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Screening of Antidepressant Activity of Mangifera indica in Mice by Performing Chronic Unpredictable Mild Stress (CUMS) Model

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ABSTRACT

A common mental illness that has a big influence on both people and society is depression. Research on mental health is still focused on finding safe and effective antidepressant medications. This study's purpose was to evaluate the potential antidepressant outcomes of Mangifera indica fruit juice and leaf extract were employed in a model of depression produced by Chronic Unpredictable Mild Stress (CUMS). In order to produce depression in the CUMS paradigm, mice were challenged to a range of unanticipated mild stressors for a week. Throughout the trial, the treatment group received oral doses of Mangifera indica fruit juice (MIFJ 1 ml/kg, 2 ml/kg) and leaf extract (MILE 200 mg/kg, 500 mg/kg). The standard group was treated with the medication fluoxetine. Models such as the Forced Swimming Test (FST), Tail Suspension Test (TST), and spontaneous locomotor activity were used to assess depressive-like behaviours. Animals exposed to CUMS showed signs of significant behavioural discomfort, as evidenced by longer immobility times in the FST and TST. In contrast to the standard group, the immobility period in these tests was markedly increased by treatment with MILE and MIFJ at both doses. Additionally, administering Mangifera indica fruit juice (MIFJ) and leaves extract (MILE) raised serotonin levels in the brain, pointing to a possible mechanism of action for the antidepressant effect.

This study concludes by demonstrating that Mangifera indica has antidepressant properties. In conclusion, this study shows that Mangifera indica has antidepressant properties extract from leaves and fruit juice from Mangifera indica. The finding suggest that MILE and MIFJ may exert its beneficial effect by modulating the serotonergic system. Further research is warranted to elucidate the specific bioactive components responsible for the observed antidepressant activity and to explore the underlying molecular mechanisms. Mangifera indica has potential as a natural antidepressant and could help create new depression treatments.

INTRODUCTION:

Depression: Depression is characterized by an ongoing feeling of hollowness, sorrow, or lack of ability to feel joy that may seem to have no evident reason. It is distinct from grief and other emotions[1]. It is distinct from grief and other emotions. Depression is one of the most prevalent forms of disability globally, as stated by the WHO. Depression is a mental illness that develops as an ongoing sense of melancholy, emptiness, and loss of joy. It is not the same as the emotional fluctuations that people experience on a daily basis. 1) Severe depression Second, 2) postpartum depression
Antidepressant drug - Preventing and counteracting

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the depression Ex. Amitriptyline, Imipramine, Nortriptyline[2,3]

Treatment:
There are ways to treat depression, albeit the specific type a person has may determine the course of action. However, about 30.9% of patients either react poorly or not at all to treatment. About 40% of people get a remission of their symptoms within a year; nonetheless, depression might persist.

- **Support:** This could involve informing family members and exploring feasible remedies and possible causes.
- **Psychotherapy:** Also known as psychological therapy, this kind of care consists of individual counselling and cognitive behavioural therapy (CBT).
- **Medication:** Antidepressants may be prescribed by a physician[4,5].
- **Marketed formulation:** 1. Mirtazapine Sol Tab (Fluoxetine), 2. Citalopram (escitalopram), 3. Venlafaxine XR (Paroxetine CR)

Fruit of Mangifera Indica was collected from Pune. There common name is Mango and belonging to family Anacardiaceous and their class is Dicotyledons. Mango leaves are large and green in colour and raw fruit is green in colour. This plant contain fruit[6]. This is used in Antidepressant, Antiseptic, Stomachic, Diuretic, Laxative. Parts used - Peels, Stembark, Leaves Seed kernels. Its fruit is sour in taste. leaves give antibacterial properties, fruit is used to lower the blood pressure. Peel is used to prevent Anemia. Depression may be serious illness generally manifested by symptoms at the psychological, behavioural and physiological leaves. Various attempts are created to develop animal models of depression[7,8].

MATERIAL AND METHODS:

I Material:

1) Animals: LACSMI Biofarms Pvt. Ltd. supplied Swiss Albino mice measuring 20–30 grams. Jagtap Dairy, Pasaydan, Samarth Colony, Pimple Nilakh, Pune 1277/PO/Re/S/07/CPCSEA. A day prior to the experiment, mice were uniformly assigned to groups and housed in standard lab settings (room temperature at 25±2°C, 12/12-hour light/dark period, open possession of food and drink). The Institutional Animal Ethical Committee (IAEC)-approved study protocol was strictly followed in all animal handling and experimental procedures. Each effort has been taken to use fewer animals in the experiment and to lessen their discomfort.

2) Drug & Chemicals: Fluoxetine (Fluoxetine 20mg) was purchased from local market. Another name for it is a selective serotonin reuptake inhibitor (SSRI). Fluoxetine has special pharmacokinetic effects; its longer half-life probably distinguishes it from other SSRIs. Most SSRIs possess a half-life of about one day. On another side, fluoxetine comes with a half-life of two to four days.

3) Preparation of Herbal Extracts:
3.1 Plant extract preparation: Dried Mangifera indica leaves were gathered from the pune local garden in Oct 2024. After being rinsed and dried under shade, Mangifera indica leaves were reduced into a thin powder using an grinding mill. Through the maceration procedure, powdered dry leaves were extracted using ethanol and water. Maceration is process in which 25gm of leaves powder + 100ml ethanol and next extract with 25gm of leaves powder + 100 ml water added in a close tight container. Shaken it 2-3 times in a day. After 7 days filtration was performed and filtrate were collected.
3.2 Making Fruit Juice: Fresh fruits were gathered, chopped to the proper size, and ground in a mixer for a short while in order to prepare the juice. After more juice was filtered, it was chilled for the antidepressant research.

II Methods:

I. Phytochemical Screening of herbal extract
Alkaloids, carbohydrates, proteins, amino acids, flavonoids, and tannins were among the phytoconstituents identified by phytochemical screening using a conventional procedure.

1) CARBOHYDRATES TEST
The Molisch test:
Following the addition of two to three drops of an alcohol-based alpha-naphthol solution to three milliliters of liquid extract, add concentrated H2SO4 from the test tube's sidewalls. A violet ring appears when two different liquids combine, indicating the presence of carbohydrates.

SUGAR REDUCTION TEST
The Fehling test:
Boil for one minute after combining one milliliter of Fehling's A and one milliliter of Fehling's B solutions. Added an equivalent volume of the test sample. cooked for five to ten minutes in a bath of boiling water. When reducing sugar is present, the precipitate first becomes yellow before it turns brick red.

Benedict's examination
In a test tube, Benedict's reagent along with the test solution were mixed in a volume that was equal. Boiled in a bath of boiling water for five minutes. The development of a red color signified the presence of decreasing sugar.
MONOSACCHARIDES TEST
Barfoed's test
The test solution and Barfoed's reagent were mixed

in the same volume. Cooled after heating for one to two minutes in a bath of boiling water. The existence of monosaccharides was verified by the formation of a red color.

PENTOSE SUGAR TEST
HCL and test solution were combined in equal amounts. This combination was heated. Added a phloroglucinol crystal. Pentose sugar is confirmed to be present when a red color forms.

Hexose Sugar Test
Seliwanoff's test (for fructose-like ketohexose)
For one to two minutes, three milliliters of Seliwanoff's reagent and one milliliter of test solution were heated in a bearing water bath. The red color of the solution indicates the presence of ketohexose, such as fructose.

2) Test for proteins
The Millon Test
Five milliliters of Millon's reagent were mixed with three milliliters of the test solution. When protein is present, warm white ppt becomes brick red.
3) Test for amino acids
Tyrosin assay
Heat three milliliters of test solution and three drops of Millon's reagent. The solution's deep red colour indicates the existence of amino acids.

Test for cysteine
A 5 ml test solution was mixed with a couple of drops of 40% NAOH and 10% lead acetate solution. Heat the mixture until it boils. Protein is present when lead sulfate precipitates black.

4) STEROID Salkowski response test
Two milliliters of pure H2SO4, two milliliters of extract, and two milliliters of chloroform were added. Shaken vigorously The chloroform layer glows red, but the acid layer fluoresces greenish yellow.

5) ALKALOIDS TEST:
Mayer's examination
Three milliliters of test solution were mixed with three drops of Mayer's reagent, and the existence of alkaloids was assessed by searching for a reddish-brown color.

Hager's test:
The presence of alkaloids was found by searching for the formation of a yellow precipitate following the addition of four to five drops of Hager's reagent to three milliliters of filtrate.

Oxalic acid test confirmation test
Two milliliters of test solution were mixed with a few drops of 5% lead acetate. The existence of oxalic acid can be observed by white precipitate.

SAPONIN GLYCOSIDES FOAM TEST
Use water to shake the medication extract. Stable, persistent foam is seen.

6) TANNINS:
Potassium Dichromate Test
Two to three milliliters of water-based extract were combined with several of droplets of potassium dichromate. The creation of red precipitate is thought to indicate the existence of tannins.

Lead Acetate Solution
Two to three milliliters of water-based extract were mixed with couple of drops of lead acetate solution. White precipitate production is regarded as a sign that tannins are present.

Test of Acetic Acid Solution
Two to three milliliters of water-based extract have been diluted with several of drops of acetic acid solution.

Gelatin Solution test
Three milliliters of water-soluble extract have been paired with couple of droplets of gelatin solution. The formation of white precipitate is thought to indicate the presence of tannins.

Glycosides of Anthraquinone
The modified Borntrager test for c-glycosides
Five milliliters of extract, five milliliters of 5% Fecl3, and five milliliters of diluted HCL are mixed. Heat for five minutes in a bath of boiling water. After cooling, add the benzene and give it a good shake. Add an equivalent volume of diluted ammonia after separating the organic layer. When anthraquinone glycosides are produced, the pinkish red color of the ammonical layer is seen as favorable.

7) FLAVONOID TEST
Test for lead acetate
A solution of 0.5 grams of extract in water was mixed with around 1 milliliter of a 10% lead acetate solution. When yellow precipitate is produced, flavonoids are thought to be present.

II. Test for Acute Toxicity
The acute toxicity tests took place out in alignment with OECD guideline 423 in order to determine a safe dose of extract.

MIFJ (Mangifera indica fruit juice) and MILE (Mangifera indica leaf extract) were tested for acute toxicity in 6+6 mice.

Methodology: An acute oral toxicity experiment was carried out on healthy, nulliparous, and non-

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pregnant adult female Swiss Albino mice (25–30gm) in compliance with OECD guideline -423 (OECD, 2001). The extract was given orally to two groups of three Swiss albino mice at a dose of 2000 mg/kg. For the first five hours, the mice were continually monitored for any obvious behavioural or neurological toxic effects. After that, they were periodically checked for toxic symptoms and mortality for up to twenty-four hours. For the next fourteen days, additional animals were watched for any indications of delayed toxicity.

Acute Toxicity Result

After Dosing (Half hour)	After Dosing (24 hour)
• Respiration Fast	• Respiration Normal
• Heart Rate Increase	• Heart Rate Normal
• Mobility Activity Less	• No Diarrhea
• No Diarrhea	• No tremor
• No tremor	• No Convulsions
• No Convulsions	• No Death

III. Procedure of Experiment:

Depression was used through Chronic Unpredictable Mild Stress (CUMS) model in all Groups except normal control. Swiss Albino Mice were separated into seven groups of five mice each. For seven days, mice in the CUMS group were exposed to a variety of stressors. [From Group II to Group VII, every exercise was carried out every day for 14 days as shown below]

The Stressors included are:

- 45 degree cage tilting for 18 hours
- Swinging cage for 10 minutes
- 5 minutes of frigid paddling at 4°C
- Pinch the tail for one minute at a distance of 1 cm from its tip, then oscillate for five minutes.

Table 1: Seven Mice Group

Sr. No.	Group	Treatment (mg/kg)	Swiss Albino Mice
1	Normal	0.5 ml/kg of water from distillation (p.o.)	5
2	Control	0.5 ml/kg of water from distillation (p.o.)	5
3	Standard	Fluoxetine 20mg/kg (p.o.)	5
4	MIFJ X1	*Mangifera indica* fruit juice 1ml/kg	5
5	MIFJ X2	*Mangifera indica* fruit juice 2ml/kg	5
6	MILE Y1	*Mangifera indica* leaves extract 200mg/kg	5
7	MILE Y2	*Mangifera indica* leaves extract 500mg/kg	5

i. Group I was administered 0.5 ml (p.o.) of distilled water from the 7th to the 14th day as a normal control.
ii. From the 7th to the 14th day, Group II, which served as a disease control, received 0.5 ml/kg of water from distillation (p.o.)
iii. Group III, which served as a standard group, gained fluoxetine 20 mg/kg (p.o.) treatment from day 7 to day 14.
iv. From the 7th to the 14th day, Group IV, a MIFJ X1 group, gained 1 ml/kg (p.o.) of Mangifera indica fruit juice.
v. From the 7th to the 14th day, Group V, a MIFJ X2, was given 2 ml/kg (p.o.) of Mangifera indica fruit juice.
vi. From the 7th to the 14th day, Group VI, a MILE Y1, received a 200 mg/kg (p.o.) Mangifera indica leaves extract.
vii. From the 7th to the 14th day, Group VII, a MILE Y2, received a 500 mg/kg (p.o.) Mangifera indica leaves extract.
viii. The mice were tested for antidepressant activity on the 1st, 7th, and 14th days of the trial. Every mouse was put through the forced swimming test, the spontaneous locomotor activity test, and the tail suspension test.

Pharmacological Screening Test

1) Determining body weight

Put a big beaker on the scale, tare it, then return it to zero. Gently put the mouse inside the beaker. Note the value that appears on the weighing balance. Put the mouse back in its cage or move on to the next mouse.

Table No. 2 Weight of Mice in gm

Groups	1st day weight					Before starting dose (7th day)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
Group1	18.76	18.79	18.68	17.50	17.89	11.53	25.63	27.73	20.25	18.80
Group2	17.98	14.45	21.08	20.50	18.37	-	14.63	19.83	19.15	20
Group3	14.7	17.07	18.93	19.48	16.37	22.93	25.73	29.73	21.05	18.28
Group4	16.08	21.09	15.95	17.38	16.26	20.43	22.13	17.83	18.50	21
Group5	18.31	13.93	18	15.40	12.50	24.13	20.53	21.63	25	22.55
Group6	13.88	14.98	18.08	16.80	15.50	21.03	23.83	22.23	24.03	20
Group7	15.03	20.07	13.98	16.56	17.67	20.33	26.43	22.63	21.30	23.50

2) Spontaneous Locomotor Activity:

The actophotometer was utilized to assess locomotor activity. Its dimensions were 30 x 30 x 30 cm. Six identical holes were arranged horizontally in each of the cage's walls, 7 cm above the ground. Six light beams from all sides of the apparatus passed through the aperture. In the middle of the cage, they crossed.

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Infrared filters were used to reduce the impact of light beams, and readings were automatically logged on the instrument's digital counters.

Table No.3 Locomotor Activity Readings

Groups	Before CUMS Induction (1st day)					After CUMS Induction (7th day)					After Dosing (14th day)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
Group1	117	135	114	105	126	6	440	162	105	98	75	74	216	109	95
Group2	91	102	124	132	116	-	86	164	146	112	-	117	181	149	105
Group3	130	230	129	124	120	172	260	149	130	105	82	213	158	168	110
Group4	153	46	138	112	98	165	95	163	128	134	119	109	182	185	201
Group5	87	136	143	130	105	145	121	161	91	103	139	126	158	105	89
Group6	75	120	114	88	101	480	75	630	145	160	153	133	215	232	148
Group7	70	93	68	102	99	123	147	88	107	125	134	132	151	95	104

3) Tail Suspension Test (TST)

Using sticky tape, each mouse from each group will be suspended separately by the tip of its tail. For a duration of six minutes, the mobility will be recorded. The total mobility time will be recorded during the last four minutes of the six-minute test phase.

Table No. 4 Mobility Activity Readings (TST)

Groups	Before CUMS Induction (1st day)					After CUMS Induction (7th day)					After Dosing (14th day)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
Group1	23	33	22	30	25	35	36	49	33	38	19	54	38	30	36
Group2	62	41	39	50	48	-	24	26	48	55	-	24	26	39	60
Group3	49	26	13	33	18	45	11	38	40	45	21	16	31	45	56
Group4	38	26	20	17	21	29	12	13	25	30	53	27	20	23	35
Group5	31	51	39	54	40	28	38	44	35	18	33	55	38	30	25
Group6	35	20	20	45	30	27	19	12	25	29	46	51	25	31	30
Group7	3	48	75	65	60	39	38	18	28	41	33	49	48	23	42

4) The FST (Forced Swim Test):

The mouse was placed for six minutes in a glass cylinder that measured 20 cm in height and 14 cm in diameter and held 15 cm of water at a temperature of 24 to 25°C. The animals exhibit a period of immobility after the first two to three minutes of intense activity, floating with little in the water and only making the movements required to maintain their heads above the water. The period of climbing, immobility, and swimming behaviours were recorded throughout the final four minutes of the swimming test.

Table No. 5 Climbing Activity Reading

Groups	Before CUMS Induction (1st day)					After CUMS Induction (7th day)					After Dosing (14th day)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5	M 1	M 2	M 3	M 4	M 5
Group1	30	33	45	20	31	25	30	40	25	38	41	35	45	30	40
Group2	-	24	60	59	40	-	50	44	61	39	-	28	53	58	41
Group3	25	24	50	58	40	20	47	52	41	45	20	25	58	67	46
Group4	28	44	55	35	43	19	45	48	33	38	31	51	53	32	47
Group5	26	18	20	15	14	22	16	24	13	15	27	16	27	13	45
Group6	60	45	57	68	59	49	41	52	50	60	63	56	62	65	69
Group7	48	38	35	20	37	40	30	15	18	26	51	42	39	19	38

RESULTS:

1. Phytochemical Analysis of Mangifera indica L. leaves extract:

Mangifera indica L. leaves extract contained carbohydrates, amino acid tyrosine, flavonoids, reducing sugar, steroids, saponin glycoside, anthraquinone glycoside, and oxalic acid, according to phytochemical study.

2. Analysis of Phytochemicals in Mangifera indica L. Fruit Juice:

Phytochemical analysis revealed that Mangifera indica L. fruit Juice included carbohydrate, reducing sugar, monosaccharides, amino acid, steroid, oxalic acid, alkaloids, anthraquinone glycoside, saponin glycoside, tannins, pentose and hexose sugar, protein, flavonoids.

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3. Flavonoids showed the antidepressant activity.
4. Effect of Mangifera indica L. leaf extract (MILE 200 mg/kg and 500 mg/kg) and fruit juice (MIFJ 1 ml/kg and 2 ml/kg) on CUMS-induced altered behaviors in spontaneous locomotor activity: Analysis of spontaneous locomotor movement from the first day of the research (before CUMS Induced) revealed significant differences in locomotor activity across the groups, whereas all of the mice in the CUMS-exposed group showed a decrease in locomotor activity (after CUMS Induced). On day 14, there was a boost in movement activity vs to the standard group (1 ml/kg and 2 ml/kg) and (200 mg/kg and 500 mg/kg). The following figures illustrate the effects of MIFJ (1 ml/kg and 2 ml/kg) and MILE (200 mg/kg and 500 mg/kg) on CUMS-induced changes in mouse locomotor activity.

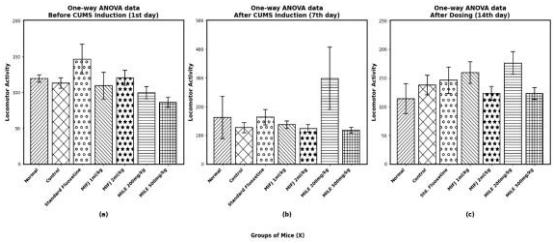

Fig. 1. Groups of Mice (X)

5. Effect of Mangifera indica L. leaf extract (MILE 200 mg/kg and 500 mg/kg) and fruit juice (MIFJ 1 ml/kg and 2 ml/kg) on CUMS-induced altered behaviors in the tail suspension test (TST):
Analysis of the tail suspension test (TST) on the first day of the experiment (before CUMS Induced) revealed significant differences in mobility activity across the groups, whereas all of the mice in the CUMS-exposed group showed reduced locomotor activity (after CUMS Induced). Mobility activity increased on day 14 (1 ml/kg and 2 ml/kg) and (200 mg/kg and 500 mg/kg) in comparison to the standard group. The following figures illustrate the effects of MIFJ (1 ml/kg and 2 ml/kg) and MILE (200 mg/kg and 500 mg/kg) against CUMS-induced alterations in mouse mobility activity.

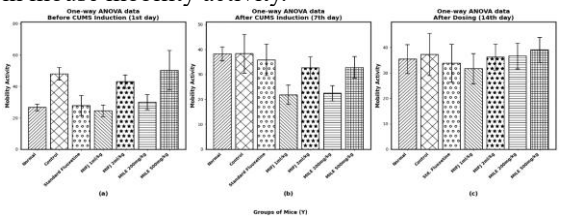

Fig. 2 Groups of Mice (Y)

6. Effect of Mangifera indica L. leaf extract (MILE 200 mg/kg and 500 mg/kg) and fruit juice (MIFJ 1 ml/kg and 2 ml/kg) on CUMS-induced altered behaviors in the forced swimming test (FST):
Analysis of the forced swimming test (FST) on the first day of the experiment (before to CUMS Induced) revealed significant differences in climbing activity across the groups, although all of the mice in the CUMS-exposed group showed reduced locomotor activity (following CUMS Induced). In comparison to the standard group, climbing activity increased on day 14 (1 ml/kg and 2 ml/kg) and (200 mg/kg and 500 mg/kg). The following figures illustrate the effects of MILE (200 mg/kg and 500 mg/kg) and MIFJ (1 ml/kg and 2 ml/kg) on CUMS-induced changes in mice's climbing activity.

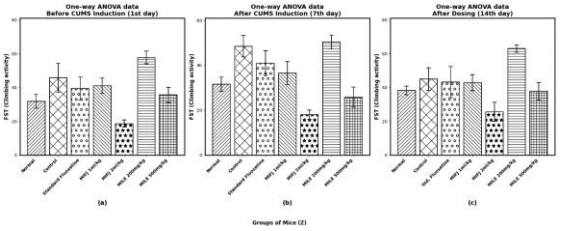

Fig. 3 Groups of Mice (Z)

DISCUSSION:

Depression is a mental disorder that manifests as an enduring sense of gloom and disinterest.

According to reports, the flavonoid has depressive properties. In India, mangifera indica is a popular indigenous fruit that is widely grown. The bark is said to include protocatechi acid, catching, mangiferin, alanine, glycine, y-amino butyric acid, kick acid, and shikimic acid. Its fruits are rich in polyphenol, flavonoids, triterpenoids, mangniferin, a xanthine glycoside, tannin, and derivatives of gallic acid. Mango has anti-inflammatory, anti-oxidant, anti-viral, cardiotonic, hypotensive, and anti-diabetic properties. Numerous effects have also been investigated, including antibacterial, antifungal, anticancer, anti-HIV, antispasmodic, antipyretic, antidiarrheal, immunomodulatory, hypolipedimic, and gastro protecting. The current study uses models such as the tail suspension test (TST) and forced swimming test (FST) to assess the antidepressant activity of Mangifera indica leaf extract and fruit juice. Both the fruit juice and leaf extract of Mangifera indica have been found to contain flavonoids and alkaloids that have been shown to have antidepressant properties. Mangifera indica fruit juice and hydroalcoholic leaf extract did not cause any harmful or significant adverse effects in mice, so dosages were chosen for the extract and juice plant. It has been observed that the TST has a higher pharmacological sensitivity and is less stressful than the FST (Fig. Group of mice X, Z). In the current study, the antidepressant effects of various fruit juice dosages and Mangifera indica hydroalcoholic extraction were examined. The

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animal's weight was measured on the first, seventh, and fourth days of the study; from the first and seventh days, the animal's weight grew. climbing behaviour, sideways mobility throughout the swimming chamber, and upward-directed forepaw movements. The mice's forelimbs moved upward more frequently in the FST on the first day prior to stress induction, and there was no discernible difference between the groups. The mice are less active on the seventh day following stress than they were on the first. Mice in the dose-treated drug groups moved their upper limbs more frequently than the standard group on the fourteenth day (Fig. Group of mice Z). TST on the first day, all groups showed an upward bend prior to stress induction. Mice in all groups were less active on the seventh day following stress induction than they were on the first. Compared to the standard group, the dose-treated groups' upward movement frequency appeared to improve on the fourteenth day. It has been said that TST is an easy way to assess possible antidepressants (Fig. Group of mice Y). Rodents' immobility in the face of an inevitable situation is indicative of behavioural despair, which may be a sign of depressive disorders in humans. When mice are suspended by their tails, clinically effective antidepressants lessen their immobility following active and unsuccessful attempts to flee.

CONCLUSION:

According to our research, Mangifera indica fruit juice and leaf extract significantly reduced depression in both FST and TST models when compared to typical medication effects. Although it appears that catecholamine inhibition is primarily responsible for this effect, more research is required to fully comprehend its antidepressant mechanism. This study presented Mangifera indica fruit juice and leaf extract as a readily available natural antidepressant.

CONFLICT OF INTEREST:

Regarding this research project, There are no competing interests of interest for the writers.

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