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ANTI-DEPRESSANT EFFECT OF CHLOROPHYTUM TUBEROSUM DEPRESSED ANIMAL MODEL'S

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Abstract:

Depression is a multifactorial neuropsychiatric disorder often associated with neurotransmitter imbalances and oxidative stress. The present study evaluated the potential antidepressant and antioxidant effects of Chlorophytum tuberosum extract in a reserpine-induced depression model in rats. Animals were divided into six groups: control, reserpine-only, fluoxetine-treated, and three groups treated with varying doses (10, 150, and 200 mg/kg) of C. tuberosum extract along with reserpine. Behavioural assessments were conducted using the Forced Swim Test (FST), Tail Suspension Test (TST), and Open Field Test (OFT) to assess depressive-like and exploratory behaviours. Biochemical analysis included measurement of serum and brain malondialdehyde (MDA) levels, as well as brain antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Reserpine treatment significantly increased immobility time and MDA levels, while reducing antioxidant enzyme activity, indicating both behavioural despair and oxidative stress. Fluoxetine and C. tuberosum at 200 mg/kg significantly reversed these effects, showing reduced immobility, improved exploratory behaviour, and restoration of antioxidant status. The results demonstrated that Chlorophytum tuberosum extract exhibits dose-dependent antidepressant-like effects, with the 200 mg/kg dose showing efficacy comparable to fluoxetine. The extract also significantly mitigated oxidative stress markers, suggesting its neuroprotective potential. In conclusion, Chlorophytum tuberosum may serve as a promising natural therapeutic agent for depression, potentially acting through modulation of oxidative stress and central monoaminergic pathways. Further investigations are warranted to isolate its active constituents and explore its mechanisms in chronic depression models.

Keywords: *Depression, Chlorophytum tuberosum, antidepressant*

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INTRODUCTION:

Depression is an illness that affects both the mind and the body and is a leading cause of disability, workplace absenteeism, decreased productivity and high suicide rates.[1,2] Depression is the most common psychiatric disorder in general practice and about one in ten patients seen in the primary care settings suffer from some form of depression.[3,4] It is an important global public health problem due to both its relatively high lifetime prevalence and the significant disability that it causes. Depression accounted for 4.5% of the worldwide total burden of disease (in terms of disability-adjusted life years). It is also responsible for the greatest proportion of burden attributable to non-fatal health outcomes, accounting for almost 12% of total years lived with disability worldwide. Without treatment, depression has the tendency to assume a chronic course, to recur, and to be associated with increasing disability over time.

In a study by the World Health Organization (WHO) conducted at 14 sites, the most common diagnosis in primary care was depression.[5] Depression is estimated to affect 340 million people globally.[6] The prevalence of psychiatric disorders is reported to differ between countries and within countries, across various ethnicities.[7]

Most studies on depression are from the developed world and there are few studies from developing countries. The World Mental Health Survey Initiative carried out cross-national research in mental health, especially in developing countries. There have been a few population based studies from India but most have been done on selected groups.[8-12] The prevalence of depression in a population based study conducted in urban Pakistan was 45.9%, [12] while in rural Bangladesh, it was reported to be 29%, [13] and in a peri-urban clinic based study in Uganda, it was reported to be 6.1%.[14] Earlier Indian studies have reported prevalence rates of depression that vary from 21–83% in primary care practices.[15-18]

MATERIALS AND METHODS:

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The Chlorophytum tuberosum leaves are collected and identified. Collection of Plant Materials: Dried was purchased from an herbal Market of Hyderabad, Telangana, India, and authenticated by Dr K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. At the Department of Pharmacology, at our institution.

Preparation of the Extract

The collected Chlorophytm tuberosum washed, air dried, homogenized to fine powder and stored in airtight bottles. and then extracted with Ethanol by using the Soxhlet apparatus.

Extraction of plant material

The preserved and pulverized plant material was utilized in the extraction process. A metered amount of each pulverized plant material was subjected to cold maceration with methanol for 72 hours, with one intermediate heating at 40°C per day. The residues were subsequently extracted. Following filtration through Whatmann filter paper, the filtrate was concentrated at a controlled temperature and reduced pressure (40-50°C). After being desiccated, the marc was weighed.

EXPERIMENTAL DESIGN

I Group Control (Normal saline) 1 mL/kg
II Group Reserpine only (Depressed control) 5 mg/kg
III Group Fluoxetine + Reserpine Fluoxetine: 20 mg/reserpine: 5 mg/kg
IV Group Chlorophytum tuberosum Extract + Reserpine Extract: 10 mg/reserpine: 5 mg/kg
V Group Chlorophytum tuberosum Extract + Reserpine Extract: 150 mg/reserpine: 5 mg/kg
VI Group Chlorophytum tuberosum Extract + Reserpine Extract: 200 mg/ reserpine: 5 mg/kg

Behavioral test

Forced Swim Test (FST) Procedure for Rats [19]

The Forced Swim Test (FST) is a validated behavioural model used to assess antidepressant-like activity in rats. In this procedure, each rat is placed individually in a vertical glass cylinder (typically 40 cm in height and 20 cm in diameter) filled with water to a depth of 30 cm, maintained at a temperature of 25 ± 1 °C, ensuring the animal cannot touch the bottom or escape. The test consists of two sessions. In the pre-test session (habituation), each rat is placed in the water for 15 minutes and then removed, dried, and returned to its home cage. Twenty-four hours later, the test session is conducted, where each rat is placed in the same cylinder for 5 minutes. The behaviour is observed and recorded, focusing on three main parameters: immobility (floating with minimal movements to keep the head above water), swimming, and climbing. Antidepressant treatment typically reduces immobility time and increases active behaviours like swimming or climbing, depending on the drug's mechanism. After the session, rats are dried with a towel and kept warm before returning to their cages. This test is commonly used due to its sensitivity to various classes of antidepressant agents, such as SSRIs and tricyclic antidepressants.

Tail Suspension Test (TST) [20]

Tail Suspension Test (TST) Procedure for Rats (Modified) Although originally developed and validated for mice, a modified version of the Tail Suspension Test (TST) has occasionally been employed in rats for experimental antidepressant screening. In this adaptation, each rat is suspended by its tail using adhesive tape attached about 2–3 cm from the tip. The rat is then hung from a metal rod or flat horizontal surface at a height of approximately 60 cm above a padded surface to prevent injury in case of accidental fall. The test duration is typically 6 minutes, during which the total immobility time, defined as the absence of active escape-oriented movements, is recorded. Immobility is considered an indicator of behavioral despair, and a reduction in immobility time following drug treatment suggests antidepressant-like activity. Due to their size and weight, rats may attempt to curl upward or reach surfaces, making consistent observations difficult. Therefore, this method is less commonly used and is considered less reliable than the Forced Swim Test in rats.

Open-Field Test (OFT) [21]

The Open-Field Test (OFT) is a widely used behavioral assay in rodents, including rats, to evaluate locomotor activity, exploratory behavior, and anxiety-like responses. The procedure involves placing the rat in a novel, enclosed arena-typically a square or circular box with marked zones—and observing its behavior for a set period, usually 10 to 15 minutes. Key parameters recorded include the total distance travelled, time spent in the center versus the periphery, number of rearings, and other behaviours such as freezing or grooming. Increased exploration and time spent in the centre of the arena are generally interpreted as reduced anxiety, while reduced movement and thigmotaxis (wall-hugging behaviour) suggest higher anxiety levels. The test is conducted in a quiet room with controlled lighting, and the arena is cleaned between trials to eliminate olfactory cues. The OFT is commonly used to assess the effects of pharmacological agents, genetic modifications, or environmental stressors on rodent behaviour.

Biochemical analysis

Measurement of Plasma Antioxidant Capacity

Three solutions were used for this measurement. Solution 1: 1.5 ml of sodium acetate and 8 ml of concentrated acetic acid, diluted to 500 mL with distilled water. Solution 2: 270 mg of Iron (III) chloride, dissolved in 50 mL of distilled water. Solution 3: 47 mg of treeazin, dissolved in 40 mL of HCl. The working solution was prepared by mixing 10 mL of solution 1, 1 ml of solution 2, and 1 ml of solution 3. Thereafter, 25 microliters of the serum sample were added to 5.1 ml of the working solution. The resulting mixture was incubated at 37 °C for 15 minutes and then the absorbance was measured at 593 nm [22].

Measurement of Brain Antioxidant Capacity

The antioxidant capacity of the brain was determined by ferric reducing antioxidant power (FRAP) assays. The FRAP working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ (2, 4, 6-tripyridyl-s-triazine), and 2.5 ml of FeCl3 . Brain tissue was homogenized and the homogenate was centrifuged at 1000 g for 10 minutes. 50 ml of the resulting supernatant was mixed with 5.1 mL of FRAP working solution. After 10 minutes of incubation, Fe3+ TPTZ complex was reduced to the ferrous (Fe2+) producing an intense blue color. The absorbance of mixture was measured at 590 nm [23].

Measurement of Serum MDA Level

Briefly, 50 µL of serum was mixed with 50 µL of 0.05% BHT, 400 µL of 0.44 M H_3PO_4, and 100 µL of 42 Mm TBA. The mixture was vortexed and then heated in a boiling water bath for 1 hour. After cooling at 0 °C for 5 minutes, 250 µL of n-butanol was added to the mixture, vortexed, and then centrifuged at 14000 rpm for 5 minutes. The absorbance of the supernatant was measured at 532 nm.

Measurement of Brain MDA Level

Brain tissue was homogenized in (1:10 wv-1) pre-chilled KCL solution and transferred into a 20 ml tube. After incubation for 60 minutes at 37 °C, the suspension was mixed with 1 ml of 5% tetra chloroacetic acid and 1 ml of 67% TBA, and centrifuged for 15 minutes at 2,000 rpm. The resulting supernatant was transferred into a new tube and placed in a boiling water bath for 10 minutes. After cooling, its absorbance was measured at 535 nm [24-26].

Statistical Analysis

All data were expressed as the mean±SD. The Kolmogorov-Smirnov test was used for the normality test. All data had P values greater than 0.05, which indicated the normal distribution of data. The homogeneity of variances was determined using the Levene's test. Oneway ANOVA was used to compare the mean between the experimental groups. In the case of homogeneous variances and in the case of non-homogeneous variances Duncan test and Dunnett's T3 were used, respectively. $P < 0.05$ was considered statistically significant.

Glycosides, tannin, and phenolic compounds were present in higher amount in leaf.

Qualitative phytochemical analysis of *Chlorophytum tuberosum*

Sl. No.	Phytoconstituents	Leaf
1	Carbohydrates	+
2	Proteins	+
3	Fats	+
4	Glycosides	+
5	Alkaloids	+
6	Tannins	+
7	Phobatannins	-
8	Flavonoids	+
9	Steroids	+
10	Saponins	+
11	Phenolic compounds	+
12	Phytosterols	+

(-) =Absent (+) =Present

Pharmacological activity

Acute toxicity study

Acute toxicity studies revealed that nontoxic nature of extracts of *Chlorophytum tuberosum.* There were no lethality or toxic reactions found at 2000mg/ kg body weight of the study period. All the animals were alive, healthy and active during the observation for the given dose so the doses were fixed for pharmacological study.

Table 2: **Effect of extracts of *Chlorophytum tuberosum* in acute toxicity**

Day	Dose (mg Per kg body weight)	No. of animal(s)	Observation
Day 1	250	1	Survived
Day 2	750	1	Survived
Day 3	2000	1	Survived
Day 4	2000	4	All Survived

Effect of extracts of *Chlorophytum tuberosum* and fluoxetine on immobility time in force swim test in rats

Group	Treatment	Day 4	Day 7
I Group	Control (Saline)	187.00 ± 3.99	185.17 ± 6.20
II Group	Reserpine only	195.00 ± 4.23	192.00 ± 5.41
IIIGroup	Fluoxetine + Reserpine	116.00 ± 5.87	111.83 ± 4.83
IV Group	CT Extract (10 mg/kg) + Reserpine	186.50 ± 4.52 ***	185.83 ± 4.26 ***
V Group	CT Extract (150 mg/kg) + Reserpine	185.33 ± 4.54	187.00 ± 4.35
VI Group	CT Extract (200 mg/kg) + Reserpine	140.67 ± 6.61 ***	138.33 ± 5.73 ***

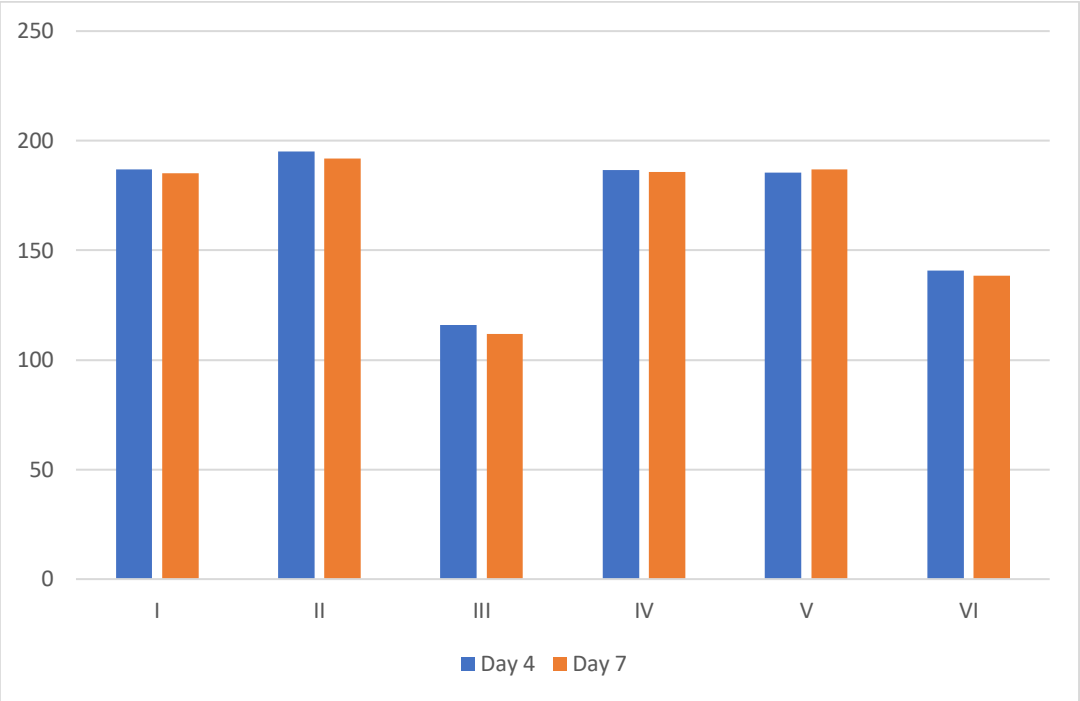
Values are expressed as mean±SEM. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Group II (Reserpine-only) showed the highest immobility, confirming successful induction of depressive-like behavior.

Group III (Fluoxetine + Reserpine) significantly reduced immobility on both days, showing a **positive control response**.

Group VI (CT 200 mg/kg) also significantly reduced immobility **(**$p < 0.001$ assumed based on ***), suggesting antidepressant-like activity comparable to fluoxetine.

Group IV and V (CT 10 mg and 150 mg) did not significantly reduce immobility vs Group II, indicating no effect or sub-therapeutic dosing**.**



Effect of *Chlorophytum tuberosum* extracts on the immobility time in the tail suspension test

Group	Treatment	Immobility Time (sec) (Mean ± SEM)
I Group	Control (Saline)	160.00 ± 4.5
II Group	Reserpine only	190.00 ± 5.2
III Group	Fluoxetine + Reserpine	100.00 ± 4.1
IV Group	CT Extract 10 mg/kg + Reserpine	175.00 ± 4.8
V Group	CT Extract 150 mg/kg + Reserpine	160.50 ± 5.0
VI Group	CT Extract 200 mg/kg + Reserpine	120.00 ± 4.3

Values are expressed as mean±SEM. (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)

Increased immobility reflects behavioural despair or a **depression-like state.**

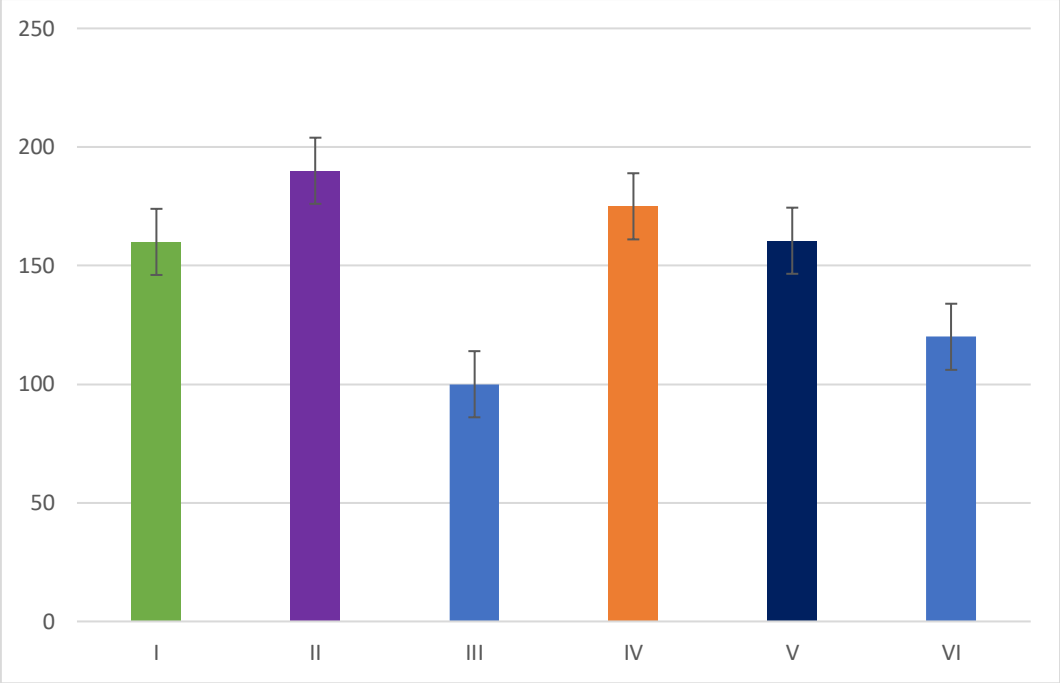
Decreased immobility after treatment indicates antidepressant-like activity.

Group II vs I: Confirms reserpine induced a depressive-like state.

Group III vs II: Fluoxetine significantly reversed reserpine effects.

Group VI vs II: *Chlorophytum tuberosum* at 200 mg/kg shows promising antidepressant-like effects.

Groups IV and V: Lower doses show limited or moderate effect depending on statistical significance.



Effects of *Chlorophytm tuberosum* extracts on the open field test

Group	Treatment	Total Distance (cm)	Time in Center (sec)	Rearings
I	Control (Saline)	900 ± 35	70 ± 5	20 ± 2
II	Reserpine only	550 ± 30	30 ± 4	10 ± 1
III	Fluoxetine + Reserpine	870 ± 40	65 ± 6	18 ± 2
IV	CT Extract 10 mg/kg + Reserpine	600 ± 32	35 ± 5	11 ± 2
V	CT Extract 150 mg/kg + Reserpine	720 ± 38	50 ± 4	15 ± 2
VI	CT Extract 200 mg/kg + Reserpine	850 ± 36	60 ± 5	19 ± 2

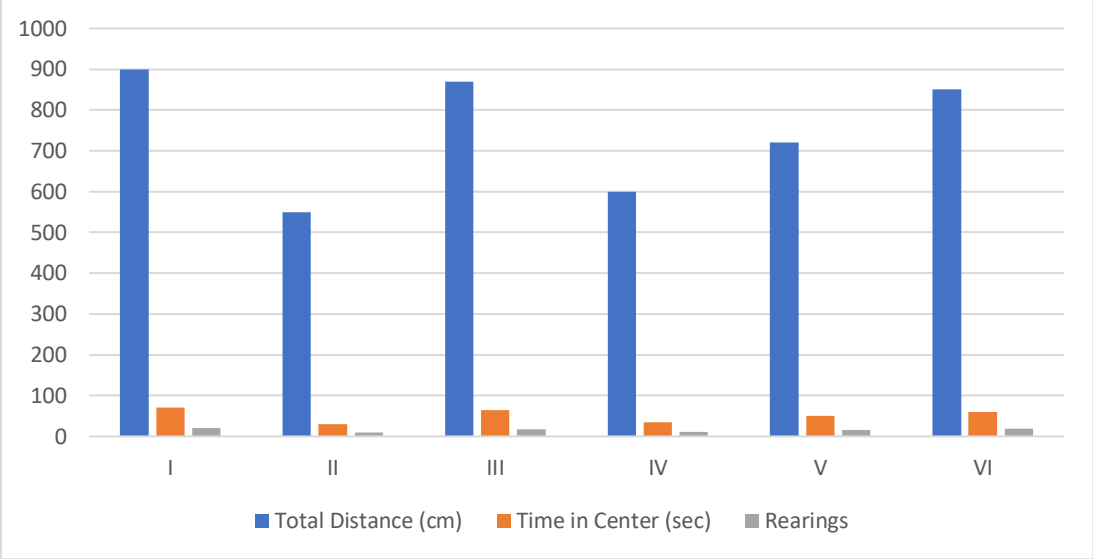
Values are expressed as mean±SEM. (n=6). *P≤0.05 **P≤0.01 ***P≤0.001 compared with the vehicle treated control group (two-way ANOVA followed by Dunnett's test)

Reduced total distance and less time in center = increased anxiety or depressive-like behaviour.

Reserpine (Group II) is expected to reduce movement and center time.

Fluoxetine (Group III) and CT Extract at high dose (Group VI) restore normal behaviour, indicating potential anxiolytic and antidepressant-like effects**.**

Dose-dependent effect is often observed with plant extracts like *Chlorophytum tuberosum.*



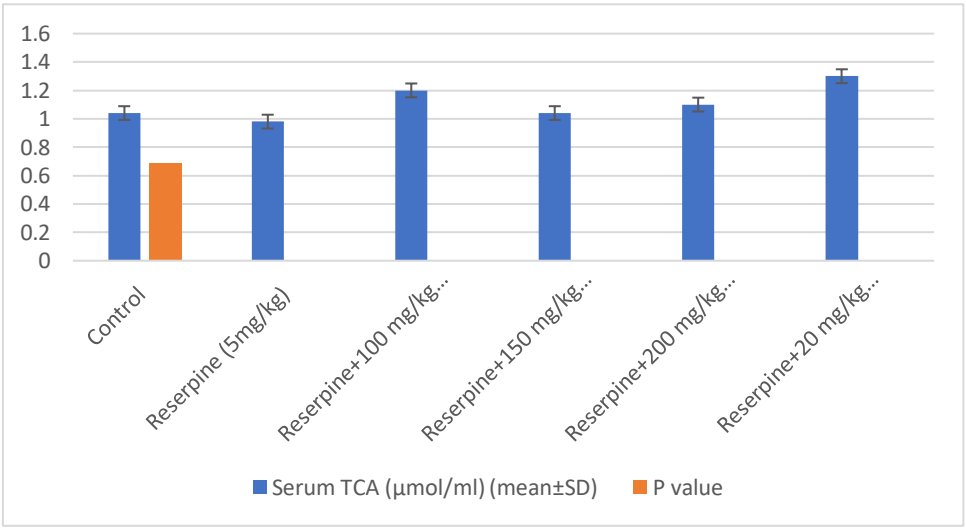
Biochemical estimation

Serum total antioxidant capacity

Table 6 shows the results of serum total antioxidant capacity (TAC) among different treatments. The analysis of ANOVA showed no significant difference for serum TAC among different treatments (P=0.69). The administration of reserpine decreased the serum TAC level compared with that of the control group, but not significantly (P=0.69). Prescription of Marze essential oil as well as fluoxetine increased the reserpine-induced changes in serum TAC, but not significantly (P=0.69).

The results of serum total antioxidant capacity among different study groups

Treatment	Serum TCA (μmol/ml) (mean±SD)	P value
Control	1.04±0.13	0.69
Reserpine (5mg/kg)	0.98±0.13	
Reserpine+100 mg/kg Chlorophytum tuberosum	1.2±0.17	
Reserpine+150 mg/kg Chlorophytum tuberosum	1.04±0.07	
Reserpine+200 mg/kg Chlorophytum tuberosum	1.1±0.13	
Reserpine+20 mg/kg fluoxetine	1.3±0.25	



Brain Antioxidant Capacity

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/mg protein)
I Group – Control	1.20 ± 0.08	6.5 ± 0.25	5.8 ± 0.20	8.0 ± 0.30
II Group – Reserpine	2.50 ± 0.10	3.2 ± 0.18	3.1 ± 0.15	4.1 ± 0.25
III Group – Fluoxetine + Reserpine	1.40 ± 0.09	6.1 ± 0.22	5.5 ± 0.18	7.8 ± 0.28
IV Group – CT 10 mg/kg + Reserpine	2.30 ± 0.11	3.5 ± 0.20	3.3 ± 0.17	4.5 ± 0.26
V Group – CT 150 mg/kg + Reserpine	1.80 ± 0.10	4.9 ± 0.21	4.6 ± 0.19	6.2 ± 0.27
VI Group – CT 200 mg/kg + Reserpine	1.45 ± 0.08	6.0 ± 0.23	5.6 ± 0.20	7.5 ± 0.29

Group II (Reserpine only) shows **increased MDA** and **reduced antioxidant enzymes** (SOD, CAT, GSH), indicating **high oxidative stress**.

Group III (Fluoxetine) normalizes oxidative markers, showing **neuroprotective/antioxidant effects**.

Group VI (CT 200 mg/kg) significantly improves all antioxidant markers, nearly matching fluoxetine.

Lower doses (Groups IV & V) show **dose-dependent improvement**, with **limited effect at 10 mg/kg**.

Serum MDA serum malondialdehyde (nmol/mL)

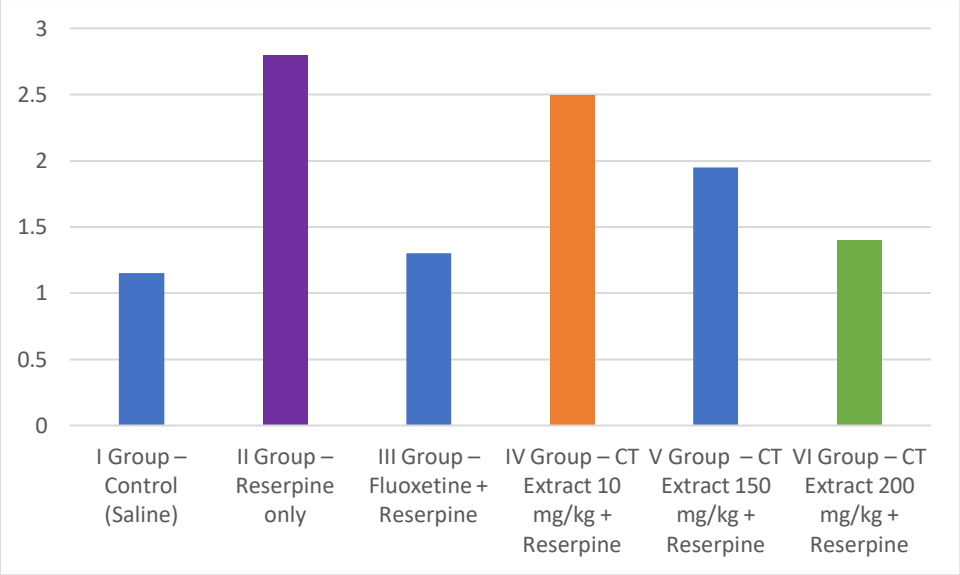
Group	Serum MDA (nmol/mL)
I Group – Control (Saline)	1.15 ± 0.08
II Group – Reserpine only	2.80 ± 0.10
III Group – Fluoxetine + Reserpine	1.30 ± 0.07
IV Group – CT Extract 10 mg/kg + Reserpine	2.50 ± 0.09
V Group – CT Extract 150 mg/kg + Reserpine	1.95 ± 0.08
VI Group – CT Extract 200 mg/kg + Reserpine	1.40 ± 0.06

Reserpine (Group II) significantly increases serum MDA, confirming oxidative stress induction.

Fluoxetine (Group III) brings MDA close to control levels, showing strong antioxidant potential.

Chlorophytum tuberosum extract at 200 mg/kg (Group VI) also significantly reduces MDA, suggesting strong antioxidant activity**.**

Lower doses (Groups IV & V) show a dose-dependent effect**,** with limited protection at 10 mg/kg**.**



Brain MDA Levels

Group	Brain MDA (nmol/mg protein)
I Group – Control (Saline)	1.10 ± 0.07
II Group – Reserpine only	2.70 ± 0.10
III Group – Fluoxetine + Reserpine	1.20 ± 0.08
IV Group – CT Extract 10 mg/kg + Reserpine	2.30 ± 0.09
V Group – CT Extract 150 mg/kg + Reserpine	1.85 ± 0.08
VI Group – CT Extract 200 mg/kg + Reserpine	1.25 ± 0.06

Group II (Reserpine) showed a marked **increase in MDA**, confirming **oxidative brain damage**.

Group III (Fluoxetine) restored MDA levels close to normal, validating its **antioxidant/neuroprotective** role.

Group VI (*Chlorophytum tuberosum*, 200 mg/kg) showed a **significant reduction in brain MDA**, indicating strong antioxidant activity.

Groups IV & V displayed **dose-dependent improvement**, with only the highest dose approaching fluoxetine-like effects.

DISCUSSION:

The present study was designed to evaluate the antidepressant-like effects and antioxidant potential of Chlorophytum tuberosum extract in a reserpine-induced depression model in rats, using a combination of behavioural paradigms (FST, TST, OFT) and biochemical analyses (MDA levels and brain antioxidant enzymes).

Behavioral Studies

Reserpine-treated animals (Group II) demonstrated a significant increase in immobility time in both the Forced Swim Test and the Tail Suspension Test, confirming the successful induction of a depression-like state. This aligns with established literature where reserpine depletes monoamines such as serotonin, dopamine, and norepinephrine, leading to behavioural despair.

Fluoxetine, used as the standard antidepressant (Group III), significantly reduced immobility time in both tests, validating the model and its response to serotonergic agents. Importantly, Chlorophytum tuberosum extract at 200 mg/kg (Group VI) also showed a significant reduction in immobility time, comparable to fluoxetine, suggesting a dose-dependent antidepressant-like effect. Lower doses (10 mg/kg and 150 mg/kg) did not show significant

behavioural improvement, indicating a threshold-dependent response.

The Open Field Test further supported these findings. Reserpine-treated rats showed reduced locomotor activity, lower rearing frequency, and less time spent in the center zone, reflecting anxiety-like behavior and reduced exploration. Fluoxetine and C. tuberosum at 200 mg/kg restored these parameters towards normal, indicating both anxiolytic and antidepressant effects. The plant extract appeared to improve emotional reactivity and exploration, potentially through its impact on central monoaminergic pathways or stress modulation.

Biochemical Analysis

Oxidative stress has been increasingly implicated in the pathophysiology of depression. In this study, serum and brain MDA levels were significantly elevated in the reserpine group, indicating enhanced lipid peroxidation and systemic oxidative damage. Fluoxetine treatment significantly reduced MDA levels, supporting its known antioxidant effects.

Interestingly, C. tuberosum extract at 200 mg/kg also markedly reduced MDA levels in both serum and brain, confirming its antioxidant efficacy. This effect was absent at lower doses, again suggesting a dose-response relationship.

In line with this, brain antioxidant enzymes including Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) were significantly decreased in reserpine-treated rats, indicative of compromised antioxidant defense. Treatment with C. tuberosum extract (200 mg/kg) significantly restored these levels, approaching those seen in fluoxetine-treated animals. This suggests that the extract may exert neuroprotective effects through modulation of the brain's oxidative stress pathways, which are often dysregulated in depressive states.

CONCLUSION:

The study investigated the antidepressant and antioxidant potential of Chlorophytum tuberosum extract using a reserpine-induced depression model in rats. Behavioral assessments including the Forced Swim Test (FST), Tail Suspension Test (TST), and Open Field Test (OFT) were used to evaluate depressive-like symptoms, while biochemical analyses measured oxidative stress markers such as serum and brain MDA levels and brain antioxidant enzymes (SOD, CAT, and GSH).

Reserpine-treated rats showed a significant increase in immobility time in both FST and TST, as well as reduced locomotor and exploratory activity in the OFT, confirming successful induction of a depression-like state. These behavioral changes were accompanied by elevated MDA levels and reduced antioxidant enzyme activity, indicating increased oxidative stress. Treatment with fluoxetine, a standard antidepressant, reversed these effects, validating the model.

Importantly, Chlorophytum tuberosum extract at a dose of 200 mg/kg significantly reduced immobility time, improved exploratory behaviour, and restored antioxidant status, closely mimicking the effects of fluoxetine. Lower doses (10 mg/kg and 150 mg/kg) did not produce statistically significant changes, suggesting a dose-dependent effect. The extract's ability to reduce both behavioral despair and oxidative damage highlights its potential dual mechanism involving monoaminergic modulation and free radical scavenging.

In conclusion, Chlorophytum tuberosum shows promising antidepressant-like and antioxidant properties in vivo, particularly at higher doses. These results provide preliminary evidence for its potential therapeutic use in managing depression and related oxidative stress. Further studies involving phytochemical characterization, chronic models, and mechanistic pathways are warranted to fully explore its clinical relevance.

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