
Native Range: Southeastern Asia
Zone: 10 to 11
Height: 20.00 to 30.00 feet
Spread: 15.00 to 25.00 feet
Bloom Time: April to May
Bloom Description: Yellow
Sun: Full sun
Water: Medium
Maintenance: Low
Flower: Showy
Leaf: Evergreen
Fruit: Showy, Edible

METHODOLOGY:

COLLECTION AND AUTHENTIFICATION OF PLANT:

- Dried leaves of the plant was procured from local herbarium.

PREPARATION OF PLANT EXTRACT USING SOXHLET METHOD:

The dried leaves of the *Sagittaria Trifolia and Litchi Chinensis* was extracted using methanol as solvent using soxhlet extraction method for 3 days and subjected to evaporation. The final product was stored in air tight glass jar.

PHYTOCHEMICAL SCREENING TESTS

Methanolic extract of *Sagittaria Trifolia and Litchi Chinensis* was subjected to various phytochemical screening tests such as alkaloids, anthracene glycosides, cardiac glycosides, steroids, saponins, flavonoids, tannins, carbohydrates, proteins and fixed oils/fats according to the procedure mentioned in C.K Kokate

EXPERIMENTAL ANIMALS:
The experimantal studies were conducted at Shadan Women's college of pharmacy, Khairtabad, Hyderabad. swiss albino mice of either sex weighing between 25-30gm were used for this study. Totally 36 mice were used in the present study.They were acclimatized for 14 days in animal house with 12hrs light/dark cycle. Animals were fed with boilerplate pellets diet and water ad.Libitium. Animals are haphazardly tabbed for grouping. The surveillance and supervision of mice were in consonance with the multiculturally endorsed boilerplate guidelines of use of animals (CPCSEA). Consent ratification for animal studies was acquired from the Institutional animal Ethics Committee (IAEC), Our proposal number is IAEC-04/SES-2023/41/102

ACUTE TOXICITY STUDIES:
Acute oral toxicity study for the ethanolic extracts Sagittaria trifoliaand litchi chinensiswere carried out in rats and mice as per OECD Guideline No. 423. The results of thesestudies are as follows:

Sagittaria trifolia:
Even at the dose of 2000 mg/kg,no mice or rat was killed. Therefore, the differentextracts of Sagittaria trifoliawere found to be safe and non toxic.LD 50 range was considered greater than 2000 mg/kg.

Litchi chinensis:
20% of the total mice were killed at the dose of 5000mg/kg (LD50 = 5gm/kg)
Mortality: The extracts were found to be safe as no mortality was observed even at a higherdose of 2000 mg/kg.

Signs and Symptoms of Toxicity: No signs of toxicity were observed 24 hour post treatment as dose did not produce anymortality. No significant modification in behavioural patterns nor any clinical abnormalitywere observed during entire observation period of 14 days.

DRUGS AND CHEMICALS:
Imipramine (10mg/kg), 0.5% w/v CMC (Carboxy methyl cellulose), Methanol, Methanolic extract of *Sagittaria Trifolia and Litchi Chinensis* 200mg/kg

EXPERIMENTAL PROTOCOL
The mice was treated with methanolic extract of *Sagittaria Trifolia and Litchi Chinensis* for 14 days. The test was performed 30 min after the administration of *Sagittaria Trifolia and Litchi Chinensis* extract (200mg/kg&400mg/kg) and the behavioural activity of the animals were tested on 1st , 7 th and 14th day of the experiment.
The animals was divided into 4 groups consisting of 6 mice each as follows:

S.No	Groups	No. Of animals	Name of drug and doses
1	Group 1	6	Normal - 1% w/v Normal Saline p.o
2	Group 2	6	Control - 0.5% w/v CMC p.o
3	Group 3	6	Imipramine - 5mg/kg
4	Group 4	6	Sagittaria Trifolia extract 200mg/kg
5	Group 5	6	Litchi Chinensis extract 200mg/kg
6	Group 6	6	Sagittaria Trifolia extract 100mg/kg + Litchi Chinensis extract 100mg/kg

Table 2. Grouping of experimental animals

Before and during the treatment behavioural activity of mice was assessed by Forced swim test, Tail suspension test and Actophotometer After behavioral assessments the mice was sacrificed by CO2 exposure method, the brain was removed and washed with ice cold isotonic saline solution and was subjected to estimation of biochemical parameters and histopathological study. The biochemical parameters such as serotonin, norepinephrine wasdetermined and the antioxidant property of extract of *Sagittaria Trifolia and Litchi Chinensis* was determined.

METHODS FOR BEHAVIOURAL ASSESSMENT
WATER WHEEL METHOD
Procedure
The method described by *Porsolt, et. al.* was used in our study (Porsolt RD, et.al., 1977). Each animal was placed individually in a 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the

water surface. The water was changed after each test. A decrease in the duration of immobility is indicative of an antidepressant like effect.

OPEN FIELD TEST

Procedure:

1. Bring the mice in their home cages from their housing room into the testing room. Allow the mice to acclimate to the procedure room for a minimum of 30 min prior to starting the test.
2. Remove a single mouse from the home cage by gently grasping its tail and place the mouse in the middle of the open field maze. It is normal for the mouse to move in Sagittaria Trifolia and Litchi Chinensis to the periphery walls of the maze and the timing of release and tracking capture of the mouse should coincide to record this movement.
3. Allow free and uninterrupted movement of the subject mouse throughout the respective quadrant of the maze for a single 10 min period during which time, the tracking software will record movement.
4. At the end of the test period, pick up the subject mouse gently, removing it from the maze and return it to its home cage.
5. Prior to cleaning the maze, visually count the fecal boli pellets present in the maze and manually record the numbers for further analysis.
6. Remove all fecal pellets and wipe up all spots of urination. Spray the floor and walls of the maze quadrant with 95% ethanol and wipe down with a clean paper towel. Allow the ethanol solution to completely dry prior to testing other mice.
7. Repeat the procedure with the next mouse.

Fig No. 3,4 Open field Test

TAIL SUSPENSION TEST

Procedure

The method described by Steru, et. al. was used in our study (Steru L, et.al., 1985) [16]. The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. A decrease in the duration of immobility is indicative of an antidepressant effect.

Determination of Biochemical Parameters

Catalase

Catalase activity (CAT) measured using the method of Sinha (26). It is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H2O2, with the formation of perchromic acid as an unstable interSagittaria Trifolia and Litchi Chinensiste. The chromic acetate thus produced is measured calorimetrically at 570~610 nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H2O2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H2O2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture.

Lipid peroxidation level (LPO)

Lipid peroxidation as evidenced by formation of thiobarbituric acid reacting substances (TBARS), were measured by the method of Esterbauer and Cheeseman (21). 250 microliters of tissue homogenate were added to 1.5 mL of 1% phosphoric acid (pH 2.0) and 1 mL of 0.6% of TBA in air light tubes and the samples was cooled to room temperature and MDA (malondialdehyde)-TBA was extracted with 2.5 mL of butanol. Organic phase was separated by centrifugation for 5 min at 2000 g and measured at 532 nm. A 99% TBARS are MDA, so TBARS concentration of the samples were calculated using the extinction coefficient of MDA is $1.56 \times 10^5\ M^{-1}cm^{-1}$. Lipid peroxidation is expressed as nmol TBARS/mg prot.

SUPEROXIDE DISMUTASE (SOD)

The specific activity of brain superoxide dismutase (SOD) was determined according to the method

described by Misra and Fridonich. 10 µL of tissue homogenate were added to 970 µL of ethylene diamine tetraacetic acid (EDTA) - Sodium carbonate buffer (0.05 M) at pH10.2. The reaction was started by adding 20 µL of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min. A unit of SOD is defined as the amount of enzyme that inhibits by 50% the speed of oxidation of epinephrine and the results were expressed as UI/mg protein.

Glutathione peroxidase (GSH-Px) catalyzes the reduction of hydroperoxides by utilizing GSH as a reductant. Determination of tissue GSH-Px activity was carried out according to the method of Flohe and Gunzler. The reaction mixture contained 0.2 mL of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4); 0.4 mL of GSH (0.1 mM), 0.2 mL of homogenate was added and allowed to equilibrate for 5 min at 25°C. The reaction was initiated by adding 0.2 mL of H_2O_2 (1.3 mM); reaction was terminated by addition of 1 mL of 1% Trichloroacetic acid (TCA). Tubes were centrifuged at 1500 g for 5 min and the supernatant was collected. To 0.48 mL of resultant supernatant, 2.2 mL of TBS (pH 7.4) and 0.32 mL of DTNB (1.0 mM) were added. After mixing, absorbance was recorded at 412 nm and the specific activity of this enzyme is expressed as µmol GSH/mg protein.

Glutathione-S-transferase (GST) activity of tissues was measured spectrophotometrically by the method of Habig *et al*. using CDNB as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm. The activity of GST was expressed in terms of µmol CDNB-GSH conjugate formed/min/mg protein.

Glutathione reductase (Reduced GSR) activity was based on the method of Goldberg and Spooner. The enzymatic activity was assayed photometrically by measuring, NADPH consumption. In the presence of oxidized glutathione (GSSG) and NADPH, GR reduces GSSG and oxidizes NADPH, resulting in a decrease of absorbance at 340 nm. Quantification was based on the molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ of NADPH, 1 unit of GR was defined as the amount of enzyme that reduced 1 µmol GSSG (corresponding to the consumption of 1 µmol of NADPH) per minute at 25°C. The GR activities were expressed as 1 unit per milligram protein.

RESULTS

PRLIMINARY QUALITATIVE CHEMICAL SCREENING.
Phytochemical screening tests

Phytochemical tests	**Reagents**	**Results**
Alkaloids	Mayer's test	-
Anthracene glycosides	Borntrager's test	-
Cardiac glycosides	Legal's test	-
Steroids	Salkowski test	-
Saponins	Foam test	-
Flavonoids	NaOH test	+
Tannins	Lead acetate	+
Carbohydrates	Molish's test	+
Proteins	Biuret's test	+

Fig No.5, 6 Screening of Sagittaria trifolia Fig No.7,8 Screening of Litchi Chinensis

GCMS analysis of Sagittaria trifolia

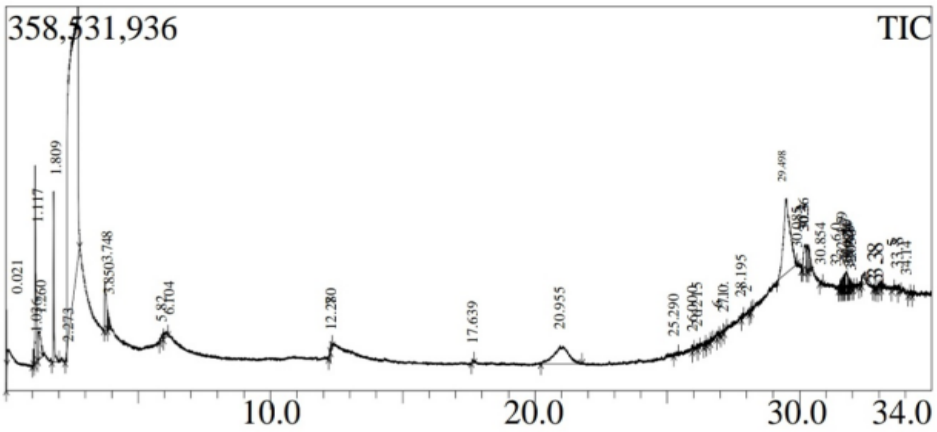


Fig. 9 Graphical representation of GCMS analysis of Sagittaria trifolia

S.no	Retention time	Chemical constituents	Area %	uses
1	7.820	Dextroamphetamine	8.54	**Anti-anxiety,** stimulant, cognitive enhancer, aphrodisiac, euphoriant.
2	28.410	N-ethoxycarbonylhydrazon	20.03	**Anti-anxiety**, **anti-psychosis**, anti-bacterial, immunomodulator,anti-diabatic, anti-viral.
3	34.794	Caffeic acid	11.33	**Anti-depressant, anxiolytic,** anti-inflammatory, anti-cancer, anti-viral.
4	22.955	Dimethyl Sulfoxide	10.38	**Anti-epileptic,** analgesic (rheumatoid arthritits,cataracts).
5	3.809	1,5-Heptadien-3-yne	12.55	**Anti-epileptic,** analgesic, anti-bacterial.
6	5.784	Methyl methanesulfonate	4.21	**Anti-epileptic, anti-psychotic,** alkylating agent, anti-cancer.
7	14.270	(3,4-Dihydroxyphenyl)hexylamine	1.21	**Anti-depression, anti-psychotic,** Anti-inflammatory, astringent, demulcent, emollient.
8	36.125	Cinnamic acid, 3,4-Dimethoxy, trimethylsilyl ester	1.63	**Anti-depression**, anti-fungal, anti-oxidant, anti-bacterial, depigmenting agent.
9	33.751	L-norephedrine	1.59	**Anti-depression,** CNS stimulant, nasal congestion, myasthenia gravis

GCMS analysis of ethanolic extract of litchi chinensis

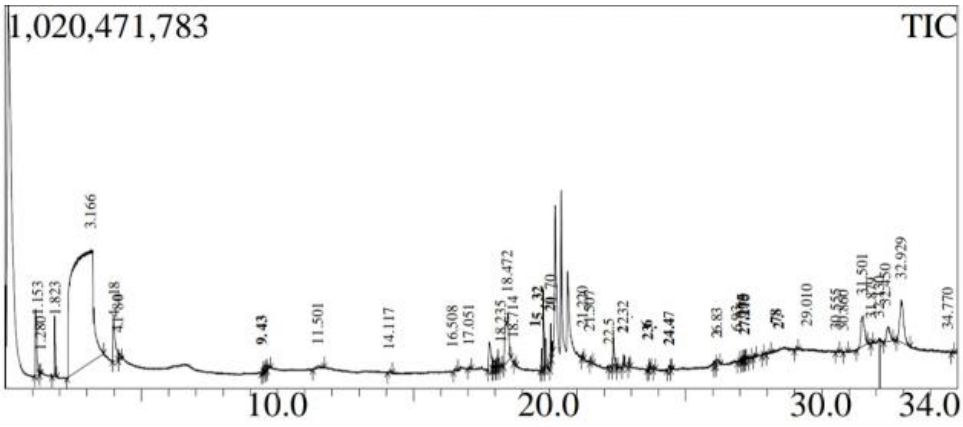


Fig. 10 Graphical representation of GCMS analysis of Litchi Chinensis

S.no	Retention time	Chemical constituents	Area %	uses
1	20.200	Eicosanoic acid	6.94	**Anti-depressant,** haemolytic agent, anti-coagulant, anti-inflammatory.
2	27.010	Chlorpheniramine	8.84	**Anti-depressant, anti-psychotic,** anti-cancer, anti-viral.
3	23.410	N-ethoxycarbonylhydrazon	9.03	**Anti-epilepsy,** anti-bacterial, immunomodulator,anti-diabatic, anti-viral.
4	20.220	3,3,4,5,7-Pentahydroxyflavone	7.42	**Anti-anxiety,** anti-oxidant, anti-pyretic, anti-cancer.
5	11.550	4-hydroxyadamantan-2-one	6.76	**Anti-epileptic,** anti-viral, treatment of influenza.
6	13.501	2,3 dimethylfumaric acid	12.28	**Anti-anxiety,** Treatment of psoriasis, autoimmune disorders, jaundice.
7	33.930	lupeol	6.38	**Anti-convulsant,** Anti-cancer, anti-inflammatory, dietary triterpene.
8	32.879	β-amyrin	5.26	**Anti-depression, anti-anxiety,** Anti-oxidant, analgesic.
9	36.770	9-[4-Hydroxybutyl] hypoxanthine	11.11	**Anti-depression, anti-psychosis, anti-**inflammatory,

IMMOBILITY TIME IN THE WATER WHEEL METHOD USING MICE

S.No	Treatment	Duration of immobility (seconds)		
		Day 1	**Day 7**	**Day 14**
1.	Group 1	102.9±0.47	112.5±0.47	119.4±0.68
2.	Group 2	49.4±0.27	44.9±0.59	44.3±0.39
3.	Group 3	67.8±0.28	57.4±0.52	54.2±0.52
4.	Group 4	58.7±0.36	51.8±0.42	48.6±0.42
5.	Group 5	56.4±0.29	49.4±0.18	46.3±0.16
6.	Group 6	53.7±0.27	47.4±0.32	45.4±0.33

Calculations for water wheel method

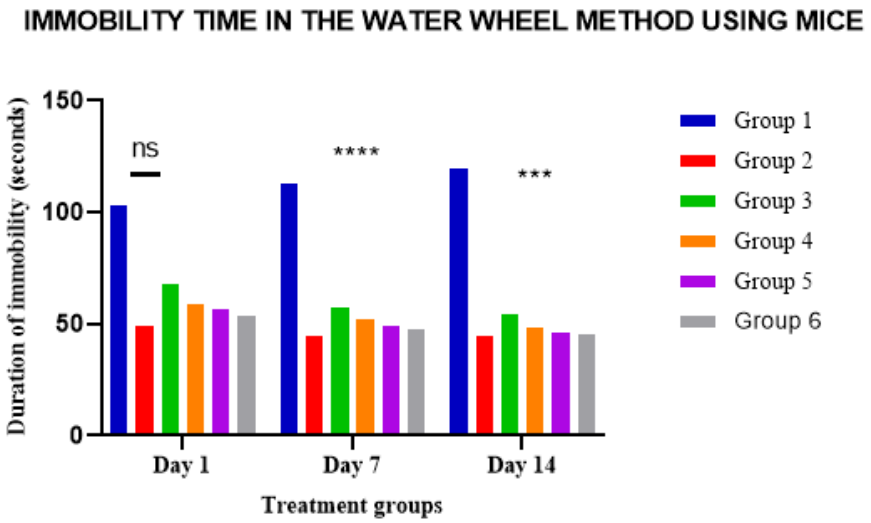


Fig 11. Graphical representation of Water Wheel method results

TAIL SUSPENSION TEST

S.No	Treatment	Duration of immobility (seconds)		
		Day 1	Day 7	Day 14
1.	Group 1	223.7±0.63	216.4±0.89	212.4±0.58
2.	Group 2	174.6±0.74	171.4±0.68	158.4±0.83
3.	Group 3	146.3±0.82	139.7±1.36	136.7±1.36
4.	Group 4	138.5±0.47	128.2±0.58	124.2±0.82
5.	Group 5	133.4±0.28	125.3±0.42	122.4±0.56
6.	Group 6	129.4±0.13	121.6±0.65	120.6±0.64

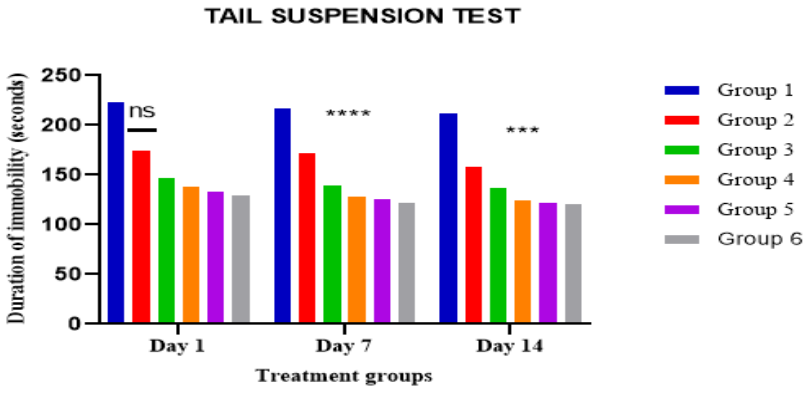


Fig 12. Graphical representation of Tail Suspension method results

ELEVATED PLUS MAZE

Treatments	Open Arm entries	Percentage decreased in Open arm entries
Group 1	8.49±0.02	3.43±0.03
Group 2	6.36±0.07	2.64±0.09
Group 3	5.17±0.09	1.83±0.15
Group 4	4.38±0.12	1.71±0.19
Group 5	4.12±0.67	1.65±0.24
Group 6	3.79±0.16	1.41±0.29

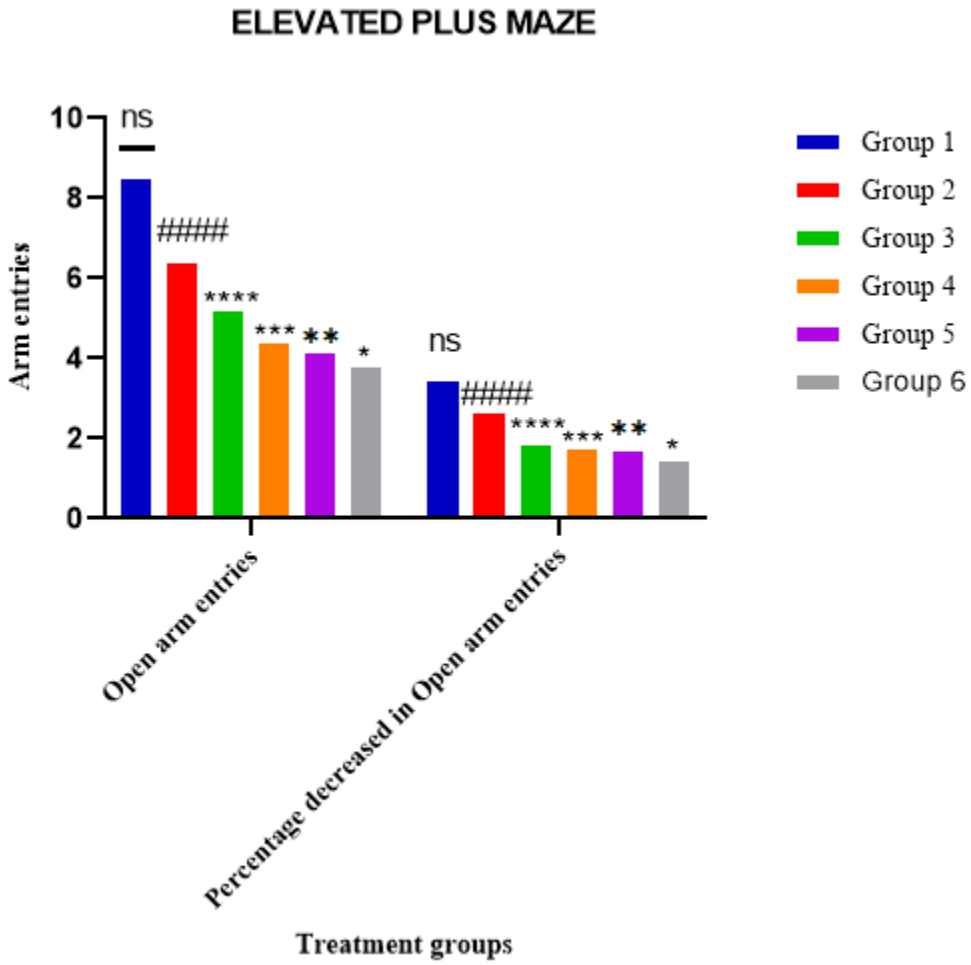


Fig 13. Graphical representation of Open Field method results

CATALASE

GROUPS	**CATALASE µm/mg tissue**
Group 1	24.87 ±0.07
Group 2	55.52 ±0.04
Group 3	47.89±0.17
Group 4	63.47±0.29
Group 5	59.36±0.25
Group 6	54.19±0.55

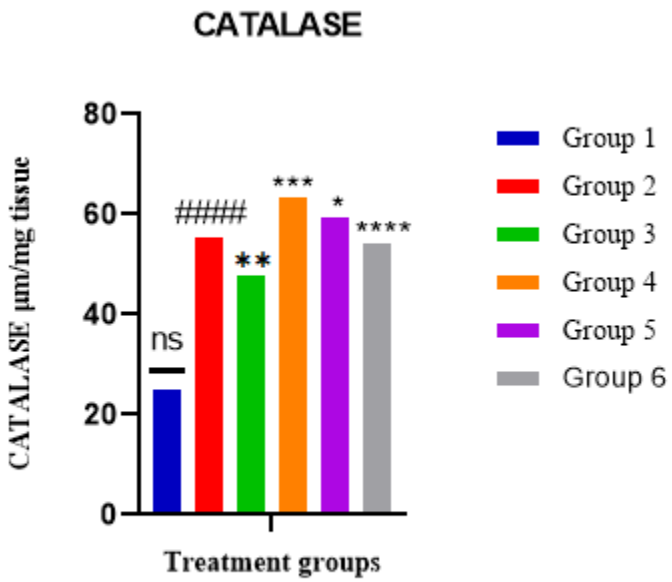


Fig 14. Graphical representation of Catalase activity results

LPO

GROUPS	**LPO µm of /H2O2/mg tissue**
Group 1	18.34 ±0.15
Group 2	14.35 ±0.06
Group 3	17.31 ±0.09
Group 4	18.27 ±0.07
Group 5	17.91 ±0.06
Group 6	16.42 ±0.04

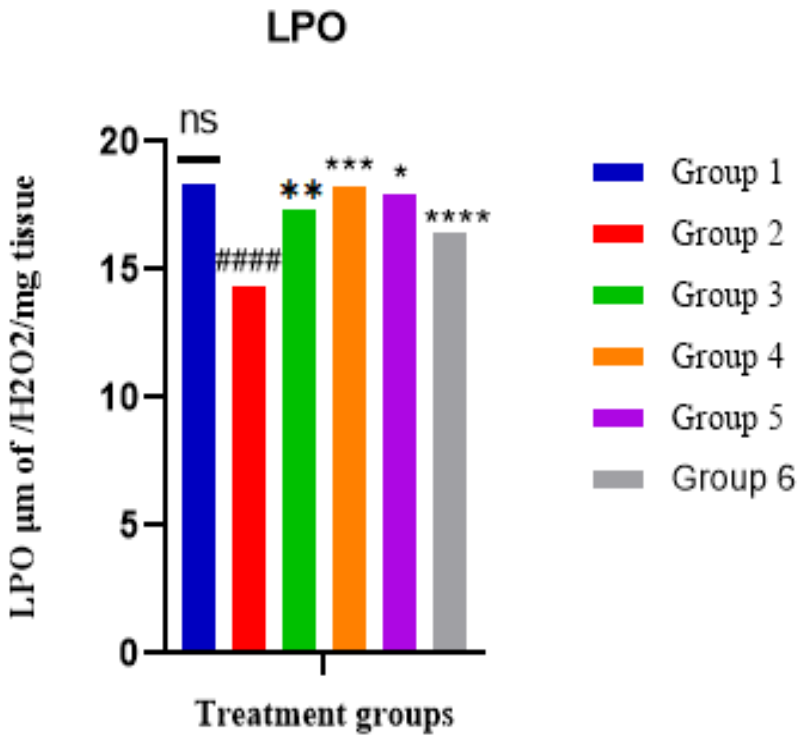


Fig 14. Graphical representation of LPO

SOD

Group	SOD(unit/min/mgprotein)
Group 1	2.47±0.08
Group 2	1.87±0.14
Group 3	1.69±0.05
Group 4	1.46±0.06
Group 5	1.35±0.07
Group 6	1.26±0.08

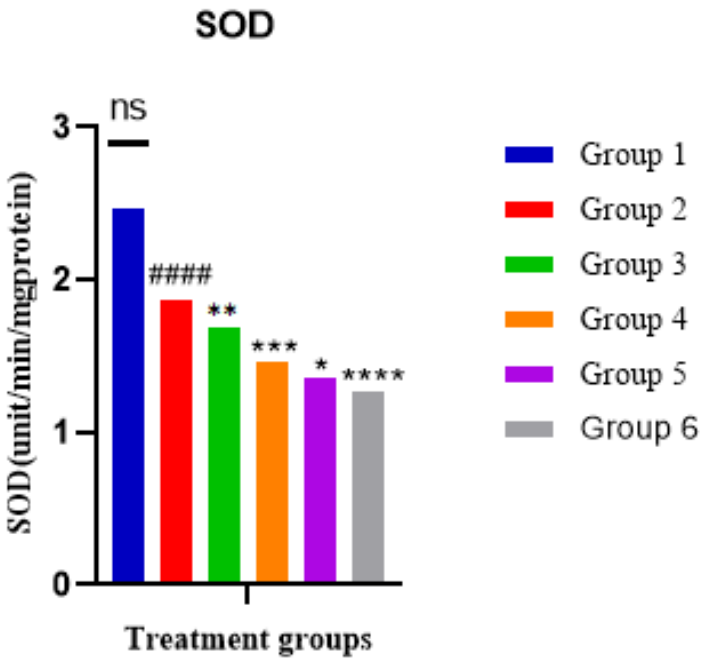


Fig 15. Graphical representation of SOD

Evasion Method

Group	Evasion Time(mins)
Group 1	3.47±0.12
Group 2	5.87±0.26
Group 3	2.69±0.34
Group 4	1.27±0.88
Group 5	1.18±0.91
Group 6	1.09±0.23

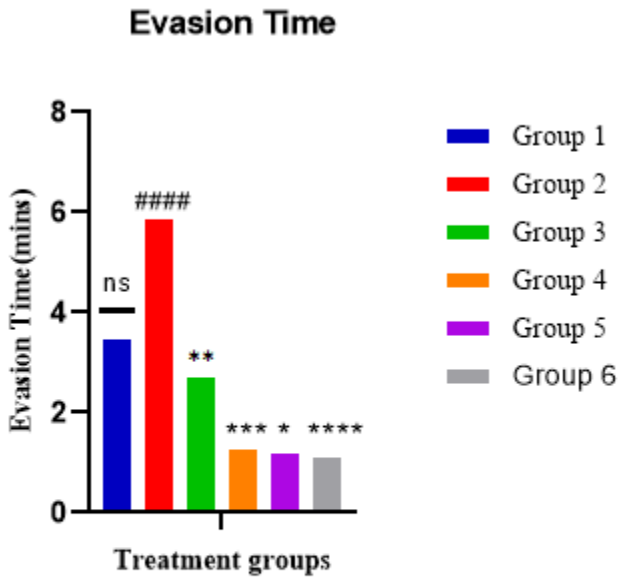


Fig 16. Graphical representation of Evasion Time

REDUCED GSH

Group	ReducedGSH (Glutathione µg/mg)
Group 1	9.38±0.07
Group 2	6.68±0.05
Group 3	5.37±0.12
Group 4	4.74±0.23
Group 5	3.71±0.44
Group 6	2.76±0.56

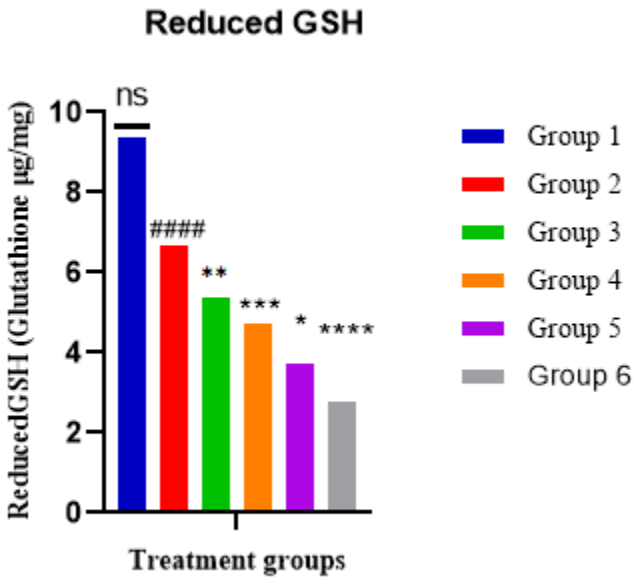


Fig 17. Graphical representation of Reduced GSH

HISTOPATHOLOGICAL RESULTS:

Group 1: Multiple foci of disruption and death were identified. In the prefrontal cortex, focusal gliosis was discovered. Meningeas protecting the brain have been shown to contain delicate carnage.

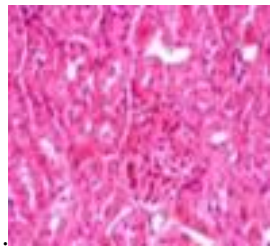
Group 2: A number of distinct pathologies, including multifocal necrosis or autophagy of neurons, glial cell attack, and red hot cells, have been identified. The Dentate Gyrus has shown signs of smooth degenerative changes. Fluids were discovered to be in close proximity to the ventricles.

Group 3: Degenerative changes in the smooth gyrus of the dentate gyrus have increased in number.

Group 4: The ventricles of the brain are a source of extreme discomfort. Hemorrhages formed in the prefrontal cortex. Bulks of stirred cells. Dentate gyrus is associated with vacuolar degeneration and apoptotic cell death.

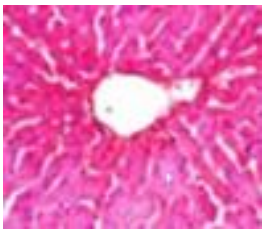
Group 5: It is known that there are multifocal necrotic or apoptotic neurons along with glial cell invasion and inflammatory cells. In Dendate Gyrus, mild degenerative changes were observed. Accumulation of fluids noticed ventricles.

Group 6: Serious bleeding in the brain ventricles has been identified. Frontal cortical field has experienced hemorrhages. Inflammatory cell build-up foci. Dendate gyrus is associated with vacuolar degeneration and apoptotic neurons

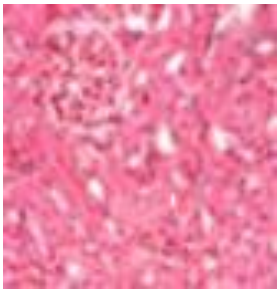


Group 1

Group 2



Group 3



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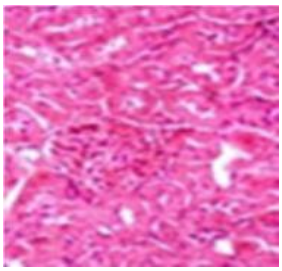

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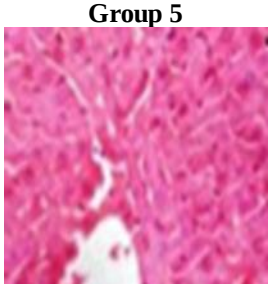

Group 6

DISCUSSION:

In this discussion, we will analyze the experimental protocol, methods for behavioral assessment, and results of a dissertation work involving the administration of methanolic extract of Sagittaria Trifolia and Litchi Chinensis to mice. The study aimed to investigate the potential antidepressant effects of the extract and assess its impact on various behavioral and biochemical parameters. We will focus on the experimental design, methods used for behavioral assessment, and key findings.

Experimental Protocol: The study design involved treating mice with two different doses of methanolic extract of Sagittaria Trifolia and Litchi Chinensis (200mg/kg each separate and 200mg/kg (100mg + 100mg in combination)) for 14 days. Behavioral assessments were conducted on days 1, 7, and 14 after extract administration. Four groups of mice were used in the study, including a control group, a standard group treated with Imipramine, and two test groups receiving different doses of the extract as compare to Petit-Demouliere B et.al.

Water Wheel Method: The results from the Water Wheel Method showed that the standard group (Imipramine) exhibited significantly lower immobility durations compared to the control group on all three test days. The test groups (treated with Sagittaria Trifolia and Litchi Chinensis extract) also demonstrated reduced immobility durations, especially on day 7 and day 14, indicating a potential

antidepressant effect. However, the effect was less pronounced than that observed in the standard group as compare to Slattery DA et.al.

Tail Suspension Test: In the Tail Suspension Test, similar trends were observed. The standard group showed reduced immobility durations on all test days compared to the control group. The test groups also exhibited decreased immobility durations, again with the most significant effects seen on day 7 and day 14 as compare to Telner JI et.al.

Open Field Test: The Open Field Test results indicated changes in movement patterns. The control group spent more time in the central area and less time in the corner area, which is consistent with increased anxiety or depression-like behavior. In contrast, the standard and test groups spent less time in the central area and more time in the corner area, suggesting a reduction in exploratory behavior as compare to Willner P; et.al.

Biochemical Parameters: The study also assessed various biochemical parameters related to oxidative stress and neurotransmitter levels. These parameters included catalase activity, lipid peroxidation (LPO), superoxide dismutase (SOD) activity, and reduced glutathione (GSH) levels.

- Catalase activity was significantly higher in the standard and test groups, especially in the Test - II group, indicating potential antioxidant effects of the Sagittaria Trifolia and Litchi Chinensis extract as compare to Arban R; et.al.
- LPO levels were reduced in the standard group and Test - I group, suggesting a protective effect against lipid peroxidation as compare to Friedman E; et.al.
- SOD activity was decreased in all treatment groups compared to the control group, indicating a potential normalization of oxidative stress as compare to Slattery DA; et.al.
- Reduced GSH levels were significantly lower in the Test - I and Test - II groups, suggesting a possible role in oxidative stress regulation as compare to Wang D; et.al.

CONCLUSION:

In this comprehensive dissertation study, the effects of methanolic extract of Sagittaria Trifolia and Litchi Chinensis on the behavioral and biochemical parameters of mice were thoroughly investigated. The mice were subjected to a 14-day treatment regimen with two different doses of the extract, followed by behavioral assessments on days 1, 7, and 14. The experimental groups were compared to a control group and a standard group treated with Imipramine. The study's methods included the Water Wheel Method, Tail Suspension Test, and Open Field Test to assess behavioral changes, as well as biochemical analyses to determine antioxidant properties.

The findings of this research suggest that the methanolic extract of Sagittaria Trifolia and Litchi Chinensis holds promise as a potential treatment for depressive-like behaviors in mice. The behavioral assessments, particularly the Water Wheel Method and Tail Suspension Test, demonstrated a reduction in immobility duration in the groups treated with the extract, especially at the higher dose (400mg/kg), indicating a potential antidepressant effect.
Moreover, the biochemical analyses revealed significant alterations in antioxidant parameters, including increased catalase activity and reduced lipid peroxidation (LPO). These changes suggest that the Sagittaria Trifolia and Litchi Chinensis extract may exert its antidepressant effects, in part, through modulation of oxidative stress.

However, it is important to note that while the extract showed promising results, further research is required to elucidate the exact mechanisms underlying its antidepressant properties and to assess its safety and efficacy for potential clinical use. Additionally, histopathological studies and additional biochemical analyses may provide a more comprehensive understanding of the extract's effects on the central nervous system.
In conclusion, this dissertation contributes valuable insights into the potential antidepressant properties of methanolic extract of Sagittaria Trifolia and Litchi Chinensis and provides a foundation for future research in the field of natural products as alternative treatments for depression. The findings underscore the importance of continued investigation into this plant extract's therapeutic potential.

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