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ELUCIDATE THE ETHANOLIC EXTRACTS OF THE BARKS OF *ANTHOCEPHALUS CADAMBA* AND *ZIZPHUS CARACUTTA* FOR PROTECTIVE EFFECT AGAINST THE DEPRESSION AND ANXIETY

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ABSTRACT

The present research were carried out to evaluate the anti-depression and anti-anxiety potential of the ethanolic extracts of *Anthocephalus cadamba* and *Ziziphus caracutta*. Depression and anxiety are major mental health concern that affect a large number of people globally. Although several synthetic medicines are available their use is limited due to unwanted side effects and dependency problems. Therefore natural herbal sources are being explored as safer and effective alternatives. The ethanolic extracts of these plants were screened for their phytochemical components, which showed the presence of flavonoids, alkaloids, phenols and tannins are known for their neuroprotective activity. *In vivo* studies were conducted using behavioral models such as the Forced Swim Test, Yail Suspension Test, Elevated Plus Maze Method, Dark and Light Model. The extracts significantly reduced immobility time and increase exploratory behavior, indicating both anti-depressant and anxiolytic activity. These findings suggest that *Anthocephalus cadamba* and *Ziziphus caracutta* possess promising neuropharmacological effects and may serve as potential herbal remedies for the treatment of depression and anxiety.

KEYWORDS

Anthocephalus cadamba, *Ziziphus caracutta*, Anti-depression and Anti-anxiety.

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INTRODUCTION

Depression

Depression is additionally referred as major depressive disorder. The disorder is a mental illness with loss pleasure or interest in the routine tasks. The depression conceivably mild or severe, in specific severe cases it promotes to suicide related cognition. The depression can adversely

repercussions the persons life the manner of thinking and exhibiting behaviour.

Anxiety

The anxiety is an emotional state which is unpleasant, fear about the prospective threats. The threats may be defined and undefined. Amygdala is superintends the emotion control. The state where the persons feels fear in disproportionate amount the amygdala are endowed with impulses and controls emotion.

Review of drug under study

Anthocephalus cadmba and Ziziphus caracutta

Taxonomy of *Ziziphus caracutta*

Phylum: Tracheophyta

Family: Rhamnaceae.

Genus: Ziziphus.

Species Ziziphus caracutta.

Synonyms: Ziziphus Xylo Pyrus.

Taxonomy of *Anthocephalus Cadamba.*

Phylum: Tracheophyta

Family: Rubiaceae

Genus: anthocephalus

Species: cadamba.

Vernaular name: Kadam, kadamba, kadam tree, bur flower tree

MATERIAL AND METHODOLOGY

Plant materials

A dried powdere bark of the *Anthocephalus cadamba* and *Ziziphus caracutta* were sruitinized and taxonomically affirmed by the Dr. K Madhava Chetty, Assistant professor, department of Botany, Sri Venkateshwara College, Tirupathi.

Extract preparation

The plant sample is extracted by the maceration technique. The ethanol (99.9%) is utilized for the extraction by the maceration process.

Maceration process

The powdered plant material was soaked in a suitable solvent and kapt undisturbed for several days with occasional stirring. The extract was then filtered and concentrated to obtain the crude extract for further analysis.

Acute Toxicity Studies

The studies were performed as per OECD guidelines no. 425 of CPCSEA. The administration of extract formulation by oral route of 100mg/kg, 200mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg has no toxic effect. The dose of 200mg/kg has determinant effect. The ethanolic extract of bark of the *Anthocephalus cadamba, Ziziphus caracutta* has LD50 value above the 2000mg/kg. Therefore the studies were accomplished on the preffered dose of 200mg/kg as a low dose and 400mg/kg as a high dose of *Anthocephalus cadamba* and *ziziphus caracutta*.

DRUGS AND CHEMICALS

Imipramine, Alprazolam, Diazepam, Phencyclidine, Ethanol, Ethanolic extracts of *Anthocephalus cadamba*, *Ziziphus caracutta*.

Screening method for depression

Forced Swim Test

Principle

The behavioral despair test in rodents were performed in an inescapable water tank the rodents in a water containers tries to escape and assist passive coping (passivity) strategy. Eventually, the rodents become immobile and deplete the time of passive coping.

Procedure

A cylindrical test arena filled with water is utilized to place the mice. The temperature of water and room must be normal to dismiss the normal stress. The animals at beginning shows behavioral despair and later increase the active coping effect. The test persists about 4-6 minutes. The duration of immobility is recorded. The mice are take out of the cylindrical tank after 15 minutes and allowed to dry. For the test and standard group the same methods were carried out and drug is bestowed 1 hour prior to the test.

Evaluation

Immobility time (in seconds): When the animal floats with minimal movement to keep its head above water.

Interpretation: Reduced immobility time indicates anti-depressant activity (less behavioral despair)

Screening methods for anxiety

Elevated plus maze method

Principle

The elevated plus maze method is naturalistic and mostly used method for screening of anxiety in rodents. The EPM test depends on susceptibility to escape the expose area.

The apparatus consists of two open arms and two closed arms contrary to each other. This is placed above the 50cm to the surface.

Procedure

The mice (18-20g) are selected from the cages and are fed with proper diet. 30 min prior to the experiment the test drug is administered through i.p route. The 5 minutes of standardized session is performed. The temporal scoring and ethogram profiling is determined. The various parameter are evaluated in the experiment, time spend in open and closed arms and all four paws on arm.

Evaluation parameters

Number of enteries in open arms

Time spent in open arms

Number enteries in closed arms

Interpretation

Increased time spent and enteries into open arms, indicates anti-anxiety activity, as the animal reduced fear or anxiety.

Interpretation

Increased time in the light area and more transitions indicate anxiolytic effect, as normal animals prefer dark areas due to fear of open spaces.

RESULTS AND DISCUSSION

Phytochemical Screening Test

The preliminary phytochemical screening was performed on the gleans of the *Anthocephalus cadamba* and *Ziziphus caracutta* by following the standard methods.

Bio chemical evaluation of sodium in non-stressed, stressed and standard blood samples of mice.

Evaluation of potassium and calcium in non-stressed, standard and stressed blood samples

Discussion

In context of the study, the anxiolytic and anti-depressant effect of *Anthocephalus cadama* and *Ziziphud caracutta* bark extract was scrutinized on the stressed and non-stressed mice.

The phytochemical profiling of the both the plant extracts were determined.

In this study the anxiolytic effect. The neurobehavioral characterization was observed while performing the Light and Dark test and EPM test. The result of the test suggest the ethanolic extract have the anxiolytic activity.

Ethanolic extract of *Anthocephalus cadamba* and *Ziziphus caracutta* was evaluated on the FST and TST, which indicates the normal neurobehavior of the animal. The methods are more reliable to perform and to determine emotional state of the animal.

The results has demonstrated and substantially decrease in the immobility time in water tank has depicted.

The result of the TST indicates the significant decrease in the immobility has determined.

In the current investigation, the tremendous effect of neuromotor coordination has depicted by the extract of *Anthocephalus cadamba* and *Ziziphus caracutta*.

The histopathology and bio chemical evaluation was performed. The biochemical evaluation on stressed mice showed the potential effect of increase in monoamine level and sodium, calcium and potassium in the blood of the 5, 7 and 8 groups of the mice.

The histopathological analysis are determined on the hippocampus and cerebral cortex of the mice, showed the presence of the large vesicular nuclei and small perineural gaps that indicates the tangled neuroglial cells.

The extract of *Anthocephalus cadamba* and *Ziziphus caracutta* have anti depression like action, the accurate mechanism is not completely comprehend.

Table No.1: Phytochemical test result of *Anthocephalus cadamba* and *Ziziphus caracutta*

S.No	Phtyo-constituents	Tests	EE of *Anthicephalus cambada*	EE of *Ziziphus Caracutta*
1	Alkaloids	Dragendorff test	+	++
		Hager's test	++	_
		Wagner's test	_	+
		Mayers test	++	++
		Barfoed's	+	_
2	Glycosides	Legals test	_	++
		NaOH 10% test	+	+
3	Carbohydrates	Benedict's test	+++	++
		Molish test	+	_
		Seliwanoff's test	+	+
4	Flavonoids	Bortrangers test	+++	+
		Shinoda test	+	+
		Ferric cl test	++	+++
5	Terpenoids	Salkowski's test	++	+++
6	Tannis	Bromine H2O test	++	++
7	Sterols	L.Burchads test	+++	+++
		Salkowski's test	+	++
8	Saponins	Foam test	++	_
9	Coumarins	Sodium OH test	+	+
10	Phenols	Iodine	+++	++
		Ferric cl test	++	+
		Pb acetate test	+	+

Table No.2: Peak report of the zizphus caracutta

S.No	Retention time	Chemical constituents	Area %	Uses
1	5820	Dextroamphetamine	3.54	Antianxiety stimulant, cognitive enhancer, aphrodisiac, euphoriant
2	26410	n-ethoxycarbonylhydrazon	2.03	Antianxiety, anti-psychosis, antibacterial, immunomodulator
3	32794	Caffeic acid	1.33	Anti-depressant, anxiolytic, anti-inflammatory, anti-cancer.
4	20955	Dimethyl sulfoxide	11.38	Antiepileptic
5	1809	1, 5 heptadiene-3-yne	4.55	Anti-epiletic, analgesic
6	3784	Methyl methane sulfonate	4.21	Anti-epileptic, analgesic, antibacterial
7	12270	3, 4 dihydroxyphenyl hexylamine	1.21	Anti-epileptic, antipsychotic, alkylating agent
8	34125	Cinnamic acid, 3, 4 dimethoxy trimethylsilyl ester	1.63	Anti-depression, antifungal
9	31751	l-norephedrine	1.59	Anti-depression, CNS stimulant, nasal congestion, myasthenia gravis

Table No.3: Peak report of the *Anthocephalus caracutta*

S.No	Retention time	Chemical constituents	Area %	Uses
1	22200	Eicosanoic acid	194	Anti-depressant, haemolytic agent, anti-coagulant, anti-inflammatory
2	29010	Chlorpheniramine	284	Anti-depressant. anti-Psychotic, anti-cancer, anti-viral
3	26410	n-ethoxycarbonylhydrazon	203	Anti-epilepsy, anti-Bacterial, Immunomodulator, anti-diabatic.
4	9550	4-hydroxyadmtan-2-one	176	Anti-epileptic, anti-viral, treatment of influenza
5	11501	2, 3 dimethylfumaric acid	328	Anti-anxiety. Treatment of psoriasis, autoimmune disorders
6	32930	Lupeol	438	Anti-convulsant. Anti-cancer, anti-inflammatory. Dietary triterpene.
7	31879	B-amyrin	226	Anti-depression, anti-anxiety.
8	34770	9-[4-hydoxybutyl] hypoxanthine	111	Anti-depression. anti-psychosis, anti-inflammatory

Animal models for anti-depressant and anxiolytic activity

Table No.4: Forced Swim Test

Groups	Treatments	Immobility time (MEAN ±SEM) SEC	
		3rd Day	6th Day
Group-1	Normal Saline	182.02±0.96	186.16±0.19
Group-2	Toxic control:Diazepam (5mg/kg)	52.03±1.002	26.29±1.055
Group-3	Standard drug: Imipramine (10mg/kg)	167.02±0.66***	176.62±0.925**
Group-4	Plant 1: E.E.AC (200mg/kg)	122.02±0.35**	153.45±0.58***
Group-5	Plant 1: E.E.AC (400mg/kg)	142.06±0.05*	147.05±0.06
Group-6	Plant 2: E.E.ZC (200mg/kg)	127.54±0.61*	141.35±0.73
Group-7	Plant 2: E.E.ZC (400mg/kg)	146.05±0.53	141.43±0.64
Group-8	Plant 1+2: E.E.AC (100mg/kg) + E.E.ZC (100mg/kg)	108.23±0.005	117.62±0.63*
Group-9	Plant1+2: E.E.AC (200mg/kg)+E.E.ZC (200mg/kg)	149.68±0.002	148.43±0.72

Tail suspension test

Table No.5: Tail Suspension Test

Groups	Treatments	Immobilityu Time (MEAN±SEM) sec	
		3rd day	6th day
Group-1	Normal saline	188.27±0.034	183.58±0.077
Group-2	Toxic control: Diazepam (5mg/kg)	32.60±0.184	40.64±1.025
Group-3	Standard drug : Imipramine (10mg/kg)	188.31±0.095**	178.74±0.15***
Group-4	Plant 1: E.E.AC (200mg/kg)	167.02±0.032	156.55±0.089**
Group-5	Plant 1: E.E.AC(400mg/kg)	171.53±0.043	160.76±0.005
Group-6	Plant 2: E.E.Z.C(200mg/kg)	175.82±0.06	163.00±0.019**
Group-7	Plant 2: E.E.Z.C(400mg/kg)	178.65±0.08	169.64±0.055
Group-8	Plant1+2: E.E.A.C (100mg/kg)+E.E.Z.C (100mg/kg)	180.0±0.180***	170.00±0.080*
Group-9	Plant 1+2: E.E.A.C (200mg/kg) + E.E..Z.C (200mg/kg)	183.03±0.15*	175.82±0.05

Dark and light model

Table No.6: Dark and light model

Groups	Treatments	Dark and light exploration			
		Parameters observed			
		Time spent in light zone(sec)	Time spent in dark zone (sec)	No. of crossings	Transfer latency
Group-1	Normal saline	125.62±0.51	125.5±0.85	35.0±0.43	29.76±0.39
Group-2	Toxic control: Phencyclidine (5mg/kg)	60.03±0.04	398.2±0.51	6.02±0.53	120.03±0.63
Group-3	Standard drug Alprazolam	138.30±0.41**	130.1±0.32**	57.9±0.03**	32.31±0.43**
Group-4	Plant 1: E.E.A.C (200mg/kg)	90.8±0.75**	285.5±0.75*	26.0±0.53**	86.21±0.25**
Group-5	Plant 1: E.E.A.C (400mg/kg)	108.4±0.32	279.3±0.42	38.4±0.42	65.3±0.47
Group-6	Plant 2: E.E.Z.C (200mg/kg)	112.63±0.07	225.5±0.51**	49.02±0.3**	60.87±039*
Group-7	Plant 2 E.E.Z.C (400mg/kg)	122.2±0.05	205.5±0.42**	50.04±0.6	49.3±0.52*
Group-8	Plant 1+2 E.E.A.C(100mg/kg) + E.E.Z.C(100mg/kg)	120.43±0.63	150.4±0.42*	55.3±0.41*	35.03±0.4*
Group-9	Plant 1+2 E.E.A.C(200mg/kg) + E.E.Z.C(200mg/kg)	132.4±0.32**	152.5±0.64	57.6±0.43*	33.64±0.43*

Elevated plus maze method

Table No.7: Elevated plus maze method

Groups	Treatments	Elevated plus maze				
		Parameters observed				
		Time spent in open arm (sec)	Time spent in enclosed arm (sec)	Entries in open arm	Entries in enclosed arm	Time spent in central zone (sec)
Group-1	Normal saline	42.62±0.51	25.5±0.85	35±0.03	25±1.39	20.54±1.03
Group-2	Toxic control: phencycli dine (5mg/kg)	8.03±0.04	78.2±0.51	5±0.53	45±0.63	10.03±0.81
Group-3	Standard drug: Alprazolam (0.08mg/kg)	39±0.41***	19.2±0.3***	30±0.85***	29±0.1***	26.5±0.8***
Group-4	Plant 1: E.E.A.C (200mg/kg)	25.4±0.3**	65.6±0.52**	15±0.32***	39±0.6**	11.9±0.73**
Group-5	Plant1: E.E.A.C (400mg/kg)	28.7±0.05*	53.5±0.02*	19±0.6	35±0.02*	15.1±0.05*
Group-6	Plant 2: E.E.Z.C (200mg/kg)	32.93±0.3*	40.7±0.72*	22±0.1**	30±0.3**	20.5±0.03*
Group-7	Plant 2: E.E.Z.C (400mg/kg)	34.9±0.07	26.2±0.01**	24±0.05*	28±0.03*	22.9±0.3
Group-8	Plant 1+2 E.E.A.C (100mg/kg) + E.E.Z.C (100mg/kg)	36.7±0.3**	25.9±0.73**	25±0.8**	26±0.32	25.5±0.41*
Group-9	Plant 1+2 E.E.A.C (20mg/kg) E.E.Z.C (200mg/kg)	70.4±0.2**	20.2±0.52**	29.4±0.05*	27±0.3*	26.6±0.02*

Biochemical estimation in blood samples

Monoamine levels in the stressed and non-stressed mice

Table No.8: The table shows the levels of monoamines in the mice

Groups	DA	5HT	NE
Group-1	0.036±0.03	2.20±0.02	2.35±0.04
Group-2	0.12±0.10	0.23±0.01	0.12±0.71
Group-3	0.45±0.12	2.38±0.15	2.28±0.04
Group-4	0.34±0.04**	0.89±0.03**	0.88±0.12*
Group-5	0.38±0.13*	1.33±0.14*	1.25±0.03**
Group-6	0.30±0.16*	1.66±0.01**	1.55±0.18*
Group-7	0.33±0.04**	2.0±0.23*	2.09±0.05**
Group-8	0.43±0.02**	2.15±0.07**	2.39±0.06**
Group-9	0.56±0.12*	2.25±0.14*	2.45±0.09**

Table No.9: The present table shows the serum sodium levels in the mice

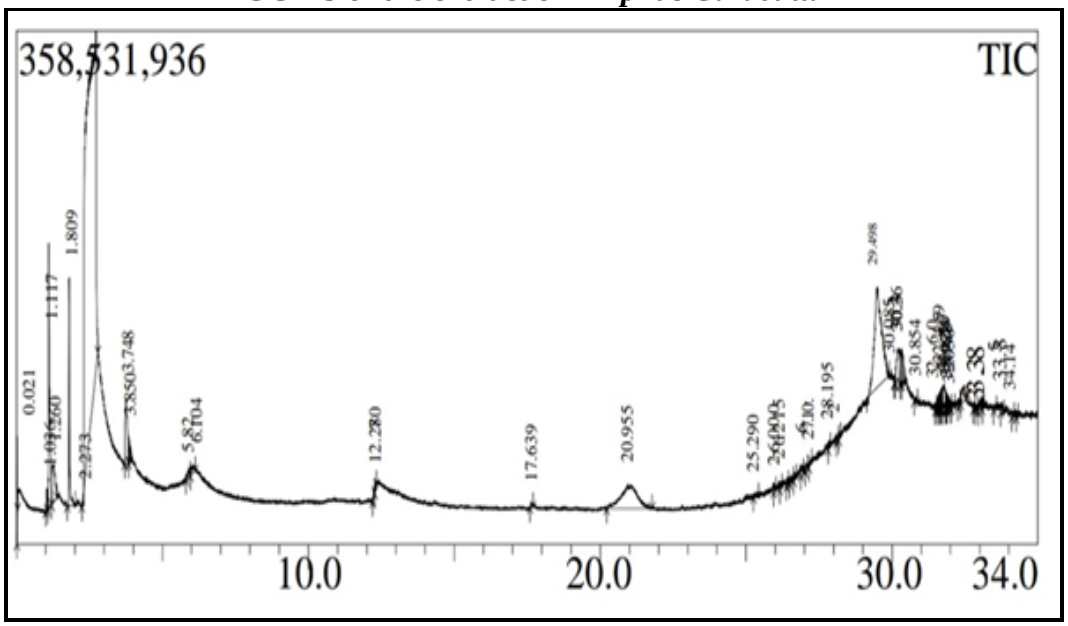
Groups	Serum Sodium (mmol/L)
Group-1	138.0±1.0
Group-2	200.3±0.06
Group-3	135±0.15
Group-4	185.6±1.5
Group-5	158±0.11*
Group-6	143.5±0.32*
Group-7	140±0.08**
Group-8	138.3±0.09**
Group-9	132.2±0.16*

Table No.10: The present table shows the serum potassium and calcium levels

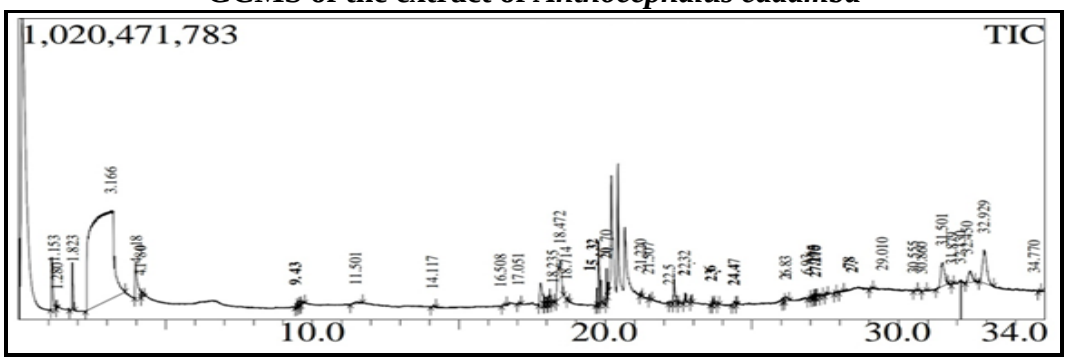
Groups	Serum potassium (mmol/L)	Serum calcium (mmol/L)
Group-1	5.10±0.20	2.48±0.01
Group-2	20.4±0.07	0.33±0.04
Group-3	5.82±0.05	2.22±0.09
Group-4	8.25±0.02**	1.31±0.2*
Group-5	7.32±0.27*	1.98±0.07**
Group-6	6.71Group±0.1*	2.0±0.12*
Group-7	5.51±0.34*	2.80±0.3**
Group-8	5.01±0.03**	2.82±0.06**
Group-9	5.71±0.32*	2.95±0.04**

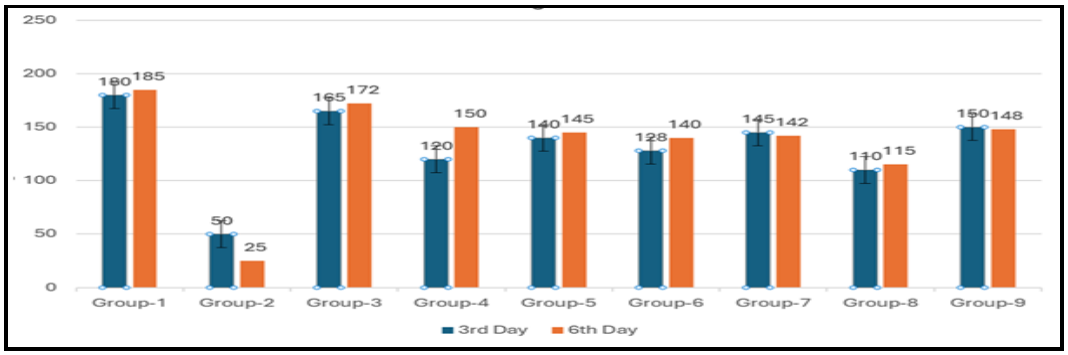
GCMS RESULTS

GCMS of the extract of *Ziziphus Caracutta*

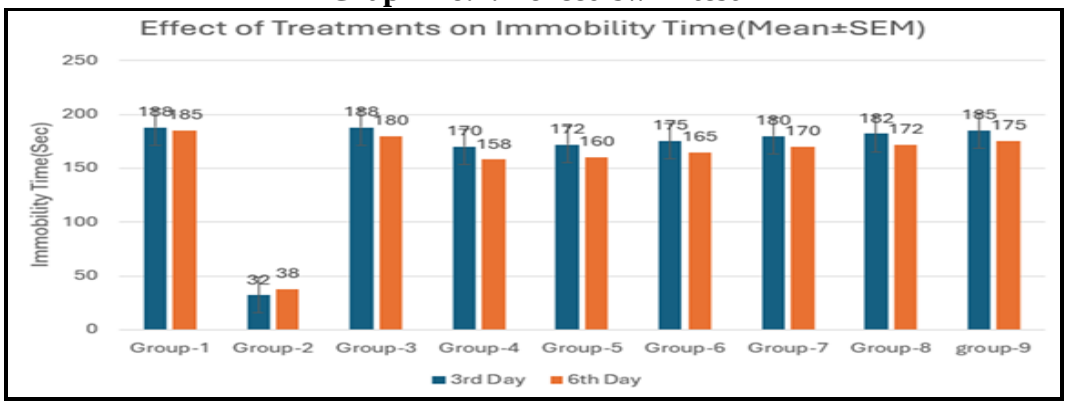


GCMS of the extract of *Anthocephalus cadamba*

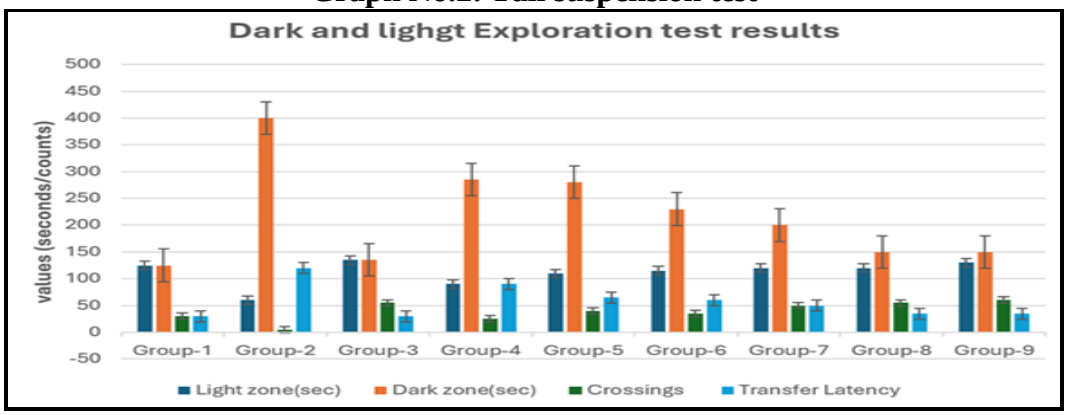




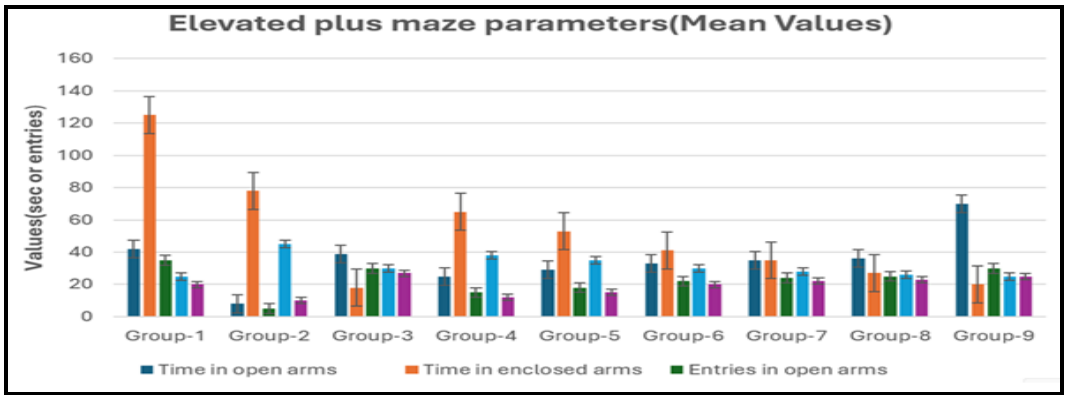
Graph No.1: Forced swim test



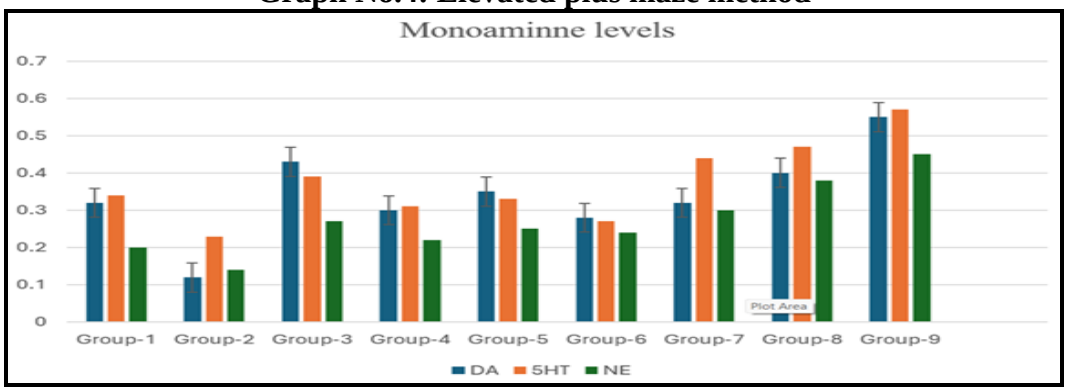
Graph No.2: Tail suspension test



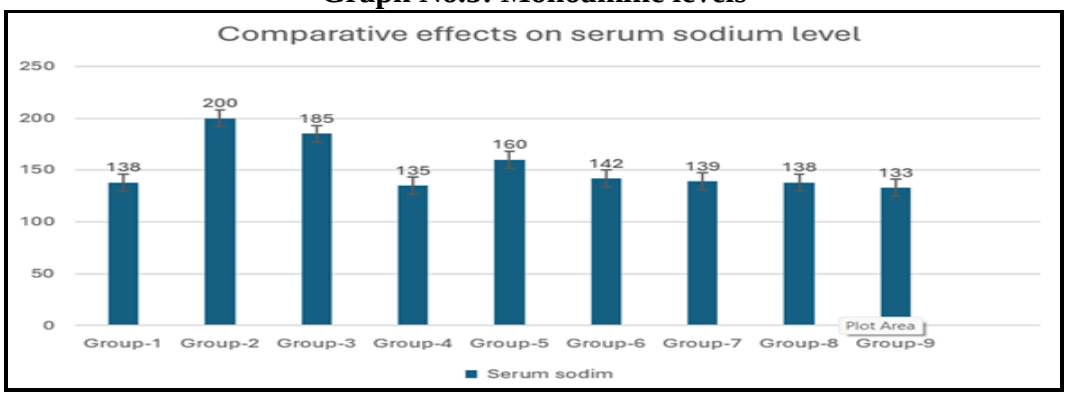
Graph No.3: Dark and light exploration



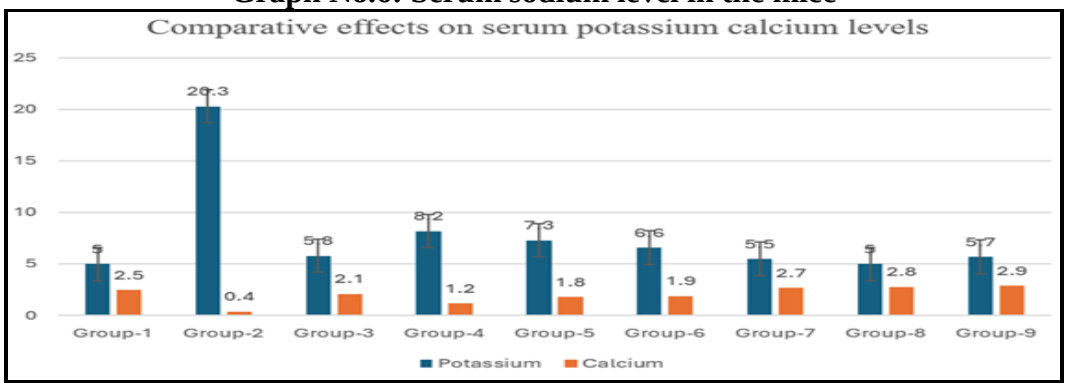
Graph No.4: Elevated plus maze method



Graph No.5: Monoamine levels

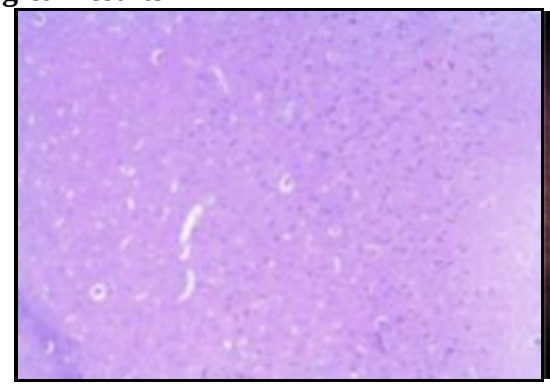


Graph No.6: Serum sodium level in the mice

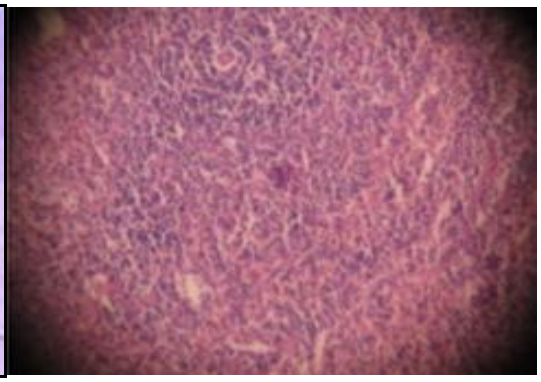


Graph No.7: The levels of serum potassium and calcium

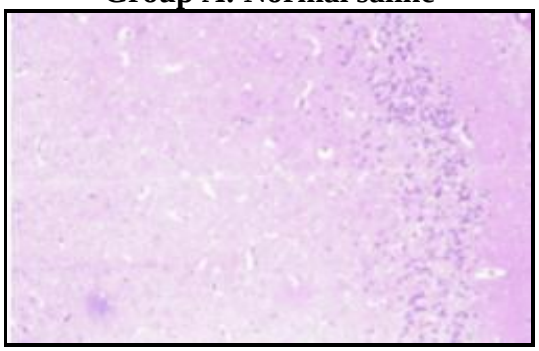
Histopathological results



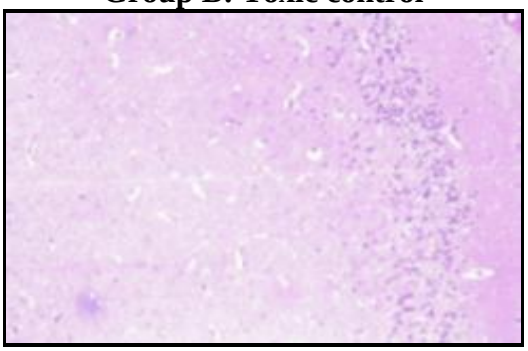
Group A: Normal saline



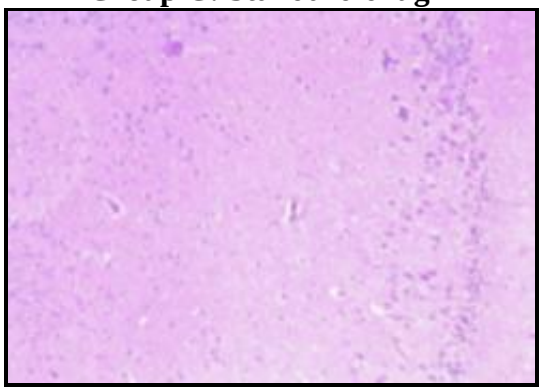
Group B: Toxic control



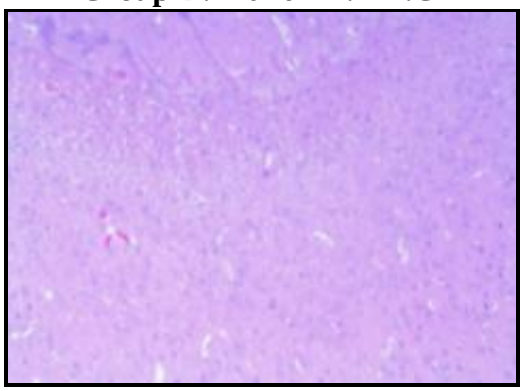
Group C: Standard drug



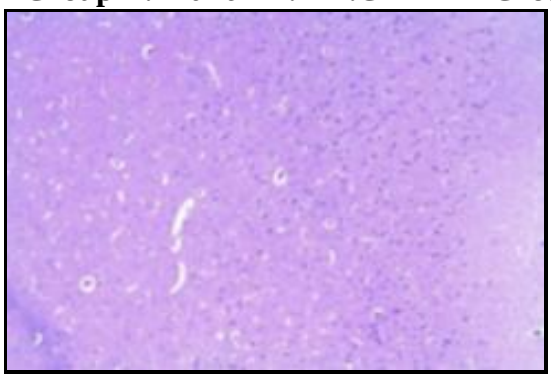
Group D: Plant 1 E.E Z.C



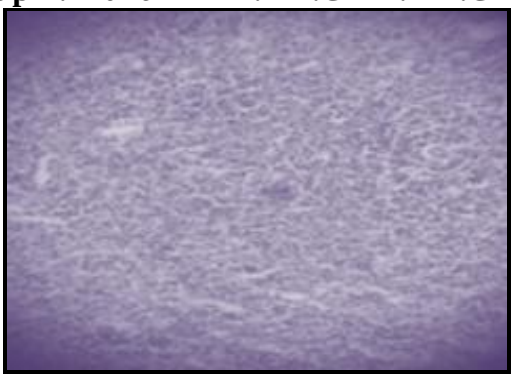
Group E: Plant 2 E.E A.C



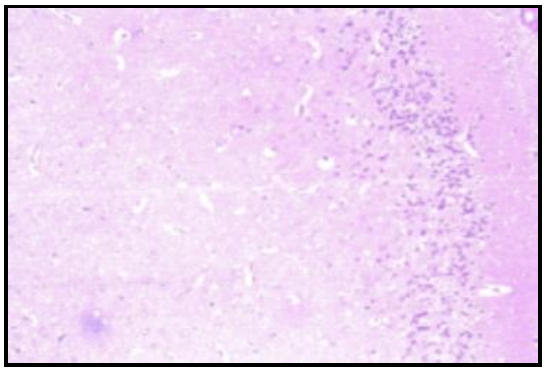
Group F: Plant 1 + 2 E.E Z.C + E.E A.C



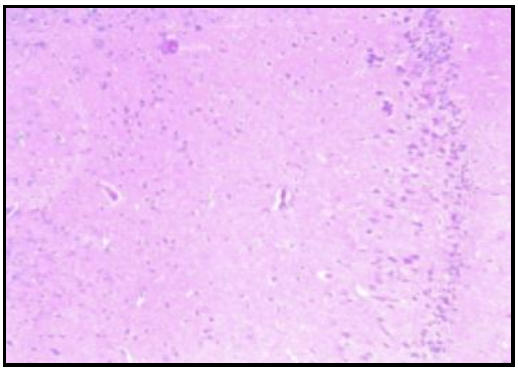
Group A: Normal saline



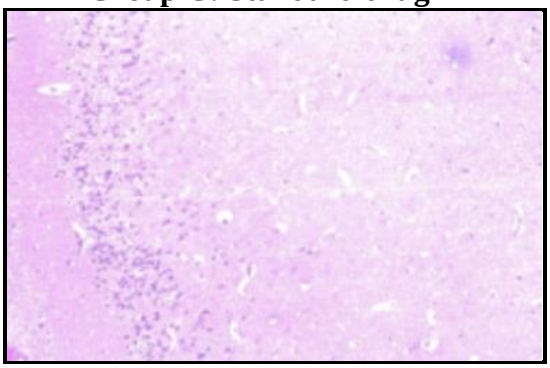
Group B: Toxic control



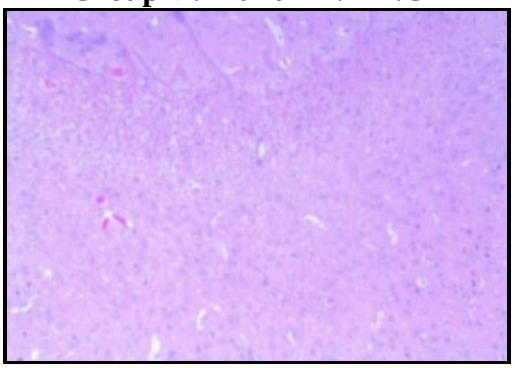
Group C: Standard drug



Group D: Plant 1 E.E Z.C



Group E: Plant 2 E.E A.C



Group F: Plant 1 + 2 E.E Z.C +E.E A.C

Figure No.1: *Zizphus Caracutta*

Figure No.2: *Anthocephalus Cadamba*

Figure No.3: Forced swim test

Figure No.4: Elevated plus maze method

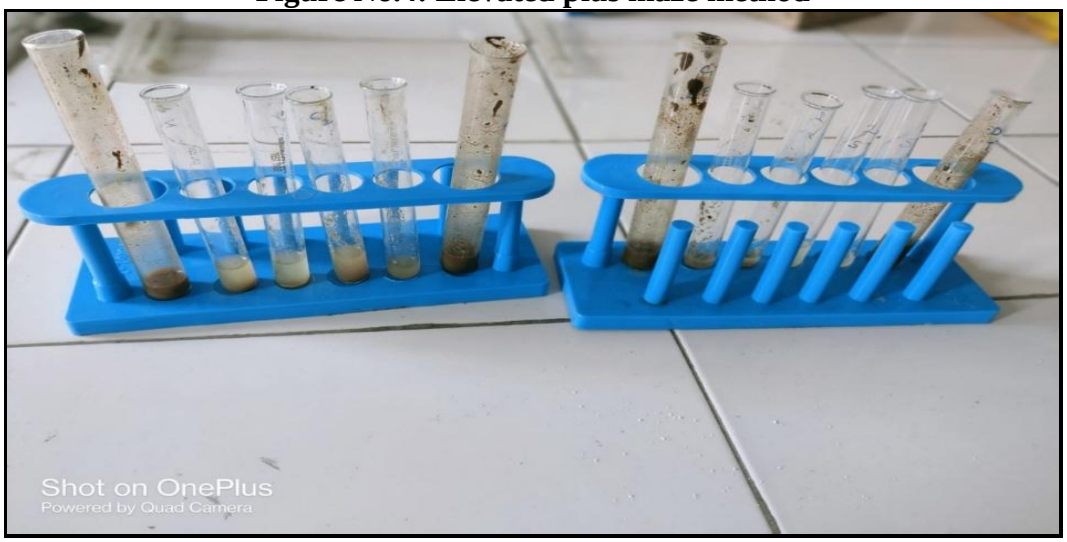


Figure No.5: Screening of the *Anthocephalus cadamba* and *Ziziphus caracutta* extracts

CONCLUSION

The purpose of this study to achieve the anti-depression and anti-anxiety effect on the experimental animals by using the extracts of the herbal plants *Anthocephalus cadamba* and *Ziziphus caracutta*. The plant extract has potential effects on the activity and can be extensively used for the treatment. The studies provides the insights for the neurobehavioral aspects of the animals while performing the behavioral test such as FST, TST, EPM.

Further investigations should be done on the extracts of the *Anthocephalus cadamba* and *Ziziphus caracutta* to strengthen present studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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