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# Antidepressant-Like and Antioxidant Effects of the Ethanolic Leaf Extract of *Mangifera Indica* Linn. in a Chronic Unpredictable Mild Stress Model in Female Wistar Rats, with Supporting Molecular Docking

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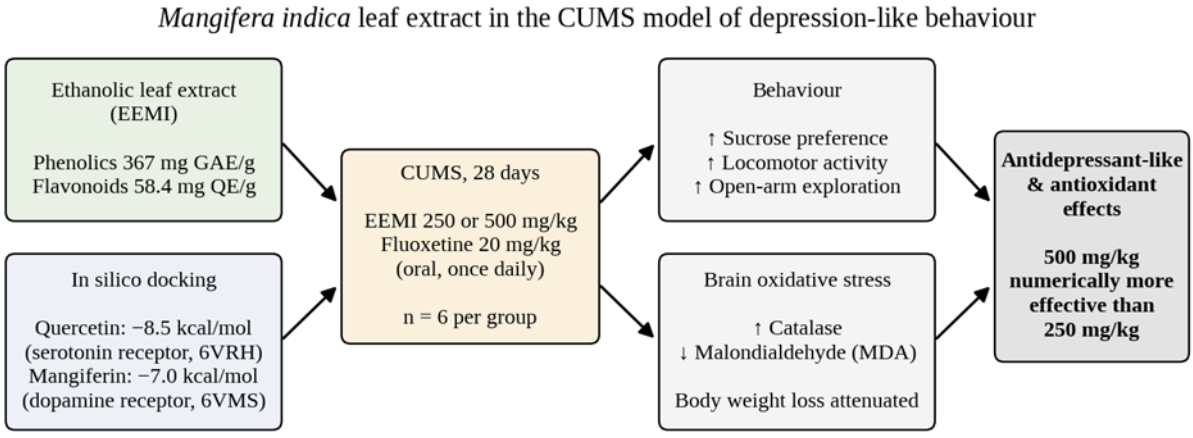
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**ABSTRACT**

Depression treatment is limited by delayed onset and adverse effects, and oxidative stress is implicated in its pathophysiology. This study evaluated the antidepressant-like and antioxidant effects of an ethanolic extract of *Mangifera indica* leaves (EEMI) in rats exposed to chronic unpredictable mild stress (CUMS). EEMI was prepared by Soxhlet extraction (70% ethanol) and analysed for phytoconstituents and total phenolic and flavonoid contents. Seven reported leaf constituents were docked against serotonin (6VRH) and dopamine (6VMS) receptor structures. Female Wistar rats (n = 6 per group) received vehicle, fluoxetine (20 mg/kg) or EEMI (250 or 500 mg/kg) orally for 28 days during CUMS; unstressed rats served as controls. Body weight, elevated plus maze, locomotor activity and sucrose preference were assessed on days 0 to 28, and brain catalase, malondialdehyde (MDA) and relative organ weights at the end. Groups were compared by ANOVA with Dunnett's test. The yield was 10.20%, with 367 mg gallic acid equivalents/g phenolics and 58.4 mg quercetin equivalents/g flavonoids. Quercetin (−8.5 kcal/mol) and mangiferin (−7.0 kcal/mol) had the lowest binding energies at the serotonin and dopamine receptor structures. CUMS reduced body weight, locomotor activity, open-arm exploration and sucrose preference (39.0% ± 0.57% on day 28), lowered brain catalase (0.46 ± 0.04 vs 1.81 ± 0.13 µmol $H_2O_2$/min/mg protein) and raised MDA (6.13 ± 0.36 vs 4.09 ± 0.04 nmol/mg protein). EEMI attenuated these changes at both doses ($P < 0.05$ to $P < 0.001$), with 500 mg/kg numerically more effective. EEMI showed antidepressant-like and antioxidant effects in CUMS-exposed rats; docking results are hypothesis-generating only.

**Keywords:** *Mangifera indica*; antidepressant-like activity; chronic unpredictable mild stress; oxidative stress; flavonoids; molecular docking

**Graphical Abstract**




## 1. Introduction

Depression is a common and multifactorial psychiatric disorder characterised by persistent low mood, anhedonia, disturbances of appetite, sleep, energy and cognition. Deficits in monoaminergic signalling (serotonin, noradrenaline and dopamine) are central to the classical hypothesis of its pathophysiology and underlie the action of selective serotonin reuptake inhibitors, tricyclic antidepressants and monoamine oxidase inhibitors. These drugs are widely

used, but delayed onset, incomplete response and adverse effects remain important limitations. These limitations have prompted a search for new antidepressant candidates from medicinal plants.[1]

*Mangifera indica* Linn. (mango; family Anacardiaceae) has a long history of use in Ayurvedic and indigenous medicine. Its leaves contain mangiferin as a major bioactive constituent, together with benzophenones, phenolic acids, quercetin and other flavonoids, and have been reported to show antioxidant, antidiabetic, immunomodulatory and other activities. Mangiferin has also been reported to protect against lipopolysaccharide-induced depressive- and anxiety-like behaviour in mice.[2]

Given the flavonoid-rich profile of the leaves and the recognised role of oxidative stress in stress-related disorders, we evaluated the antidepressant-like activity of an ethanolic leaf extract of *M. indica* (EEMI) in the chronic unpredictable mild stress (CUMS) model in rats. Behavioural, oxidative-stress and organ-weight endpoints were assessed and a molecular docking analysis was added to explore possible monoaminergic targets.

## 2. Materials and methods

### *2.1. Plant material and extract preparation*

Leaves of *M. indica* Linn. were collected from a local garden in Chitradurga, Karnataka, and authenticated by Dr. Sudhama V. N., Associate Professor and Head, Department of P.G. Studies in Botany, Government Science College, Chitradurga (authentication dated 27 January 2026; voucher no. GSC-H-28). Leaves were washed, shade-dried, powdered and passed through sieve no. 10 and 40. The powder (240 g) was extracted in a Soxhlet apparatus with 70% ethanol (about 10 mL per gram of powder) for 5–6 h. The extract was filtered and concentrated under reduced pressure at 40–50°C. To remove chlorophyll and non-polar constituents, the concentrate was redissolved in distilled water and partitioned with petroleum ether in a separating funnel. The aqueous layer was concentrated again and dried at 45°C to a constant mass and the dried extract (EEMI) was stored in airtight containers at 4°C. Percentage yield was calculated as (weight of dried purified extract/weight of powdered leaves) × 100.[3]

### *2.2. Preliminary phytochemical screening*

EEMI was screened by standard qualitative tests for alkaloids (Dragendorff's, Hager's, Mayer's, Wagner's), carbohydrates (Molisch's, Benedict's, Barfoed's, Fehling's, iodine, anthrone), cardiac glycosides (Keller–Kiliani, Baljet, Legal, modified Borntrager's), proteins and amino acids (xanthoproteic, Millon's, biuret, ninhydrin, aldehyde, sulphur), flavonoids (alkaline reagent, lead acetate, Shinoda, ferric chloride), phenols and tannins (gelatin, ferric chloride, lead acetate), saponins, steroids, triterpenoids (Liebermann– Burchard, Salkowski), resins, starch, quinones and anthraquinones (Borntrager's, modified Borntrager's).[4]

### *2.3. Total phenolic and total flavonoid content*

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method using gallic acid standards (10–100 µg/mL), with absorbance read at 760 nm and expressed as mg gallic acid equivalents (GAE) per g of extract. Total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method using quercetin standards, with absorbance read at 510 nm and expressed as mg quercetin equivalents (QE) per g of extract. Content was calculated as $C = cV/m$, where c is the concentration read from the calibration curve, V the volume of extract and m the weight of extract. Linear regression and $R^2$ were obtained in Microsoft Excel.[5]

### *2.4. Molecular docking*

Seven constituents reported from *M. indica* leaves (mangiferin, quercetin, kaempferol, rutin, caffeic acid, ferulic acid and gallic acid) were docked against the serotonin receptor (PDB ID 6VRH) and the dopamine receptor (PDB ID 6VMS). Ligand structures were retrieved from PubChem and converted to PDB format using BIOVIA Discovery Studio. Receptor structures were obtained from the RCSB Protein Data Bank. Docking was performed using PyRx with AutoDock Vina and interactions were examined in BIOVIA Discovery Studio Visualizer. Binding energies below −5 kcal/mol were taken to indicate favourable predicted binding.

### *2.5. Animals and ethics*

Healthy adult female Wistar albino rats (150–200 g) were obtained from Liveon Biolabs Pvt. Ltd., Tumkur. Animals were acclimatised for 10 days and housed at 26 ± 2°C, 45–55% relative humidity and a 12 h light/dark cycle with standard pellet diet and water ad libitum, in accordance with CCSEA guidelines. The study was approved by the Institutional Animal Ethics Committee, S J M College of Pharmacy (ref. no. 3B/SJMCP/IAEC-02/July 2024-25).

### *2.6. Dose selection and treatment*

Doses were selected from OECD 420 (fixed dose procedure) safety data for *M. indica* leaf extract (Chaurasiya et al., 2025), taking one-tenth (250 mg/kg) and one-fifth (500 mg/kg) of the reference dose. Fluoxetine (20 mg/kg) was the reference antidepressant. Fluoxetine and EEMI were suspended in 1% carboxymethyl cellulose (CMC) and given orally once daily for 28 days.[6]

### *2.7. Experimental design and CUMS procedure*

Thirty rats were allocated to five groups (n = 6; Fig. 1): Group 1, unstressed control (distilled water, 10 mL/kg, orally, no stress); Group 2, CUMS control (CUMS plus vehicle); Group 3, CUMS plus fluoxetine (20 mg/kg); Group 4, CUMS plus EEMI (250 mg/kg); and Group 5, CUMS plus EEMI (500 mg/kg).

Stressed groups were exposed to one mild stressor per day for 28 days, using seven stressors :tail squeezing (2 min), cage shaking (30 min), food deprivation (24 h), restraint (3 h), cage tilt at 45° (1 h), oscillation (5 min) and overnight water deprivation (18 h) followed by an empty water bottle for 1 h. Behavioural tests were performed on days 0, 7, 14, 21 and 28. After the final test, animals were euthanised by cervical dislocation and brains and organs were collected.[7,8]

### *2.8. Behavioural assessments*

#### *2.8.1 Body weight*

Body weight was recorded on days 0 and 28, and percentage change was calculated as [(day 28 − day 0)/day 0] × 100.

#### *2.8.2 Elevated plus maze (EPM)*

The maze had two open (length 30 cm × width 5 cm) and two closed arms (length 30 cm × width 5 cm × height 15 cm) joined by a 5 × 5 cm central platform and raised 45 cm above the floor. One hour after dosing, each rat was placed on the central platform heads facing towards an open arm and observed for 5 min. Open- and closed-arm entries (all four paws) and the time spent in each arm were recorded. The maze was cleaned with 10% ethanol between animals. %Open arm entries= (Number of entries into open arm)/ (Total number of arm entries) ×100.[9]

#### *2.8.3 Locomotor activity*

Spontaneous locomotor activity was recorded for 5 min in an actophotometer (30 × 30 cm field with photocells), and total beam interruptions were counted.[10]

#### *2.8.4 Sucrose preference test (SPT)*

After a week of habituation to 2% sucrose solution, animals were given a free choice between 2% sucrose solution and drinking water for 24 h. Sucrose preference (%) = Sucrose intake/ (Sucrose intake + Water intake) × 100.[11]

### *2.9. Biochemical estimations and relative organ weights*

Forebrain tissue was homogenised (10% w/v) in 0.1 M phosphate buffer (pH 7.0).

#### *2.9.1 Catalase assay*

Catalase activity was determined by the method of Beers and Sizer, following $H_2O_2$ decomposition at 240 nm and expressed as µmol $H_2O_2$/min/mg protein.

#### *2.9.2 Lipid peroxidation assay*

Lipid peroxidation was estimated as thiobarbituric acid-reactive malondialdehyde (MDA) by the method of Satoh, with absorbance read at 532 nm and expressed as nmol/mg protein (Sarma et al., 2019).[12]

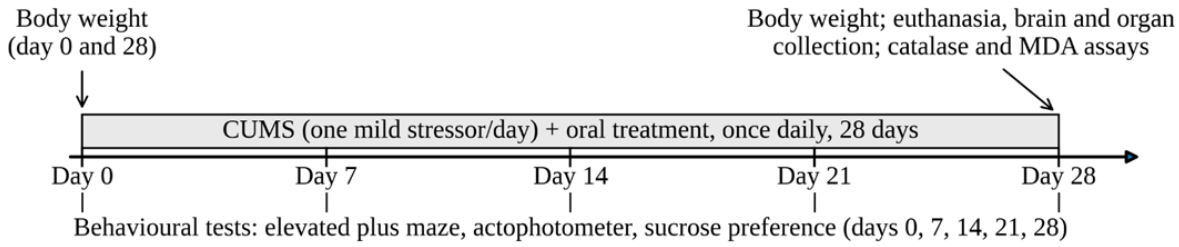
### *2.10 Morphometric parameters*

Liver, brain, spleen, stomach and adrenal glands were weighed, and relative organ weight (%) was calculated as organ weight/final body weight × 100.[13]

### *2.11. Statistical analysis*

Data are presented as mean ± SEM (n = 6). Groups were compared by one-way ANOVA followed by Dunnett's multiple comparisons test against the CUMS control group, using GraphPad Prism 11.0.2. $P < 0.05$ was considered significant.

**Fig. 1.** Experimental timeline and study plan. CUMS, chronic unpredictable mild stress; EEMI, ethanolic extract of *Mangifera indica* leaves.




## 3. Results

### *3.1. Extract yield and phytochemical profile*

EEMI was obtained as a sticky, greenish-black mass with a yield of 10.20% (24.489 g from 240 g of powder). Screening indicated the presence of alkaloids, carbohydrates, cardiac glycosides, proteins and amino acids, flavonoids, phenols and tannins, saponins, steroids, triterpenoids and quinones, whereas resins, starch and anthraquinones were absent (Table 1).

**Table 1:** Preliminary phytochemical screening of EEMI

| **Constituent** | **Tests +/total** | **Result** |
|---|---|---|
| Alkaloids | 4/4 | Present |
| Carbohydrates | 4/6 | Present |
| Cardiac glycosides | 3/4 | Present |
| Proteins, amino acids | 3/6 | Present |
| Flavonoids | 3/4 | Present |
| Phenols and tannins | 2/3 | Present |
| Saponins | 1/2 | Present |
| Steroids | 2/3 | Present |
| Triterpenoids | 1/2 | Present |
| Quinones | 1/2 | Present |
| Resins | 0/1 | Absent |
| Starch | 0/1 | Absent |
| Anthraquinones | 0/2 | Absent |

### *3.2. Total phenolic and flavonoid content*

Calibration curves were linear for gallic acid ($y = 0.0098x + 0.001$; $R^2 = 0.9995$) and quercetin ($y = 0.0051x + 0.0117$; $R^2 = 0.9998$). EEMI contained 367 mg GAE/g extract of total phenolics and 58.4 mg QE/g extract of total flavonoids. (Table 2,3 and Fig.3,4).

**Table 2:** Absorbance value for gallic acid and quercetin measured for calibration curve

| Concentration (µg/mL) | Absorbance, gallic acid | Absorbance, quercetin |
|---|---|---|
| 10 | 0.10 | 0.06 |
| 25 | 0.26 | 0.14 |
| 50 | 0.50 | 0.27 |
| 75 | 0.73 | 0.39 |
| 100 | 0.99 | 0.52 |

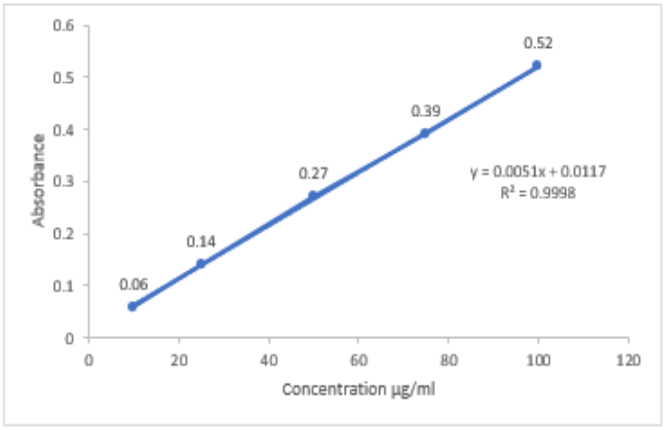



**Fig.3.** Calibration curve for gallic acid

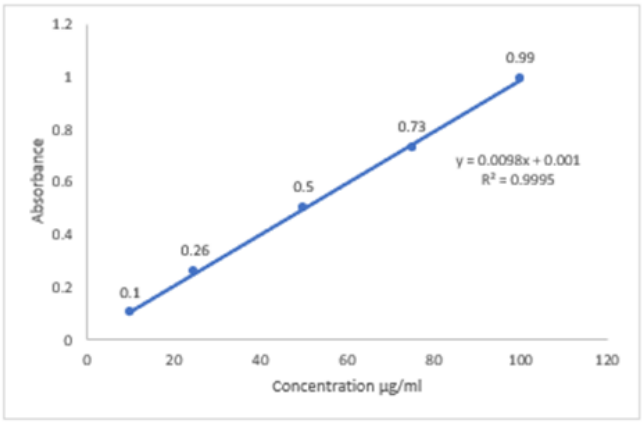



**Fig.4.** Calibration curve for quercetin

**Table 3:** Total Phenolic and Total Flavonoid Content in different extract.

| Plant parts | 70% ethanol mg GAE/ g Phenolic content | 50% ethanol QE mg/ g flavonoid content |
|---|---|---|
| Leaves | 367 mg/ GAE / g sample | 58.4 mg/ QE / g sample |

### *3.3. Molecular docking*

All seven compounds showed predicted binding energies of −5.0 kcal/mol or lower at both targets (Table 2); more negative values indicate stronger predicted binding. At the serotonin receptor (6VRH), binding energies ranged from −6.6 to −8.5 kcal/mol, with the strongest binding predicted for quercetin (−8.5), rutin (−8.2) and mangiferin (−7.9). At the dopamine receptor (6VMS), the range was −5.0 to −7.0 kcal/mol, with the strongest binding

predicted for mangiferin (−7.0), quercetin (−6.5) and kaempferol (−6.2). Rutin and ferulic acid (−5.0 kcal/mol each) were at the threshold at 6VMS (Table 4).

**Table 4:** Predicted binding energies (kcal/mol) of *M. indica* leaf constituents.

| Compound | Serotonin receptor (6VRH) | Dopamine receptor (6VMS) |
|---|---|---|
| Mangiferin | −7.9 | −7.0 |
| Quercetin | −8.5 | −6.5 |
| Kaempferol | −7.4 | −6.2 |
| Rutin | −8.2 | −5.0 |
| Caffeic acid | −7.3 | −5.4 |
| Ferulic acid | −7.2 | −5.0 |
| Gallic acid | −6.6 | −5.5 |

### *3.4. Body weight*

Body weight increased by 3.55 ± 0.08% in unstressed rats but fell by -4.55 ± 0.06% in the CUMS control group. Fluoxetine, EEMI-250 and EEMI-500 produced changes of +2.32 ± 0.06% ($P < 0.001$), +2.60 ± 0.04% ($P < 0.05$) and +3.03 ± 0.04% ($P < 0.01$), respectively, relative to the CUMS control (Table5 and Fig. 5).

**Table 5:** Effect of EEMI on percentage change in body weight in CUMS-Exposed rats

| Group | Treatment | Body weight change (%) |
|---|---|---|
| Group 1 | Unstressed control | 3.55 ± 0.08 |
| Group 2 | CUMS control | −4.55 ± 0.06 |
| Group 3 | Fluoxetine 20 mg/kg | 2.32 ± 0.06*** |
| Group 4 | EEMI 250 mg/kg | 2.60 ± 0.04* |
| Group 5 | EEMI 500 mg/kg | 3.03 ± 0.04** |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the CUMS control group (one-way ANOVA followed by Dunnett's test).

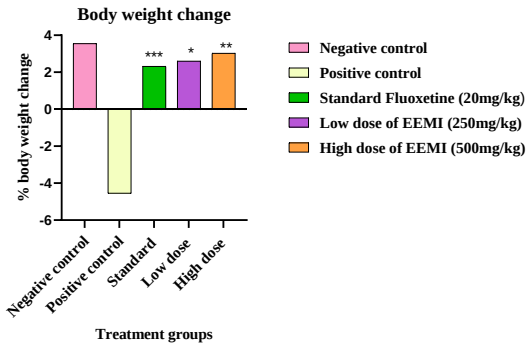



**Fig. 5.** Effect of EEMI on percentage change in body weight Values are mean ± SEM (n = 6). *P < 0.05, P < 0.01*, **P < 0.001 compared with the CUMS control group (Dunnett's test).

### *3.5. Behavioural effects*

In the CUMS control group, open-arm entries fell from 8.16 on day 0 to 3.33 ± 0.33 on day 28, open-arm time from 45.00 s to 35.00 ± 0.73 s, closed-arm entries (10.17 to 12.83 ± 0.87) and closed-arm time (207.0 to 236.0 ± 0.57 s) (Table 6 and Fig. 6).

Locomotor activity from 198.0 to 98.33 ± 2.78 counts. Sucrose preference declined from 81.00% to 39.00 ± 0.57%, while unstressed rats stayed between 80% and 83% throughout (Table 6 and Fig. 7). Fluoxetine and both EEMI doses significantly reversed these changes relative to the CUMS control from day 7 onwards, with the effect increasing through day 28(Table 6). For example, on day 28 EEMI-250 and EEMI-500 raised locomotor activity to 146.3 ± 0.91 and 165.5 ± 1.17 counts (fluoxetine: 183.5 ± 0.99)

Sucrose preference to 58.00 ± 0.57% and 70.00 ± 0.57% (fluoxetine: 79.00 ± 0.57%). The 500 mg/kg dose gave numerically larger improvements than 250 mg/kg on every behavioural measure, but the two doses were not compared directly, and fluoxetine was numerically more effective than either dose.(Table 6 and Fig.7)

**Table 6:** Behavioural parameters on day 28 (mean ± SEM, n = 6).

| Group | Open-arm entries | Closed-arm entries | Open-arm time (s) | Closed-arm time (s) | Locomotor activity (counts/5 min) | Sucrose preference (%) |
|---|---|---|---|---|---|---|
| Unstressed control | 8.50±0.22 | 10.83±1.01 | 49.00±0.73 | 211.0±0.57 | 204.0 ± 0.96 | 83.00 ± 0.57 |
| CUMS control | 3.33±0.33 | 12.83±0.87 | 35.00±0.73 | 236.0±0.57 | 98.33 ± 2.78 | 39.00 ± 0.57 |
| Fluoxetine 20 mg/kg | 9.66±0.21*** | 5.50±0.99*** | 141.0±0.73*** | 111.0±0.57*** | 183.5±0.99*** | 79.00±0.57*** |
| EEMI 250 mg/kg | 6.00 ± 0.36** | 8.33 ± 0.42** | 93.00 ± 0.73* | 143.2 ± 0.60* | 146.3 ± 0.91** | 58.00 ± 0.57** |
| EEMI 500 mg/kg | 8.00±0.25*** | 7.50 ± 0.76** | 110.0 ± 0.73** | 131.8 ±0.47** | 165.5±1.17*** | 70.00±0.57*** |

*P < 0.05, P < 0.01,* **P < 0.001 compared with the CUMS control group (one-way ANOVA followed by Dunnett's test).

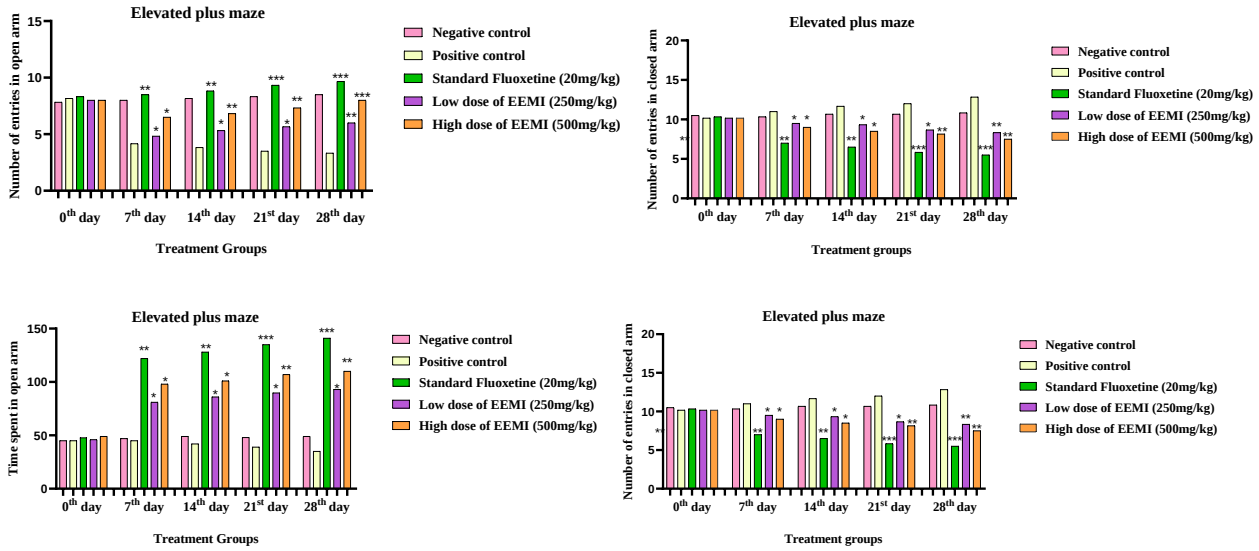



**Fig. 6.** Effect of EEMI on elevated plus maze parameters in CUMS-exposed rats: open-arm entries, closed-arm entries, time spent in open arms and time spent in closed arms. Values are mean ± SEM (n = 6). *P < 0.05, P < 0.01,* **P < 0.001 compared with the CUMS control group (Dunnett's test).

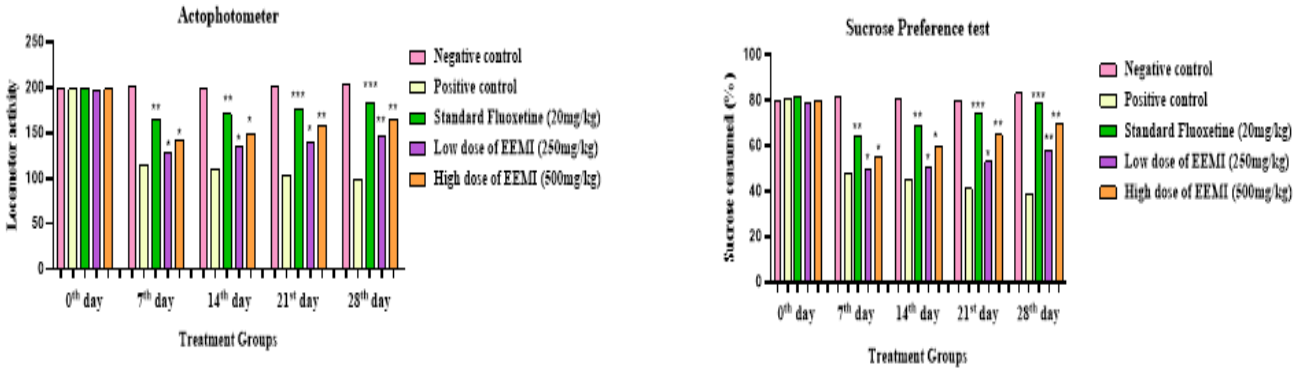



**Fig. 7.** Effect of EEMI on (a) locomotor activity and (b) sucrose preference in CUMS-exposed rats. Values are mean ± SEM (n = 6). *P < 0.05*, *P < 0.01*, **P < 0.001 compared with the CUMS control group (Dunnett's test).

### *3.6. Brain oxidative stress markers*

CUMS reduced brain catalase activity to 0.46 ± 0.04 (unstressed: 1.81 ± 0.13 µmol $H_2O_2$/min/mg protein) and increased MDA to 6.13 ± 0.36 nmol/mg protein (unstressed: 4.09 ± 0.04). Catalase was raised by fluoxetine (3.89 ± 0.16; *P* < 0.001), EEMI-250 (1.53 ± 0.11; *P* < 0.05) and EEMI-500 (2.26 ± 0.10; *P* < 0.01). MDA was lowered by fluoxetine (3.65 ± 0.28; *P* < 0.001), EEMI-250 (4.18 ± 0.03; *P* < 0.05) and EEMI-500 (3.91 ± 0.02; *P* < 0.01) (Table 7 and Fig. 8).

**Table 7:** Brain catalase activity and malondialdehyde (MDA) level in each group (mean ± SEM, n = 6).

| Group | Treatment | Catalase (µmol $H_2O_2$/min/mg protein) | MDA (nmol/mg protein) |
|---|---|---|---|
| Group 1 | Unstressed control | 1.81 ± 0.13 | 4.09 ± 0.04 |
| Group 2 | CUMS control | 0.46 ± 0.04 | 6.13 ± 0.36 |
| Group 3 | Fluoxetine 20 mg/kg | 3.89 ± 0.16*** | 3.65 ± 0.28*** |
| Group 4 | EEMI 250 mg/kg | 1.53 ± 0.11* | 4.18 ± 0.03* |
| Group 5 | EEMI 500 mg/kg | 2.26 ± 0.10** | 3.91 ± 0.02** |

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the CUMS control group (one-way ANOVA followed by Dunnett's test).

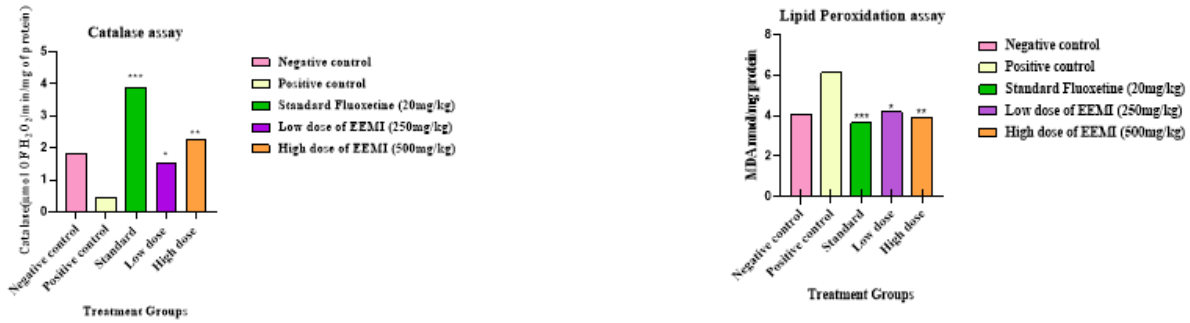



**Fig.8.** Brain catalase activity and brain malondialdehyde (MDA) in CUMS-exposed rats. Values are mean ± SEM (n = 6). *P < 0.05*, *P < 0.01*, **P < 0.001 compared with the CUMS control group (Dunnett's test).

### *3.7. Relative organ weights*

CUMS lowered relative brain (0.45 ± 0.01%), spleen (0.18 ± 0.01%) and liver (2.24 ± 0.03%) weights and raised relative adrenal gland (0.037 ± 0.001%) and stomach (1.00 ± 0.01%) weights compared with unstressed rats (Table 4). EEMI (250 and 500 mg/kg) significantly changed all five organs relative to the CUMS control (*P* < 0.05 to *P* < 0.01). Brain and spleen weights moved toward unstressed values, and adrenal weight fell. Liver weight rose above, and stomach weight fell below, the unstressed values. (Table 8 and Fig.9)

**Table 8:** Relative organ weights (% of body weight; mean ± SEM, n = 6).

| Group | Brain | Spleen | Liver | Adrenal gland | Stomach |
|---|---|---|---|---|---|
| Unstressed control | 0.58 ± 0.01 | 0.22 ± 0.01 | 2.41 ± 0.03 | 0.01 ± 0.001 | 0.68 ± 0.01 |
| CUMS control | 0.45 ± 0.01 | 0.18 ± 0.01 | 2.24 ± 0.03 | 0.037 ± 0.001 | 1.00 ± 0.01 |
| Fluoxetine 20 mg/kg | 0.57±0.01*** | 0.22 ±0.01** | 2.35±0.03*** | 0.009±0.001*** | 1.70 ± 0.02*** |
| EEMI 250 mg/kg | 0.51 ± 0.01** | 0.19 ± 0.01* | 2.52 ± 0.03** | 0.007 ± 0.001* | 0.54 ± 0.01* |
| EEMI 500 mg/kg | 0.52 ± 0.01** | 0.20 ± 0.01* | 2.53 ± 0.03** | 0.008 ± 0.001** | 0.60 ± 0.01* |

*P < 0.05, P < 0.01,* **P < 0.001 compared with the CUMS control group.

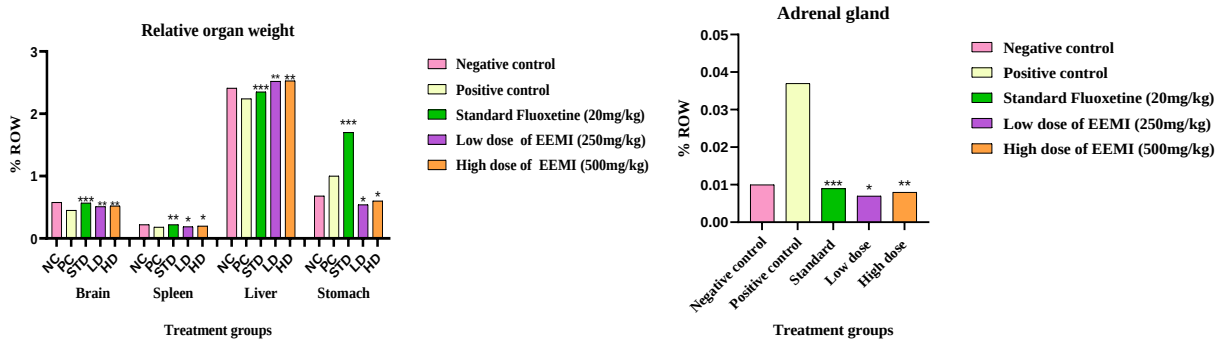



**Fig.9.** Effect of EEMI leaves on relative organ weights in experimental animals Values are mean ± SEM (n = 6). *P < 0.05, P < 0.01,* **P < 0.001 compared with the CUMS control group (Dunnett's test).

## 4. Discussion

In this study, an ethanol extract of *Mangifera indica* leaves reduced the harmful effects of four weeks of chronic unpredictable mild stress (CUMS) in female Wistar rats. It improved their behaviour, their oxidative stress markers and some organ weights. CUMS caused the changes we expected: the rats lost weight, drank less sucrose solution (a sign of reduced pleasure-seeking), moved less and avoided the open arms of the elevated plus maze. Other studies using CUMS to test antidepressants report similar changes. Fluoxetine reversed these changes, which shows that the model worked.

Both extract doses significantly increased sucrose preference. This is the test that best reflects the loss of pleasure seen in depression. The improvements in movement and open-arm exploration need more careful reading. The elevated plus maze mainly measures anxiety-like behaviour and a change in how much the rats moved could affect the other behaviour results. Stress had reduced their movement and the extract brought it back toward normal, so this is more likely a recovery from stress than a stimulant effect. However, we did not include unstressed rats given the extract alone, so we cannot be sure. The 500 mg/kg dose gave slightly larger improvements than 250 mg/kg, but we did not compare the two doses statistically, so we cannot say the effect depends on dose.

CUMS lowered catalase (an antioxidant enzyme) and raised malondialdehyde (a marker of damage to fats) in the brain. This agrees with the idea that oxidative stress is linked to depression (Bondy, 2002; Fekadu et al., 2016). The extract partly corrected both markers. It was rich in phenolic compounds (367 mg GAE/g) and also contained flavonoids (58.4 mg QE/g). Mango leaves are a known source of antioxidants such as mangiferin and quercetin (Kumar et al., 2021; Mohan et al., 2013) and mangiferin has been reported to reduce depression-like and anxiety-like behaviour in an inflammation model. These results fit the idea that the extract works partly through antioxidant action. However, we did not measure other antioxidant enzymes, glutathione or inflammation markers, and the two chemical tests we used do not identify individual compounds. Fluoxetine raised catalase above the level in unstressed rats, so its effect may be more than a return to normal.

The docking study predicted that the selected leaf compounds could bind to serotonin and dopamine receptors, with quercetin and mangiferin showing the strongest predicted binding at the two targets. This is consistent with an effect on the brain's monoamine systems, which are important in depression. Treat it only as a lead for further work. Docking scores do not measure actual binding strength or whether a compound activates or blocks a receptor. The compounds were taken from earlier studies, and we did not confirm that they are present in our extract. We also did not test receptor activity in cells or animals. Future work should state which receptor subtypes the two structures represent.

Long-term stress is known to over-activate the body's stress hormone system. The larger adrenal glands in the CUMS rats fit this and all treatments reduced adrenal weight. Brain and spleen weights moved toward the unstressed values with the extract. Liver and stomach weights in the extract groups

went past the unstressed values, with liver weight rising above and stomach weight falling below. These data cannot show whether the extract protects or harms the organs. Examining the tissue under the microscope and measuring blood markers would be needed.

This study has limitations; The groups were small (six rats each), no sample-size calculation was reported and only female rats were used, so the results may not apply to males. Each time point was analysed separately against the CUMS control rather than with a repeated-measures analysis and the unstressed group and the two doses were not compared statistically. There was no group of unstressed rats given the extract alone. We did not measure brain chemicals (monoamines, BDNF, corticosterone), run the forced swim or tail suspension tests, or examine tissue under the microscope. The extract was also not standardised by HPLC or LC-MS.

## 5. Conclusion

The ethanolic leaf extract of *Mangifera indica* (250 and 500 mg/kg, orally for 28 days) attenuated CUMS-induced loss of body weight, reduced locomotor activity, anhedonia-like behaviour and open-arm avoidance and partly normalised brain catalase and MDA in female Wistar rats, with the higher dose generally producing larger numerical effects. The activity may be linked to the phenolic and flavonoid content and antioxidant properties of the extract, while docking suggests possible interaction with serotonergic and dopaminergic targets. Standardised extracts, mechanistic experiments and additional behavioural models are needed to establish its antidepressant potential.

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