IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 6 Ser. 1 (Nov. – Dec. 2023), PP 01-20
www.Iosrjournals.Org

PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL SCREENING OF ETHANOLIC EXTRACT OF LEAVES OF ADIANTUM INCISUM FORSK AND YUCCA ALOIFOLIA FOR PYSCHOPHARMACOLOGICAL ACTIVITIES IN WISTAR RATS

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ABSTRACT :

Psychopharmacology is the branch of pharmacology which deals with the mind and mental health, and the effect of drugs that would modify the behavior of the organisms. This study evaluated the antidepressant antianxiety and antipsychotic effect of ethanolic extract of Adiantum incisum forsk and Yucca aloifolia indisease inducedwistar rats. 24 rats were taken into consideration and divided intofour groups six rats each. Group-I consisted of normal rats that were given normal sterile saline solution and served as control group. Group-II consisted of rats induced with disease either through drug or through physical induction. Group III consisted of rats which were induced with depression, anxiety and psychosis. Oral administration of combined extracts of leaves of the plants resulted in significant antidepressant, antianxiety and antipsychotic activity. Group IV consisted of rats induced with depression, anxiety, psychosis and were treated with standard drugs, the effects produced by the extracts were found to be closely similar to the standard drugs. In conclusion, the evaluated study indicates that the ethanolic extract of Adiantum incisum forsk and Yucca aloifolia combined appear to exhibit antidepressant, anti anxiety and antipsychotic activity in disease induced wistar rat.

Keywords–*Psychopharmacology, Phytochemical, Adiantum, Yucca, Wistar rats, Pathophysiology*

Date of Submission: 24-10-2023 Date of Acceptance: 04-11-2023

I. INTRODUCTION

In the evaluation of extracts of leaves of plants Adiantum incisum forsk and Yucca aloifolia were evaluated for their use in the management of diseases like depression, anxiety, psychosis. From the detailed study and investigation of the available literature of both the plants that have shown that serve as an important source of many therapeutically efficient chemicals like flavonoids, alkaloids, tannins saponins, carbohydrate, and other constituents. The ethanolic extract of leaves of adiantum incisum forsk and Yucca aloifolia are evaluated for various psychopharmacological disorder in wistar rat, the disease was induced either by drugs or through physical methods.

II. REVIEW OF DRUG UNDER STUDY

ADIANTUM INCISUM FORSK[1]

Adiantum incisum is a medicinal plant also known as aromatic ornamental fern. It is found naturally in shady regions that are humid and can also grow indoor for beautiful fronds. Adiantum incisum is known as Nilakantha- shikhaa,Mayurshikha, Vahrishika in Ayurveda (there are some other species of plants also referred as Mayurshikha in Ayurveda). It has properties like astringent tonic, febrifuge. Medicinally, the leaves are a source of cure forcough, fever, chest infection. It is also proved to be helpful in diabetes, externally the paste of the leaf is used to cure skin diseases. Adiantum incisum is used as a substitute for Adiantum capillus-veneris.

YUCCA ALOIFOLIA[2]

Yucca aloifolia belongs to Asparagaceae family, It grows in sandy soils, especially on sand dunes along the coast. It is widely planted in hot climates and arid environments.Commonly known as dagger plant or aloe Yucca. Its roots are used as soap and Shampoo, this has been widely found in natives of Atlantic and gulf coasts of the United states from the southern Virginia south to Florida andYucca has steroidal saponins, both spirostanol

and furostanol type are isolated from the past species. Methanolic extracts also showed that there is a presence of constituents such as alkaloids, tannins, sesquiterpene glycosides, steroids, steroidal glycosides, saponins and flavonoids.

III. MATERIALS AND METHODS

Collection and preparation of extract

Collection of drugs

Dried leaves of the plants were collected. The plant wastaxonomically identified and authenticated by Dr. K MadhavaChetty assistant professor of botany,department of pharmacognosy, Sri Venkateshwara University, Tirupati.

Chemicals

Ethanol, Distilled water, Diazepam- 3mg/kg, (Anti-anxiety drug), Haloperidol- 5mg/kg, antagonist(Anti-psychotic), Imipramine-20mg/kg, (Antidepressant).

Figure 1: Ethanol, Distilled water, Diazepam, Haloperidol, Imipramine (From left to right)

Preparation of plant extract

The leaves of the plants were subjected to shade drying. Oncomplete drying the leaves were powdered and stored inair tight containers at room temperature. The powder ofleaves was macerated with ethanol and distilled water for7 days and then filtered. The filtrate was evaporated toobtain dried extracts. The extracts obtained were subjectedfor evaluation of antidepression, antianxiety and antipsychotic activity. The plant extract was prepared by maceration.The filtrate thus obtained was ethanolically extracted.

Figure 2: Dried powder of leaves of A.I and Y.A

Preliminary phytochemical screening

Phytochemical screening test of the extract was performed for various plant constituents. The crude extract was screened and tested for the presence of or absence of secondary metabolites and chemical groups such as alkaloids, polyphenols, aromatic oil, tannins, sterols, carbohydrates, protein and glycosides following their standard procedures.

Figure 3: Preliminary phytochemical screening

Gas Chromatography – Mass Spectrometry(GC-MS)[3]

Principle behind the working of GC-MS is that these are two different techniques combined in to form a more efficient instrument where GC helps separate the chemical constituent into individual substances upon heat Providence. The gas upon heating is carried away through the column with an inert gas(called helium). The separated substances meet at the opening of the column, which flows into to MS for further process. Where mass spectrometry separates the compounds with respect to the mass of analyte.The GC/MS process, has a sample which is first injected into a gas chromatograph, and the components are separated according to size, polarity. The components then passes into a device known as a mass selective detector. At this stage a mass spectrum is compared against standard reference to identify unknown sample.In case of other sample detectors, it sometimes results in false positives. GC/MS, however, is a specific test and can produce results which are relevantand

identify the actual presence of unknownin sample. Any gaseous or liquid samples even with in micro-liters, can be analyzed. The process of GC/MS might take atleast an hour, depending on the complexity.The two components of GC/MS include the gas chromatograph and the mass spectrometer. With the help of a capillary column with critical dimensions and properties of phase, the gas chromatograph helps the different molecules of a sample to isolate as the sample moves through the column. The mass spectrometer, after the gas chromatograph, breaks molecule into ionized separates, and then detected according to their ratio of mass-to-charge. As they work together, the substance identification is possible accurately. It decreases the activity errors and possible to cause any wrongs decreases to minimum or nil.

Toxicity study[4]

After scrutiny of protocolthe permission has been accorded by the Institutional Animal Ethics Committee of Centralized Experimental Animal Division, Shadan Educational Society, Khairtabad, Hyderabad. The approval number is IAEC-04/SES-2023/41/110 .

Acute oral toxicity

The acute oral toxicity studies of all the three extracts were undertaken as per the (OECD) guidelines for testing of chemicals by up-and-down procedure. The rats were fasted overnight, and the weight of each rat used was recorded just before use. Animals were divided randomly into acontrol and three treatment groups for each extract, eachgroup consisting of four rats (two males and two females). Control group received only the vehicle and each treatment group received orally the EEAI and EEYA of the studied plant in the limit test at a rate of 2000mg/kg body weightwas conducted and terminated after four survivals out offour animals. Animals were kept under close observation for 4 h after administering the extracts, and then they were observed daily for 3 days for any change in generalbehavior and other physical activities and mortality.

Experimental animals

Wistar albino rats (180-250 g) of both sexes were selected. Before and during the experiment rats were fed with standard diet. After randomization to various groups andbefore initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle.

Figure 4: Experimental animals

Experimental design

GROUPS	EXPERIMENTAL DESIGN
Group I	Normal control rats received normal saline as a vehicle p.o
Group II	Rats received control drug
Group III	Rats received control drug + combination of extract of leaves of Adiantum incisum forsk+ Yucca aloifolia(100+100 mg/kg b.w)200mg/kg b.w in normal saline.
Group IV	Rats received control drug + standard drugs

Figure 5: Experimental design

SCREENING METHODS

Head dip test[5]

A specially designed rectangular-shaped head dip box having 15 holes in each side was used in this study. The number of head dips by rats through these holes in a specified time was counted. Rats of groups III receivedcombination of test drugs. The groups II and IV received control and standard drug Diazepam (2 mg/kg, i.p) and group I received normal saline. The control and drug-treated animals were placed individually in the head dip box and the observations were made for 30 min

Figure 6: Head dip test

Locomotor Activity[6]

It can be studied with the help of an Actophotometer. Each ratwas placed individually in actophotometer and the basal activity score of the animals were recorded after 30, 60 & 120 min of drug treatment. Groups IIIcombination of test drugs. The groups I, II and IV received Normal saline, control and standard drug Diazepam (3 mg/kg, i.p). Activity on eachrat was retested for 10mins. Difference in the activity was recorded considering before treatment values & after treatment values. Thus, Percentage decrease in locomotor activity was evaluated.

Figure 7: Locomotor Activity

Rota-rod test (Thread-mill device test)[7]

The study was carried out according to the method described by Perez et al., (1998). Rota rod treadmill device was used for this experiment. Rats trained to remain on slowly moving (16 rpm) rods of 5 cm diameter for 180 seconds or longer wereselected.Groups III received combination of test drugs. The groups II and IV received control and standard drug Diazepam (10 mg/kg,i.p). One hour after administration of test drugs, the rats were placed singly on the rod for 3 minutes, at 30 minutes intervals for 3 h. If animal failed more than once to remain on the rod for 3 minutes, the test was considered positive, meaning that there is lack of motor co-ordinations.

Figure 8: Rota-rod test

Hole cross test[8]

The method was adopted as described by Takagi et al. (1971). A partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 6 cm diameter was made at a height of 7.5 cm in the centre of the cage. The number of passage of a rat through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of Normal saline(Group I),combinationoftest drugs 1 and 2(Group III) and standard drug(Diazepam 1mg/kgb.w)(Group IV). (Takagi et al., 1971).

Figure 9:Hole cross test

Elevated plus Maze test[9]

Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze Consist of two open arms, 50 × 10 cm, and two closed arms, 50 × 10 × 40 cm. The maze was elevated to a height of 15 cm above the floor. Groups III received combination of test drugs 1 and 2. The groups II,I and IV received control, Normal saline and standard drug Diazepam (35 mg/kg, i.p). Each rat was placed on the central platform facing a closed arm. During 5 min test period, the following measures were taken by an observer, number of open arms entries, time spent in open arms, number of closed arm entries, time spent in closed arms. Entering into an arm was noted only when all paws had crossed out central area. (Kurt, M.,2004).

Figure 10:Elevated plus Maze test

Evasion test[10]

The method of Turner (1965) was used. The animals were introduced into a rectangularbox with an inclined plane by which the rat can escape from the box, and the rat that escaped within 5min from the rectangular box were selected for this test. 15 minutes after administration of normal saline(Group I), diazepam(0.5mg/kg) as standard and combination of both(Group IV), the animals were placed in the box again and the number of rat remaining in the box after 15 min in each group was noted.

Figure 11: Evasion test

Forced Swim Test (FST)[11]

In this test rats are forced to swim in a restricted space from which there is no escape and become immobile. Individual rats were forced to swim in an open cylindrical container containing 7 cm of water at 22.0 ±1.0ºC; the duration of immobility or struggling in a period of 6 minutes was recorded. Immobility was evaluated as when rat ceased to struggle and remained floating in the water, making only necessary movements necessary to keep its headabove water. At the end of the session, the animal was removed from water and dried gently. Rats of Group I, II, III, IV received Normal saline, Toxic control, combination of test drugs 1 and 2 and standard drug (Imipramine 10mg/kg) respectively.

Figure 12:Forced Swim Test (FST)

Catalepsy in rats[12]

Five groups of 4 albino rats each weighing between 20-50g are used. They are treated with the test drug or the standard given intraperitoneal i.e. Groups III received combination of test drugs 1 and 2 respectively. The groups I and IV received Normal saline and standard drug Resperidone (1 ml/kg, i.p). After 30 min they are placed individually into translucent plastic boxes with a dowel mounted horizontally 15cm from the floor and 5cm from the end of the box. The floor of the box is covered with approximate 8cm of the bedding material. The animals are allowed to adapt to the box for 5 min. Thereafter each animal is grasped gently around the shoulders and the forepaws are placed carefully on the dowel. The amount of time spent with at least one forepaw on the bar is determined. When the animal removes its paw, the time is recorded and the rat is repositioned on the bar. Four trials are conducted for each animal at 30, 60, 90, 120mins. Ananimal is considered cataleptic if it remains on the bar for 60 seconds or more. Thepercentage of cataleptic animals is calculated. The phenomenon of catalepsy predicts antipsychotic activity as well as the potential of producing extrapyramidial side effects.

Figure 13:Catalepsy in rats

Tail suspension test[13]

Rats of Group I, II, III, IV received Normal saline, control drug, combinationof test drugs 1 and 2 and standard drug (Imipramine 10mg/kg) respectively. The total duration of immobility induced by tail suspension was measured according to the method described by (Steru et al.). Rats were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded for a total 6 min.

Figure 14:Tail suspension test

HISTOPATHOLOGICAL STUDY[14]

The animals were euthanized using anesthetic ether and their brains were dissected out. Theisolated brains were stored in labeled containers containing neutral formalin (10% solution).These brains were studied for furtherhistopathological studies and the results were attached.

IV. RESULTS

Table 1: Phytochemical results of ethanolic extracts of *Adiantum insicum forsk* and *Yucca aloifolia*

Assay	Ethanolic extract of *Adiantum insicum forsk*	Ethanolic extract of *Yucca aloifolia*
Carbohydrates	+	+
Alkaloids	+	-
Sterols	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Proteins and amino acid	+	+
triterpenoids	+	+

observation table of preliminary phytochemical test of ethanolic extracts of *Adiantum insicum forsk and Yucca aloifolia*.

Ethanolic extract of the leaves of *adiantum insicumforsk*showed presence of carbohydrates, alkaloids, flavonoids, saponins, tannins, sterols, phenols, proteins and triterpenoids. Whereas, Ethanolic extract of the leaves of *yucca aloifolia*hadshown the presence ofcarbohydrates, sterols, phenols, saponins, flavonoids, tannins, triterpenoids and proteins and amino acids.

Graph 1:GCMS analysis of adiantum insicum forsk

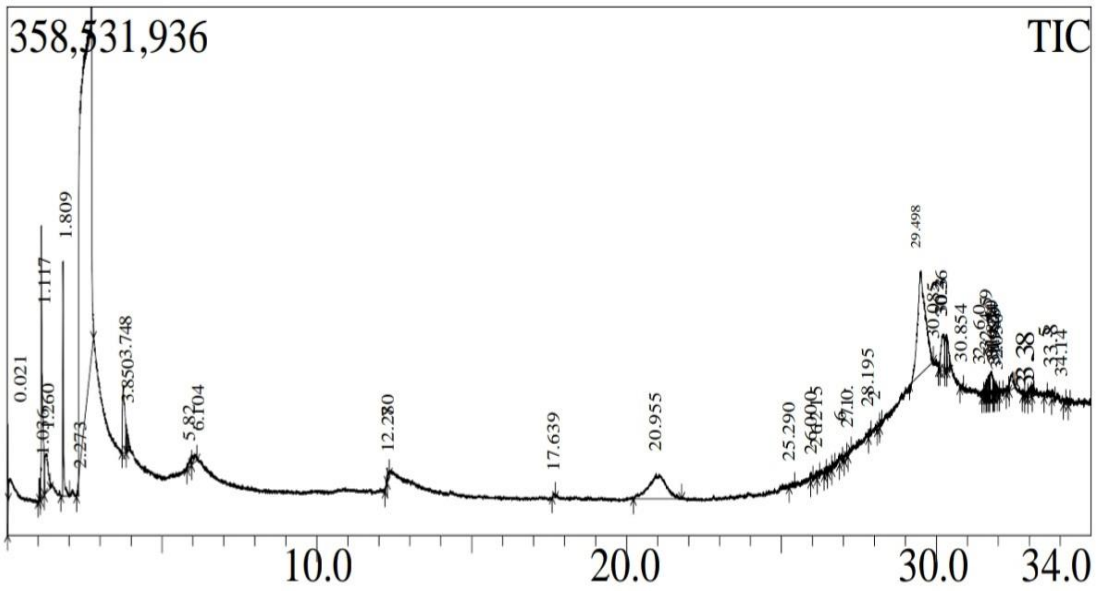


Table 2: GCMS analysis of adiantum insicum forsk

S.no	Retention time	Chemical constituents	Area %	uses
1	10.820	Dextroamphetamine	3.54	**Anti-anxiety,** stimulant, cognitive enhancer, aphrodisiac, euphoriant.
2	23.410	N-ethoxycarbonylhydrazon	2.03	**Anti-anxiety**, **anti-psychosis**, anti-bacterial, immunomodulator,anti-diabatic, anti-viral.
3	30.794	Caffeic acid	1.33	**Anti-depressant, anxiolytic,** anti-inflammatory, anti-cancer, anti-viral.
4	6.784	Methyl methanesulfonate	4.21	**Anti-epileptic, anti-psychotic,** alkylating agent, anti-cancer.
5	14.270	(3,4-Dihydroxyphenyl)hexylamine	1.21	**Anti-depressant, anti-psychotic,** Anti-inflammatory, astringent, demulcent, emollient.
6	38.125	Cinnamic acid, 3,4-Dimethoxy, trimethylsilyl ester	1.63	**Anti-depressant**, anti-fungal, anti-oxidant, anti-bacterial, depigmenting agent.
7	25.751	L-norephedrine	1.59	**Anti-depressant,** CNS stimulant, nasal congestion, myasthenia gravis

Graph 2: GCMS analysis of ethanolic extract of *yucca aloifolia*

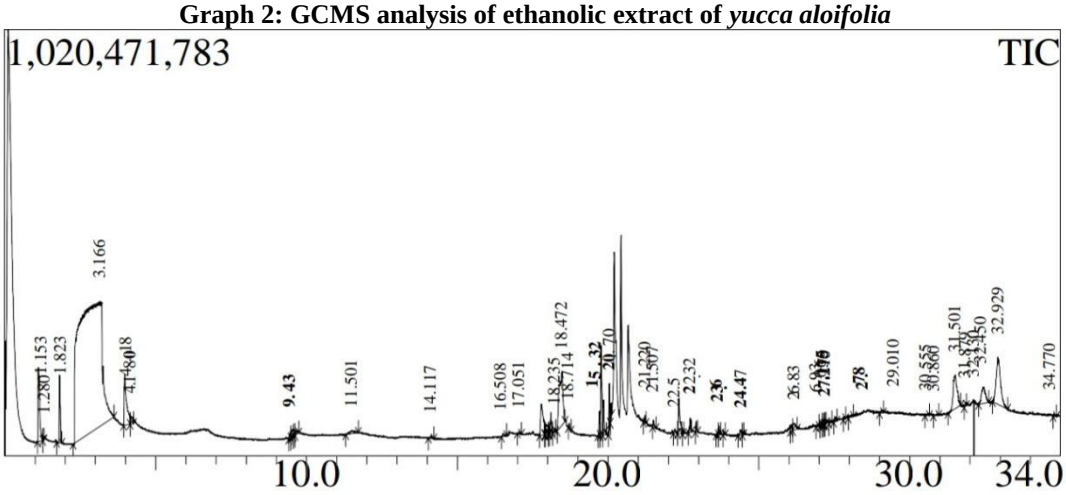


Table 3: GCMS analysis of ethanolic extract of *yucca aloifolia*

S.no	Retention time	Chemical constituents	Area %	uses
1	20.200	Eicosanoic acid	1.94	**Anti-depressant,** haemolytic agent, anti-coagulant, anti-inflammatory.
2	32.010	Chlorpheniramine	2.84	**Anti-depressant, anti-psychotic,** anti-cancer, anti-viral.
3	14.220	3,3,4,5,7-Pentahydroxyflavone	1.42	**Anti-anxiety,** anti-oxidant, anti-pyretic, anti-cancer.
4	10.501	2,3 dimethylfumaric acid	3.28	**Anti-anxiety,** Treatment of psoriasis, autoimmune disorders, jaundice.
5	33.879	β-amyrin	2.26	**Anti-depression, anti-anxiety,** Anti-oxidant, analgesic.
6	36.770	9-[4-Hydroxybutyl] hypoxanthine	1.11	**Anti-depression, anti-psychosis, anti-**inflammatory,

Acute toxicity study

Acute oral toxicity study for the ethanolic extracts adiantum insicum forsk and yucca aloifolia were carried out in rats asper OECD GuidelineNo.423.The results of the studies are as follows:

Adiantum insicum forsk and Yucca Aloifolia even at the dose of 2000mg/kg, no rat was killed. Therefore, the different extracts of Adiantum insicum forsk were found to be safe and nontoxic.LD_{50} range was considered greater than 2000 mg/kg.Yucca aloifolia20% of the total rats 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 higher dose of 2000 mg/kg.

SignsandSymptomsofToxicity:

No signs of toxicity were observed 24 hour post treatment as dose did not produce any mortality. No significant modification in behavioural patterns nor any clinical abnormality were observed during entire observation period of 14 days.

Evaluation of behavioural parameters.

Anti-depressant activity

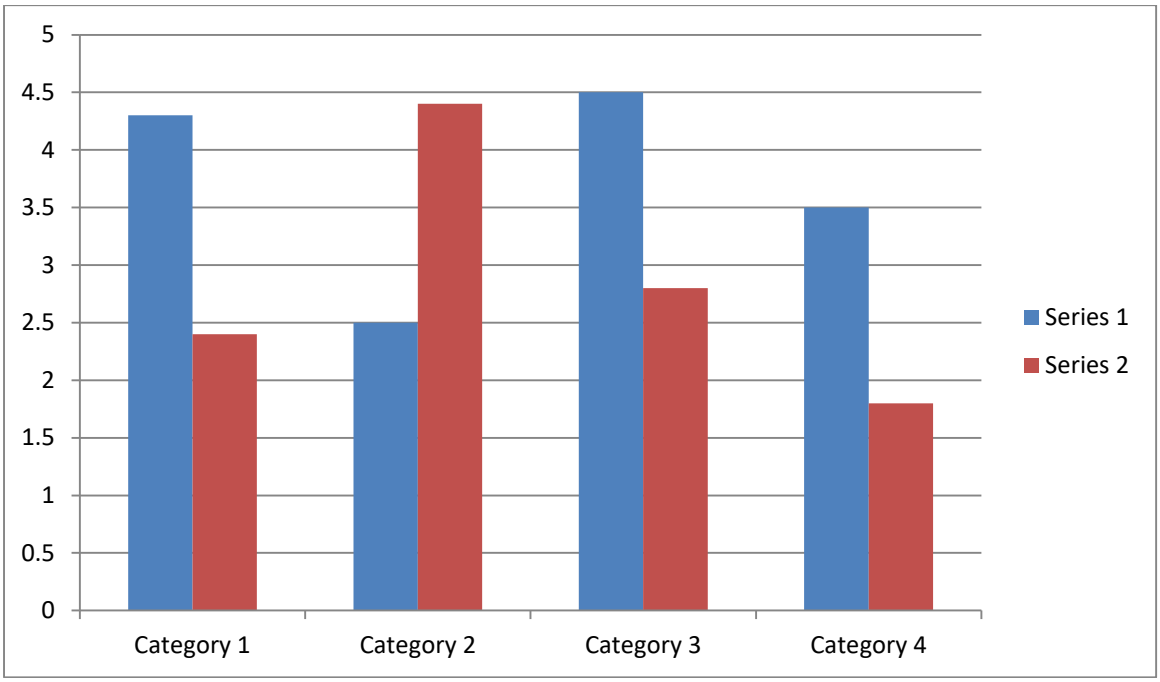
Effects of adiantum insicum forsk and yucca aloifolia extracts on the immobility time in the Force swim test.

The ethanolic extracts of A.I and Y.A prompted a significant antidepressant effect in the Forced swim test on rat, as it significantly reduced the immobility time when compared to the vehicle treated group (182.18±0.21s). The standard group treated with Imipramine (10mg/kg) i.p exhibited powerful activity (162.01±0.88s). No significant difference observed in immobility time of A.I extracts and Y.A extracts on 3rd day and 6th day in FST. No significant difference observed in immobility time of A.I + Y.A extracts on 3rd day and 6th day in FST (103±0.004, 109.68±0.67).

Table 4: Effect of extracts of leaves of E.E.A.I + E.E.Y.A extracts and imipramine on immobility time in force swim test in albino rat.

Sample	Immobility time (sec.) (Mean±SEM)	
	3rd day	6th day
Normal control	184.01±0.99	183.18±0.21
Negative control: Diazepam 5mg/kg	50.02±1.004	28.31±1.033
Standard: Imipramine (10mg/kg)	163.01±0.88***	170.84±0.827**
Plant 1+2: E.E.A.I 100mg/kg + E.E.Y.A 100mg/kg	103.51±0.004	114.68±0.67*

Graph 3: Effect of extracts of leaves of E.E.A.I + E.E.Y.A extracts and imipramine on immobility time in force swim test in albino rat.



Values are expressed as mean±S.E.M. (n=6). *P≤0.05, **P≤0.01, ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test).

Effect of adiantum insicum forsk and yucca aloifolia extracts on the immobility time in the tail suspension test

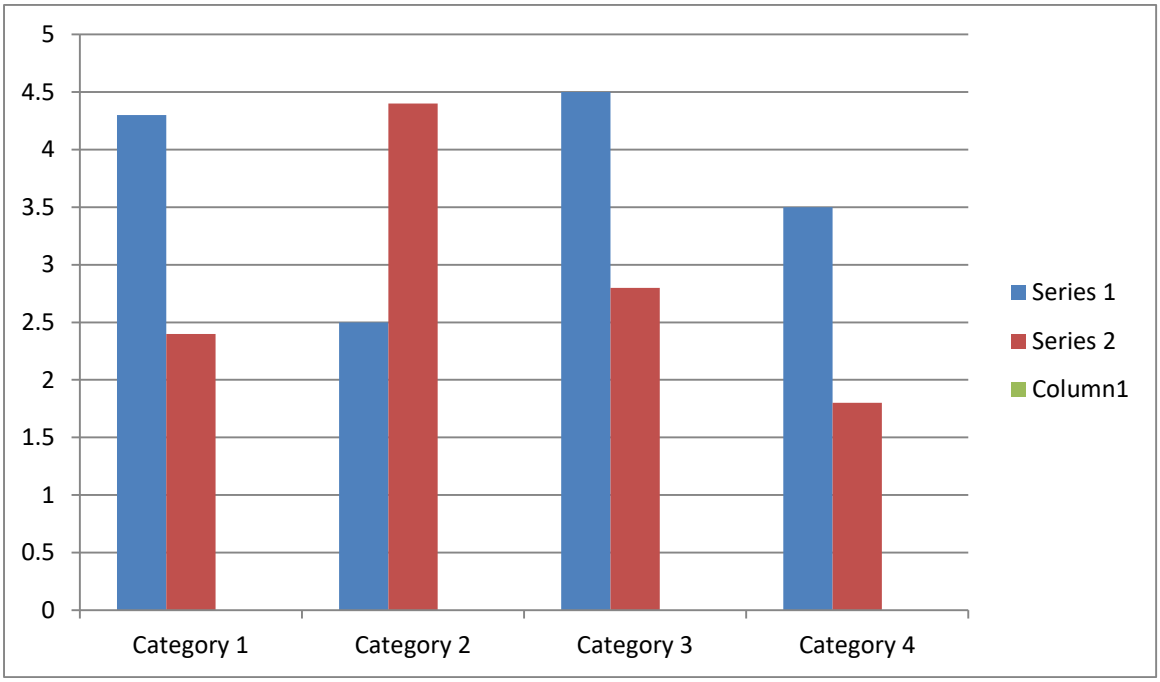
In the TST, the ethanolic extract of A.I +Y.A showed a significant effect on decreasing the immobility time, when compared to the vehicle-treated control group (183.21±0.036sec.). The standard group treated with imipramine (10 mg/kg), also significantly diminished the immobility time (190.21±0.098, 173.68±0.11sec.).No significant difference observed in immobility time of A.I+Y.A extracts on 3rd day and 6th day in FST (111.00±0.160 and 120.00± 0.083sec.) .

Table 5: Effect of extracts of A.I+ Y.A and imipramine on immobility time in the tail suspension test in albino rats.

Sample	Immobility time (sec.) (Mean±SEM)	
	3rd day	6th day
Normal control	182.21±0.036	178.49±0.033
Negative control: Diazepam 5mg/kg	23.54±0.175	40.97±1.023
Standard dose: Imipramine (10mg/kg)	190.21±0.098**	173.68±0.11***
Plant 1+2: E.E.A.I 100mg/kg + E.E.Y.A 100mg/kg	109.00±0.160	120.00± 0.083*

Values are expressed as mean±S.E.M. (n=6). *P≤0.05, **P≤0.01, ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Graph 4: Effect of extracts of A.I+ Y.A and imipramine on immobility time in the tail suspension test in albino rats.



Anti-anxiety activity

Pharmacological evaluation of anxiolytic activity of E.E.A.I and E.E.Y.A by using elevated plus maze (EPM)

Anxiolytic effect of E.E.A.I + E.E.Y.A has been studied by using elevated plus maze in terms of time spent (in seconds) in open arms, enclosed arms and central area along with entries in enclosed arms and open arms.The group of rats treated with vehicle spent 41.68 sec in open arms, 17.6 sec in the enclosed arms and 15.68 sec in the central area, rodents treated with phencyclidine(10 mg/ml) andE.E.A.I(100 mg/kg) + E.E.Y.A (100mg/kg) showed significant rise in the count of entries in theopen arms (7.84) and decrease in the number of entries to theenclosed arms (9.00).

Table 6: Effects of ethanol extract of A.I and Y.A usingElevated plus Mazemodel.

Sample	**ElevatedPlusMaze**				
	Parametersobserved				
	Time spentin open arm(sec)	**Time spentin enclosedarm (sec)**	**Entries inopen arm**	**Entries in enclosed arm**	**Time spentin centralzone (sec)**
Normal control	41.68±0.31	17.6±0.90	27.17±0.02	24.68±1.48	15.68±1.05
Negative control: phencyclidine 10mg/kg	7.21±0.01	78.88±0.54	2.01±0.42	3.42±0.9	65.01±0.94
Standard dose: Alprazolam 2mg/ml	33.4±0.31***	14.18±0.20***	23.34±0.90***	32.84±1.01***	7.68±0.97***
Plant 1+2: E.E.A.I 100mg/kg +E.E.Y.A 100mg/kg	51.9±0.44**	26.5±0.64**	7.84±0.72	9.00±0.25	33.84±0.57*

Values are expressed as mean ± SEM (n = 6). ***$P < 0.001$,**$P < 0.01$, *$P < 0.05$ vs. Vehicle (One-wayANOVA followed by Dunnett's test).

Graph 5: Effects of ethanol extract of A.I and Y.A usingElevated plus Mazemodel.

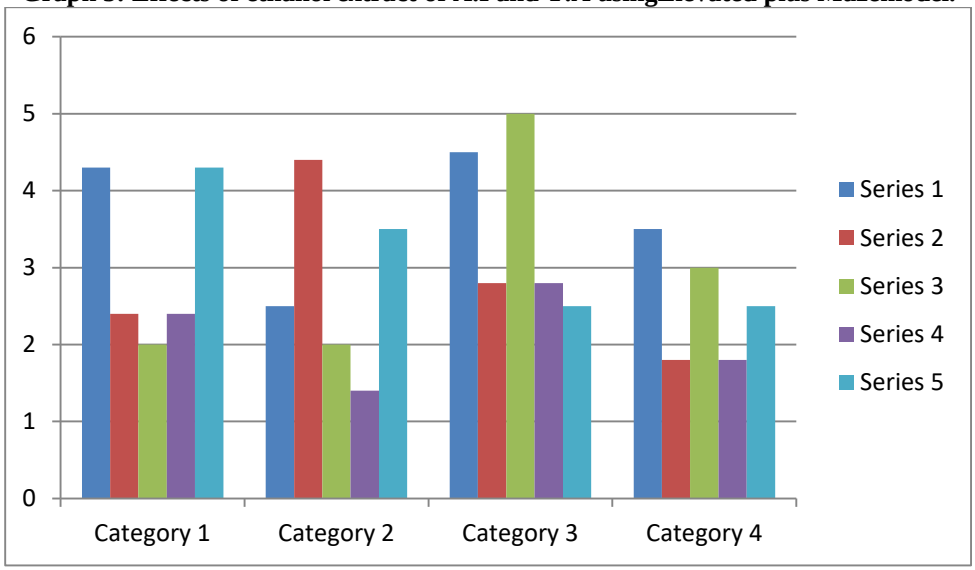
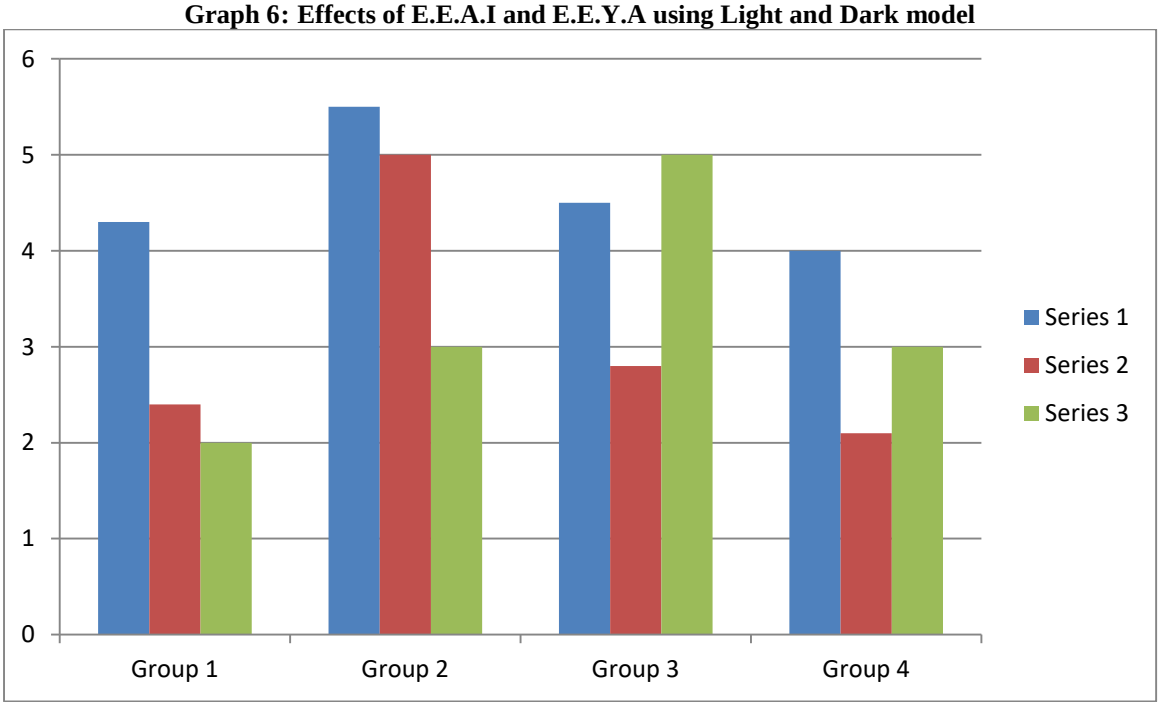


Table7: Effects of E.E.A.I and E.E.Y.A using Light and Dark model

Sample	Light and Dark exploration			
	Parameters observed			
	Time spent inLightzone(sec)	**Time spentinDark zone(sec)**	**No. ofCrossings**	**TransferLatency**
Normal Control	125.18±0.58	215.6±0.52	33.51±0.57	27.18±0.43
Negative control: Phencyclidine 10mg/kg	349.04±0.07	493.81±0.17	4.11±0.21	109.00±0.88
Standard dose: Alprazolam 2mg/ml	133.90±0.35**	178.01±0.67**	48.84±0.04**	46.51±0.32**
Plant 1+2: E.E.A.I 100mg/kg + E.E.Y.A **100mg/kg**	81.34±0.59	196.6±0.33*	24.7±0.71*	10.01±0.2*

Values are expressed as mean ± SEM (n = 6). $^{**}P < 0.001$, $^{*}P < 0.05$ vs. Vehicle (One-wayANOVA followed by Dunnett's test).

Graph 6: Effects of E.E.A.I and E.E.Y.A using Light and Dark model



Anti-psychotic activity

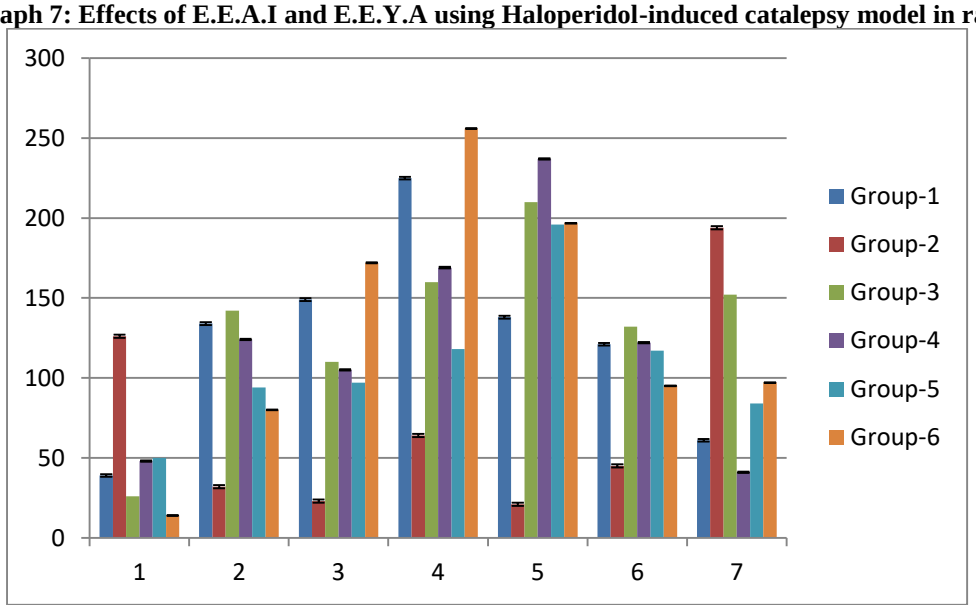
a) Pharmacologicalevaluationofantipsychoticactivityof E.E.A.I and E.E.Y.A usingHaloperidol-inducedcatalepsymodel

Table 8: EffectsofE.E.A.I and E.E.Y.A usingHaloperidol-induced catalepsymodelin rats

	Normal control:	Negative control: Haloperidol 2mg/kg	Standard dose: Chlorpromazine (10mg/kg)	Plant 1+2: E.E.A.I 100mg/kg + E.E.Y.A 100mg/kg
00	36.81±0.83	123.09±0.85	23 ± 0.23	11.61±0.62
30	129.41±0.40	31.89±0.99	138± 0.24***	78.39±0.42**
60	146.61±0.33	20.31±0.02	107.23 ± 0.23**	169.79±0.40
90	223.61±0.41	61.90±0.12	157.10 ±0.45***	256.19±0.02*
120	135.21±0.96	18.45±0.98	207.29 ±0.42***	193.80±0.04*
150	117.81±0.62	42.88±0.01	129.31 ±0.59***	92.20±0.70**
180	58±0.19	191.72±0.24	149 ± 0.9**	94.4±0.70

Each point indicates mean ± SEM (n = 6). ***P* < 0.001, ****P* <0.05) vs. Vehicle (One-way ANOVA followedbyDunnett'stest).Vel-Vehicle,Hal-Haloperidol,i.p-Intraperitonial

Graph 7: Effects of E.E.A.I and E.E.Y.A using Haloperidol-induced catalepsy model in rats



pharmacological evaluation of anti-psychotic activity of E.E.A.I and E.E.Y.A using pole climbing model.

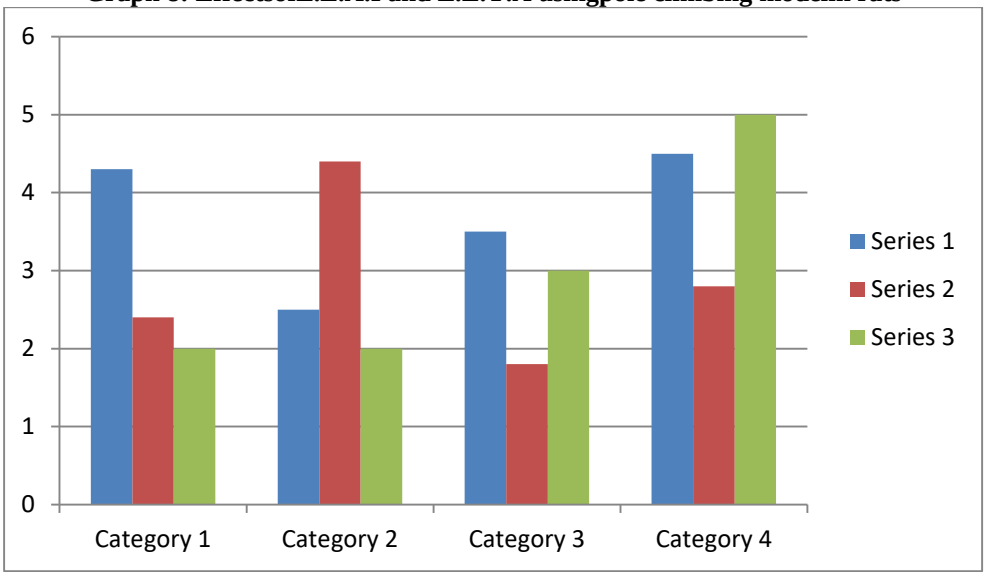
Administration of 200mg/kg of E.E.A.I and E.E.Y.A inhibited the conditioned avoidance response in rats as indicated by increased time spent on the grid floor of the chamber. However, the E.E.A.I 100mg/kg together with E.E.Y.A 100mg/kg failed to be effective in inhibiting the conditioned avoidance response.

Table 9: EffectsofE.E.A.I and E.E.Y.A usingpole climbing modelin rats

Sample	drug	dose	Latency to climb the pole
Control	Distilled water	2mg/kg p.o	2±0.28
Negative control	haloperidol	2mg/kg p.o	32±0.92
Standard dose	Chlorpromazine	10mg/kg i.p	3.1±0.19**
Plant 1+2	E.E.A.I + E.E.Y.A	200 mg/kg p.o	9±0.46

Values are expressed as Mean±SEM, n=6; One way ANOVA followed by Dunnett's Multiple Comparison Test; *P < 0.05 and **P < 0.001.

Graph 8: EffectsofE.E.A.I and E.E.Y.A usingpole climbing modelin rats



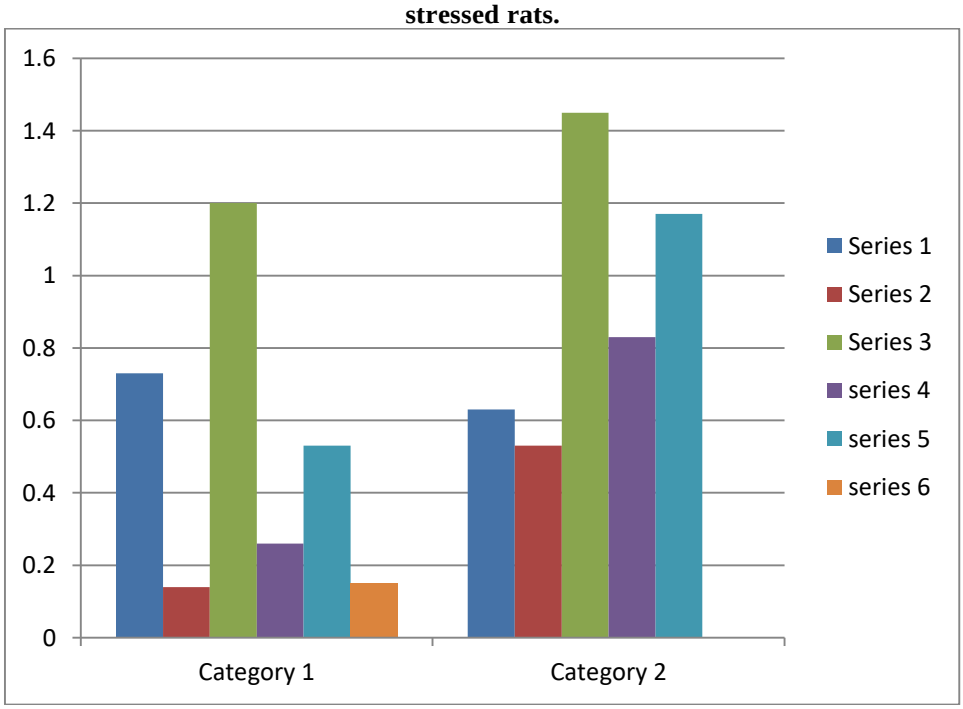
In hippocampus

Table 10: Effect ofE.E.A.I and E.E.Y.A on monoamines level in hippocampus of Non-stressed and stressed rats.

Sample	5-HT		DA		NE	
	(µg/goftissue,Mean±SEM)					
	Non- stressed	stressed	Non-stressed	stressed	Non-stressed	stressed
Control: Vehicle	0.73±0.08	0.14±0.02	1.20±0.23	0.26±0.04	0.53±0.08	0.15±0.04
Plant 1 + 2 : E.E.A.I (100mg/kg)+ E.E.Y.A (100mg/kg)p.o	0.63±0.09	0.53±0.04*	1.45±0.39	0.83±0.07*	1.17±0.05	0.57±0.03*

Experimental data was analyzed by two-way ANOVA test and expressed asmean±SEM (n=6), $^{*}p<0.01$ compared to nonstressed vehicle group, $^{**}p<0.001$ compared to stressed + vehiclecontrolgroup.

Graph 9: Effect of E.E.A.I and E.E.Y.A on monoamines level in hippocampus of Non-stressed and stressed rats.



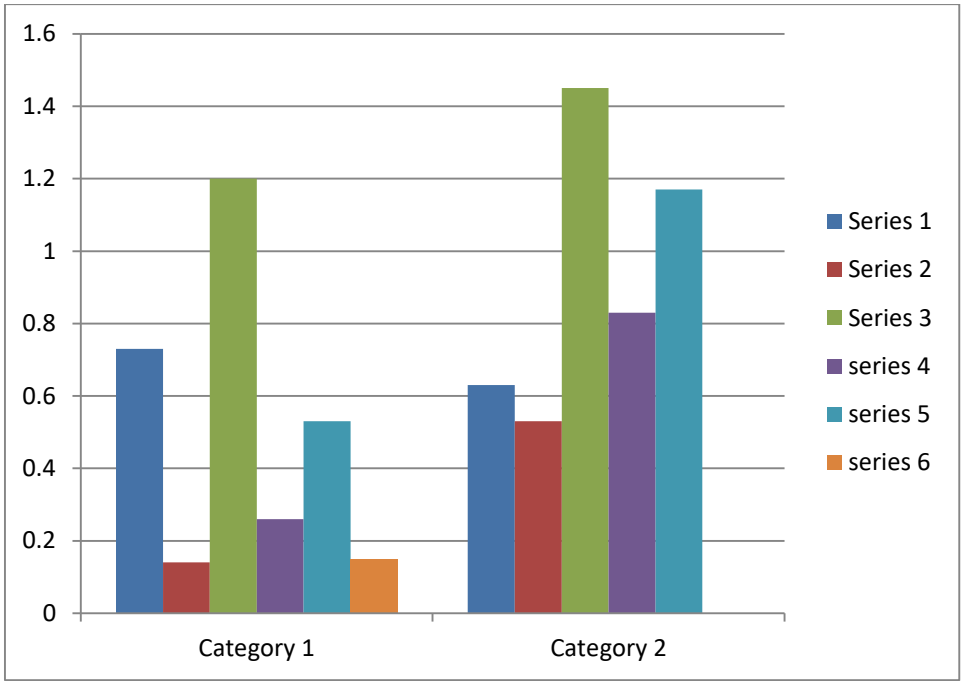
In cerebral cortex

Table 11: Effect ofE.E.A.I and E.E.Y.A on monoamines level in cerebral cortex of Non-stressed and stressed rats.

Sample	5-HT		DA		NE	
	(µg/goftissue,Mean±SEM)					
	Non- stressed	stressed	Non-stressed	stressed	Non-stressed	stressed
Control: Vehicle	0.93±0.08	0.42±0.05	1.64±0.32	0.29±0.04	0.94±0.08	0.20±0.02
Plant 1 + 2 : E.E.A.I (100mg/kg)+ E.E.Y.A (100mg/kg)p.o	0.90±0.11	0.69±0.01	1.86±0.41	1.54±0.04*	1.01±0.09	0.65±0.08**

Experimental data was analyzed by two-way ANOVA test and expressed asmean±SEM (n=6), $^{*}p<0.01$ compared to nonstressed vehicle group, $^{**}p<0.001$ compared to stressed + vehiclecontrolgroup.

Graph 10: Effect of E.E.A.I and E.E.Y.A on monoamines level in cerebral cortex of Non-stressed and stressed rats.



Effects of *adiantum insicum forsk* and *yucca aloifolia* on monoamines level in hippocampus and cerebral cortex of Non-stressed and stressed rat.

Effect of E.E.A.I (100mg/kg)+ E.E.Y.A (100mg/kg)p.o or vehicle(10ml/kg) p.oon5 HT,DAandNElevelinhippocampusandcerebral cortexinnonstressedandstressed rats.

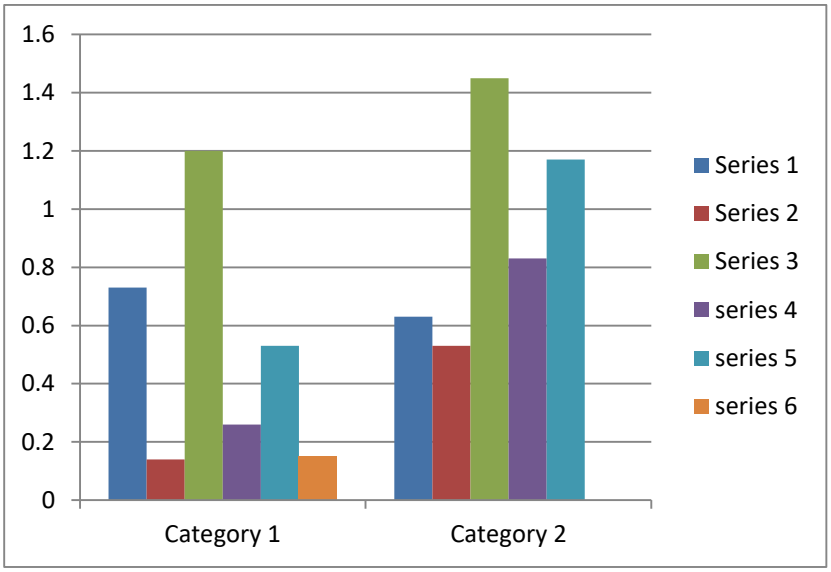
In hippocampus

Table 12: Effect ofE.E.A.I and E.E.Y.A on monoamines level in hippocampusof Non-stressed and stressed rat.

Sample	5-HT		DA		NE	
	(µg/goftissue,Mean±SEM)					
	Non-stressed	stressed	Non-stressed	stressed	Non-stressed	stressed
Control: Vehicle	0.26±0.01	0.26±0.01	0.33±0.01	0.41±0.01	0.21±1.5	0.19±1.3
Plant 1 + 2 : E.E.A.I (100mg/kg)+ E.E.Y.A (100mg/kg)p.o	0.70±0.01	0.28±0.03	1.21±0.30	0.93±0.09**	0.70±0.07	0.42±0.02

Experimental data was analyzed by two-way ANOVA test and expressed asmean±SEM (n=6), $^{*}p<0.01$ compared to nonstressed vehicle group, $^{**}p<0.001$ compared to stressed + vehiclecontrolgroup.

Graph 11: Effect of E.E.A.I and E.E.Y.A on monoamines level in hippocampus of Non-stressed and stressed rat.



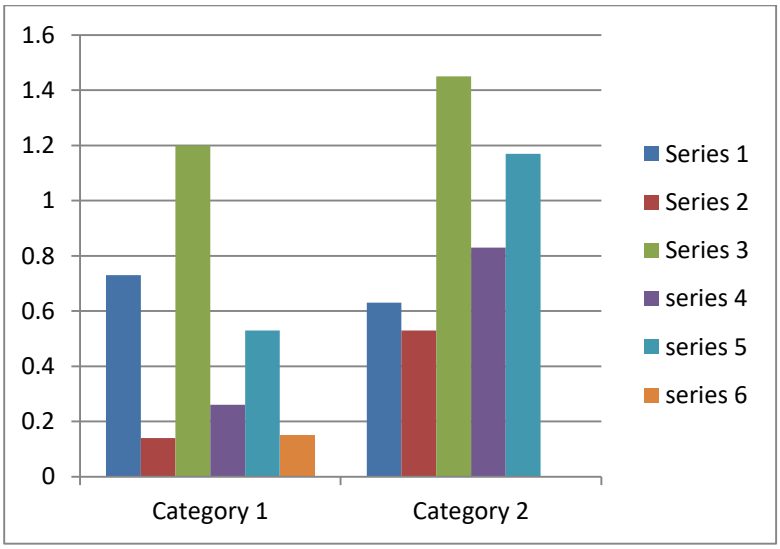
In cerebral cortex

Table 13: Effect ofE.E.A.I and E.E.Y.A on monoamines level in cerebralcortex of Non-stressed and stressed rat.

Sample	5-HT		DA		NE	
	(µg/goftissue,Mean±SEM)					
	Non- stressed	stressed	Non-stressed	stressed	Non-stressed	stressed
Control: Vehicle	0.80±0.07	0.31±0.04	1.54±0.25	0.30±0.05	0.93±0.09	0.17±0.02
Plant 1 + 2 : E.E.A.I (100mg/kg)+ E.E.Y.A (100mg/kg)p.o	0.83±0.08	0.34±0.07**	1.65±0.28	0.44±0.05	0.85±0.07	0.34±0.04*

Experimental data was analyzed by two-way ANOVA test and expressed asmean ± SEM (n=6), $^{*}p<0.01$ compared to nonstressed vehicle group, $^{**}p<0.001$ compared to stressed + vehiclecontrol group.

Graph 12: Effect of E.E.A.I and E.E.Y.A on monoamines level in cerebral cortex of Non-stressed and stressed rat.



Histopathological studies
Extraction of rat brain for histopathological studies
Cerebellar cortex

A. Normal control**B.**Negative control

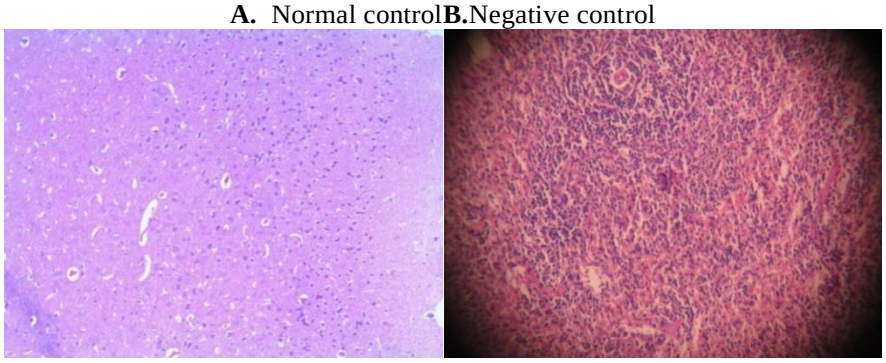


Fig A: Normal control **FigB: Negative control**

C. Standard control**D.** Plant 1+2(E.E.A.I+E.E.Y.A)

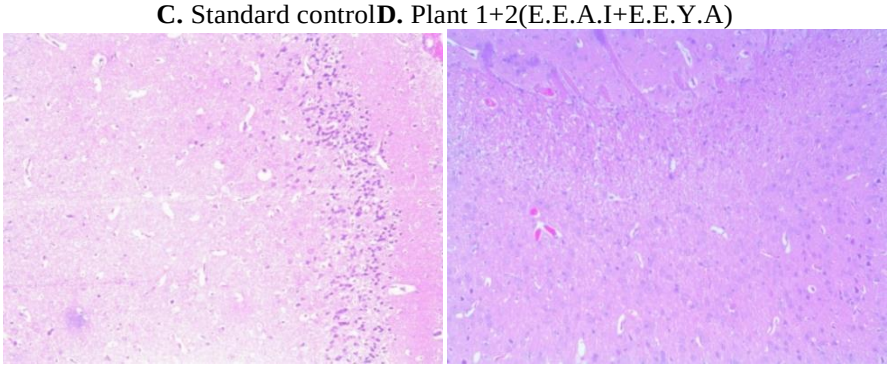


Fig C: Standard control **Fig D: Plant 1+2(E.E.A.I+E.E.Y.A)**

Histopathology slides of cerebral cortex and hippocampus of albino wistar rats

Photomicrographs illustrating stained sections (x400, scale bar = 50 μm) of Wistar rats. (A-D): Hippocampal CA1 sections. (A): Control sections representing normal architecture, neurons having large vesicular nuclei, and small dense neuroglial cells. (B): Negative control groups channeling shrunken degenerated neurons with perineural spaces that also exhibit areas of neurons loss, tied up surrounding neuroglial cells, wide neuropil and congested capillary. (C, D) shows treated groups normal neural architecture with large vesicular nuclei with minute perineural spaces indicating few tethered neuroglial cells. (C,D) demonstrating treated neural cells with reduction in the thickness of pyramidal layer and granular cell layer. The dentate gyrus representing pleomorphic layers, molecular and granular cells.

V. CONCLUSION

Plant sources continue to serve as viable and beneficial sources of drugs for the world population andother plant-based products are in extensive clinical use. This studyflags attention for further research to understand the depth of constituents responsible for the plants psychopharmacological activity, as the combination of ethanolic extract of leaves of Adiantum incisum forsk and Yucca aloifolia showed antidepressant, antianxiety and antipsychotic activity similar to the standard drugs considered and compared. Further studies must be undertaken to elucidate the proper mechanism of action by which extractsexhibit their psychopharmacological effect.Exploitation of the plants can be doneon making the different pharmaceutical products, which will be widely available due to thehigh availability of herbs (natural source) for‘making full advantage out of it’.

VI. ACKNOWLEDGEMENTS

Thanking someone is a way to show the gratitude for the kindness they contribute and praise someone for their character of being good and kind. The most deserving of praise and thanks is the Almighty Allah(SWT).

This M.Pharmacy thesis is a result of a challenging and a roller coaster journey, in which many people have contributed and extended their support. I cannot thank my parents enough, for showing their unlimited support and encouragement throughout the ups and downs of this research thesis. My utmost and sincere gratitude and appreciation to Dr. MehnoorFarheen, for persevering with me as my guide, advisor and friend throughout the time it took me to complete this research and write the dissertation. She was there for me throughout the journey.

With great pleasure, I acknowledge my profound and sincere thanks to Principal Dr. D Ramkrishna,Shadan Women 's College of Pharmacy, Hyderabad for constant encouragement throughout the period, which helped me in the successful completion of this project. I must acknowledge as well the many friends, lab assistants, and librarians who assisted, advised, and supported my research andwriting efforts. Last but not the least; I would like to thank our Lab helpers, Sultan, who was always flexible with their work timings, just to help me through myresearch.

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