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Evaluation of the Cytotoxic Effect and Antioxidant Potential of *Carpolobia lutea* Leaf Extract in a Reserpine-Induced Mouse Model of Depression

Agboola, J. B.[1]*; Ogunruku, O. O.[1]; Bolaji, A. O.[2]; Ogunsanya, S. T.[3]; Oke, J. O.[4]; Joshua, A. R.[1]; Ajayi, O. S.[5]

[1] Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria
[2] Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria
[3] Department of Anatomy, Benjamin S. Carson (Snr) College of Health and Medical Sciences, Babcock University, Ikenne, Ogun State, Nigeria
[4] Department of Cell Biology and Anatomy, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria
[5] Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

*Correspondence: Email: busayoagboola387@gmail.com; Tel.: +2347063129994

Abstract

Carpolobia lutea is traditionally used for various CNS disorders, but its multitarget potential in depression-associated genomic and oxidative damage remains poorly understood. This study investigated the antioxidant and cytotoxic effects of *Carpolobia lutea* (100, 200, and 400 mg/kg) in a reserpine-induced mouse model of depression. Animals (49 mice) were divided into two groups: a basal control group and a reserpine-treated group (0.2 mg/kg for 15 days). On day 16, they were split into seven groups to evaluate the effect of the ethanolic extract of *Carpolobia lutea* (CL-E) on oxidative stress. In a separate study, the cytotoxic safety of the extract was evaluated using the *Allium cepa* assay. This study demonstrated that the reserpine-induced group exhibited a marked elevation in intracellular concentrations of superoxide anion ($O_2^{\cdot -}$) and nitric oxide (NO) ($P < 0.05$) compared to the control group. However, treatment with CL-E (100, 200, and 400 mg/kg) significantly lowered these levels, bringing $O_2^{\cdot -}$ and NO concentrations to near-baseline levels. In contrast, the levels of intracellular peroxynitrite/hydroxyl radicals ($ONOO^-/\cdot OH$) did not change between the experimental groups ($P > 0.05$) when compared to the basal control. This study also showed elevated lipid peroxidation, followed by decreased brain glutathione content ($P < 0.05$) in reserpine-induced group when compared with the control. This process was attenuated after treatment with CL-E at 100 – 400 mg/kg, comparable to moclobemide and reserpine-induced groups ($P < 0.05$). The cytological analysis further confirmed that CL-E at low concentrations below 0.6 mg/ml did not affect the mitotic index of *Allium cepa* roots, but at higher concentrations has the potential to induce cell proliferation by increasing the mitotic index significantly, though with no chromosomal aberrations in the various stages of cell division. In conclusion, CL-E demonstrates significant antioxidant properties by mitigating reserpine-induced oxidative stress, while the reduction in mitotic index suggests potential cytotoxic activity at higher concentrations.

Keywords: *Carpolobia lutea*, reserpine-induced depression, antidepressant-like effect, antioxidant activity, chromosomal aberrations

INTRODUCTION

Depressive disorders are commonly described as a silent epidemic and a debilitating psychiatric disorder that affects 5% of the global population (Zebley and Cristan, 2024). The condition is marked by a range of symptoms, such as suicidal tendencies, sadness, lack of motivation, struggles with sleep and cognitive deficits (Lievanos-Ruiz and Fenton-Navarro, 2024). There are currently few treatment options for this disorder; synthetic antidepressants, mainly Selective Serotonin Reuptake Inhibitors (SSRIs), are the most commonly prescribed medications, though inhibitors of monoamine oxidase activity are also used (de Oliveira *et al*., 2019; Samad *et al*., 2021; Shafiee *et al*., 2025). Most of these drugs come with undesirable side effects; thus, there is a need for further investigation in the ongoing global quest for safe and effective antidepressants.

It has been hypothesised that monoamine oxidase (MAO) plays a significant role in the pathogenesis of depressive disorder (de Oliveira *et al*., 2019; Suchting *et al*., 2021; Duarte *et al*., 2020). This enzyme's activity produces reactive oxygen species (ROS) (de Oliveira *et al*., 2019), which exacerbate neurological damage (Hritcu *et al*., 2017; Czarny *et al*., 2018). For example, H_2O_2 and superoxide ($O_2^{\cdot -}$) are generated during the oxidative degradation of monoamine neurotransmitters catalyzed by MAO. These reactive species can diffuse across the mitochondrial membrane and interact with Fe^{2+}, leading to the formation of highly reactive hydroxyl radicals ($^{\cdot}OH$) via the Fenton reaction (Hritcu *et al*., 2017; Czarny *et al*., 2018). This leads to lipid peroxidation, protein degradation, fatty acids oxidation and DNA damage in the substantia nigra, indicating the role of oxidative damage in depression (Ege and Şelimen, 2021; Ji *et al*., 2023). Thus, reducing oxidative stress might serve as a useful target in managing depression. Therefore, we assessed the beneficial effects of *Carpolobia lutea* in mitigating oxidative stress dysfunction in reserpine-induced depression.

Recent years have witnessed the academic interest in the use of plant-based therapies as a potential treatment for depression and various behavioural disorders (Hritcu *et al*., 2017; Ige and Şelimen, 2021; Agboola *et al*., 2024). *Carpolobia lute*a, commonly referred to as *C. lutea*, is a well-known medicinal herb in Nigeria, whose infusions are used to treat mental disorders and headaches (Olayinka *et al*., 2019; Nwidu *et al*., 2012). The previously reported pharmacological properties of this plant include antidiarrhoeal, antibacterial and antiparasitic activities (Nwidu *et al*., 2012). Traditional uses of *C. lutea* include chewing or drinking the plant concoctions to relieve tension and desolation (Olayinka *et al*., 2019; Nwidu *et al*., 2014). The herb was used as a mood enhancer in ancient times. Although *C. lutea* has been widely used in Nigeria for CNS-related illnesses, a current mechanistic evaluation of its actions remains limited (Nwidu *et al*., 2012). The results of the High-Performance Liquid Chromatography-Ultraviolet fingerprint analysis revealed multiple variations of polyphenols and flavonoids, along with the detection of saponins and glycosides (Nwidu *et al*., 2014). In contrast, the majority of compounds identified through the gas chromatographic technique appeared to be fatty acids, esters, and alcohols (Rhuada *et al*., 2019).

Despite research elucidating the therapeutic benefits of *C. lutea* (Omeiza *et al*., 2022; Abiodun and Oshinloye, 2017), there is a dearth of information on its antidepressant mechanism, as well as its ability to provide neuroprotection at the cellular level. Additionally, the safety and neuroprotective efficacy of the extract necessitate further study to substantiate its therapeutic application. The study evaluates the antioxidant potential of *C. lutea* extract (CL-E) in a reserpine-induced mouse model and its cytotoxic safety using the standard *Allium cepa* assay, contributing to the search for safe and effective neuroprotective agents.

To evaluate the antidepressant potential of *C. lutea,* we used a reserpine-induced model. Moclobemide was selected as the standard reference drug because it is a reversible inhibitor of monoamine oxidase A (RIMA) with a well-documented pharmacological profile and the ability to

elevate monoamine neurotransmitters (serotonin, norepinephrine, and dopamine) in the central nervous system (Albayrak *et al*., 2015; Adams, 2024).

METHODS

Preparation of the Plant Extracts

The fresh leaves of *Carpolobia lutea* G. Don (Polygalaceae) were collected from the botanical garden, Obafemi Awolowo University, Ile-Ife, Osun State and were verified at the botany department. The leaves were subsequently air-dried and ground into fine powder using a laboratory mill grinder from the Pharmacy Faculty, Obafemi Awolowo University. Afterwards, the grounded powder was soaked in 80% ethanol for 72 h with continuous stirring and shaking and thereafter filtered. The filtrate underwent concentration using a rotary evaporator. The concentrated paste was kept and refrigerated at a temperature range of 4 - 8 degrees Celsius until it was needed for subsequent bio-guided assays.

Experimental Animals

Male mice (n = 49) weighing approximately 20 - 24 g were acquired from the Pharmacy Faculty animal house at Obafemi Awolowo University in Osun State, Nigeria. Before the study, all the animals were kept at room temperature for two weeks. All animals were supplied with a standard chow diet and had unlimited access to fresh water. Ethical clearance for this study was granted by the Health Research and Ethics Committee of the Institute of Public Health, Obafemi Awolowo University (HREC No.: IPH/OAU/12/2050). All experimental procedures were carried out in full compliance with international guidelines for the care and use of laboratory animals.

Experimental Protocol

The mice were distributed at random into seven groups (n = 7 per group). The study was conducted in two distinct phases:

Induction Phase (Days 1–15): The initial phase of this experiment consisted of two groups: the control group, which had free access to the vehicles until the end of the experiment and the reserpine-induced groups (injected with 0.2 mg/kg of reserpine for 15 days to establish a depression model as described by Antkiewicz-Michaluk *et al*. (2014). To induce a depressive-like state, a higher dose of 0.2 mg/kg reserpine was given during the induction phase. This dosage has been shown to effectively induce neurochemical and behavioural deficits in rodent models of depression (Antkiewicz-Michaluk *et al*., 2014).

Treatment Phase (Days 16–30): At the second phase of the experiment, which was on day 16, the reserpine-induced animals were further split into seven groups:

Group 1 (Basal Control): vehicle only (n = 7)
Group 2 (Reserpine Control): reserpine + vehicle (n = 7)
Group 3 (Standard): reserpine + moclobemide (n = 7)
Group 4 (CL-E 100): reserpine + *C. lutea* 100 mg/kg (n = 7)
Group 5 (CL-E 200): reserpine + *C. lutea* 200 mg/kg (n = 7)
Group 6 (CL-E 400): reserpine + *C. lutea* 400 mg/kg (n = 7)
Group 7 (Extract Safety): *C. lutea* alone (n = 7)

Reserpine administration was reduced to 0.1 mg/kg in the second phase of the experiment to maintain a depressive state while ensuring animal survival throughout the treatment duration, in accordance with the established protocol by Antkiewicz-Michaluk *et al*. (2014).

Reserpine induced groups: (receiving 0.1 mg/kg of reserpine for another 15 days to maintain the animal's depression state; Standard group: receiving 0.1 mg/kg of reserpine and treated with 0.2 mg/kg of monobemide; Treated groups: received 0.1 mg/kg of reserpine followed by the treatment with 100, 200, and 400 mg/kg of *C. lutea* respectively for another 15 days, the last groups received just the extract alone from the initial control. The standard and the treated groups received 0.1

mg/kg of reserpine, followed by 2-h gaps, treatment with moclobemide and the extract, respectively, for 15 days.

C. lutea and moclobemide were dissolved in distilled water and administered via oral gavage using the already reported dosage from previous studies (Albayrak *et al*., 2015; Akinola *et al*., 2022). Reserpine was suspended in glacial acetic acid solution (1 µg/µl) and then made up to 25 ml with distilled water. *C. lutea* ethanol extract was diluted in deionised water and given orally at doses of 100, 200, and 400 mg/kg, according to the graded dosages derived from the LD50 results and previous investigations (Akinola *et al*., 2022; Omeiza *et al*., 2022).

At the end of the treatment period, the brain tissues were harvested. The biochemical analyses detailed in this manuscript used n = 5 per group. The residual brain tissues (n = 2 per group) were allocated for a separate immunohistochemistry study and are not included in this manuscript.

Cytotoxicity Assay on Allium cepa (A. cepa) root Meristematic Cells

The cytotoxicity assay was carried out following standard procedures (Bolaji *et al*., 2019). Equal-sized brown variety onion bulbs (*Allium cepa* L., 3n = 18) purchased from Oja-tuntun, Ile-Ife, Osun State, Nigeria, were sun-dried for 2 weeks, after which the outer scales and old roots were peeled off and the onion bulbs thoroughly rinsed. Thereafter, the bulbs were grown in distilled water for four days to allow new roots to sprout. They were then transferred into clean vials containing distilled water (negative control) and different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of *C. lutea* leaf extracts for 24 h.

The roots were excised at 9.00 a.m. and fixed immediately in 1:3 acetic acid: ethanol for 24 h. The fixed roots were hydrolysed with 18% hydrochloric acid for 10 min, squashed and stained with modified Orcein (Bolaji *et al*., 2014) for 20 min. The cytological examination involved calculation of the mitotic index (MI) per treatment, scoring of aberrant cells at anaphase and telophase and recording of micronuclei in interphase cells. The MI was calculated in percentage (%) as the number of dividing cells divided by the total number of cells, multiplied by 100. Photomicrographs of selected mitotic cells were documented with an AmScope MT microscope camera version 3.0.0.1 attached to an Olympus light microscope.

$$\text{Mitotic index (\%)} = \frac{\text{number of cells in mitosis}}{\text{total number of cells scored}} \times 100$$

Brain Homogenate Preparation

The animals were sacrificed by cervical dislocation. Before cervical dislocation, the animals were euthanised using a diluted solution of sodium pentobarbitone administered intraperitoneally. The brain was extracted, and the animals were confirmed dead in a humane manner before disposal. The brain was sectioned on ice and rinsed with ice-cold isotonic saline. The tissues were homogenised in 10 volumes of 0.1 M phosphate-buffered saline (pH 7.4). The homogenate was thereafter centrifuged at 4000 rpm for 15 mins, and the supernatant was collected and stored at -20 °C for subsequent biochemical assays.

A total of seven animals per group were used. Five brains per group (n = 5) were processed for biochemical analysis, while the remaining two brains (n = 2) were fixed for immunohistochemistry and histopathological studies.

Protein Concentration

Protein concentration was determined in the brain according to the method of Bradford (1976), using Bovine Serum Albumin (BSA) as the standard.

Quantification of Brain Cells, ROS and RNS Production

Cellular production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) was assessed via flow cytometry using a primary cell suspension derived from mice brain tissue, in accordance with Dias *et al*. (2014). The extracted brain tissue was homogenised in phosphate-buffered saline (PBS) and subsequently filtered through a 70 µm nylon cell strainer to remove

tissue debris, resulting in a single-cell suspension. The resulting cell suspension (1.1×10^6 cells/mL) underwent treatment with 260 µM dihydroethidium (DHE) for superoxide anion ($O_2 \cdot^-$) detection and 40 µM hydroxyphenyl fluorescein (HPF) for peroxide nitrite/hydroxyl radicals ($ONOO \cdot^-/\cdot OH^-$) detection at 37 °C for 40 min in a darkened environment. Following the incubation process, cells were subsequently washed and reconstituted in PBS and kept on ice and centrifuged at 300 g for 7 min to eliminate surplus and unbound probes. The cell pellets were subsequently resuspended in 600 µl of PBS solution and stored on ice for further analysis.

The study made use of a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA) with a 487 nm blue laser for data acquisition. DHE fluorescence was observed in the FL2 channel at 580/40 nm, while HPF fluorescence was observed in the FL1 channel at 523/30 nm. Approximately 10,000 events were collected for each sample. The gating principle involves the identification of cell populations by forward scatter (FSC-H) and side scatter (SSC-H) to exclude debris and cell doublets from the population. The viability was evaluated through the gating of the morphological integrity of the cells on the FSC/SSC (Forward Scatter vs. Side Scatter) plot. Single-stained control samples were employed for standard instrument compensation. The anions of superoxide and peroxide nitrite/hydroxyl radicals were examined solely by measuring alterations in median fluorescence intensity (MFI) using DHE and HPF probes, respectively. Data were analysed with BD Accuri C6 software, and the findings were presented as median fluorescence intensity (MFI a.u.) in arbitrary units.

Determination of Total Thiol Level

The total thiol level in the cerebral cortex was determined using the method of Ellman (1959). Aliquots of homogenate were mixed with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in phosphate buffer, and the absorbance of the yellow-coloured product was read at 412 nm. Total thiols were expressed as µmol/mg protein.

Determination of Reduced Glutathione

GSH level was determined using Moron *et al*. (1979). The method is based on the development of a yellow colour when DTNB is added to compounds containing sulfhydryl groups. Briefly, brain homogenates were deproteinized with sulfosalicylic acid, centrifuged, and the supernatant was reacted with DTNB in phosphate buffer. The absorbance of the resulting solution was measured at 412 nm.

Determination of Lipid Peroxidation

This study makes use of the method described by Ohkawa *et al*. (1979) to measure the level of lipid peroxidation. The reaction mixture contained 100 µl homogenate, 30 µl of 0.1 M Tris HCl buffer (pH 7.4), and was incubated at room temperature (25 ± 2 °C) for 1 h before adding 300 µl of 8.1 % sodium dodecyl sulphate, 500 µl acetic acid/HCl (pH 3.4), and 500 µl % TBA (Thiobarbituric acid). The reaction mixture was incubated at 100 °C for 1 h to promote colour development. At 532 nm, the absorbance of thiobarbituric acid reactive species (TBAR) produced was measured. The level of malondialdehyde (MDA) was then determined by calculating mol/mg protein (= 1056 x 105 $M^{-1}cm^{-1}$).

Determination of Nitric Oxide

Nitric oxide (NO) was measured spectrophotometrically as established by Moshage *et al*. (1995). The test involves the reaction of nitrate with Griess reagents, forming a purple-pink compound that was measured at 540 nm. Samples were first deproteinized, then incubated with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride, and the absorbance was read.

Statistical Analysis

All data were analysed with GraphPad Prism version 9.2 (GraphPad Software, San Diego, CA, USA). The data were first analysed using the Shapiro-Wilk test to ensure that the data were normally distributed. The statistical comparisons across all the groups were conducted using one-

way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test to identify the differences between the groups. Results are presented as mean ± standard error of the mean (SEM) with the sample size of n = 5. For all comparisons, *P* values < 0.05 were considered significant.

The cytological data obtained were subjected to General Linear Model (GLM) Analysis of Variance (ANOVA), and the significant means were subjected to Duncan Multiple Range Test at $P < 0.05$ using System Analysis Software (SAS) version 9.0.

RESULTS

Effect of C. lutea on the mitotic index and cytotoxic endpoints in the meristematic root cell of A. cepa

The cytological analysis revealed that the mitotic index of the meristematic *Allium cepa* root cells increased significantly as the concentration of the leaf extracts of *C. lutea* was increased to 0.6 mg/ml and above (Table 1). There was no significant increase in the mitotic index at lower concentrations (i.e. at 0.2 mg/ml and 0.4 mg/ml). Also, no chromosomal aberration was recorded at the various stages of cell division during the cytological investigation (Figure 1).

Table 1: Cytological effect of *Carpolobia lutea* leaf extracts on *Allium cepa* root tips.

Conc (mg/ml)	Interphase (mean ± S.E)	Prophase (mean ± S.E)	Metaphase (mean ± S.E)	Anaphase (mean ± S.E)	Telophase (mean ± S.E)	Total NO CELLS(mean ± S.E)	MIT INDEX (mean ± S.E)%
Control	85.66± 11.05^{C}	568.33 ± 65.84^{A}	9.33 ± 5.20^{A}	9.33 ± 1.20^{A}	5.66 ± 0.66^{A}	592.66 ± 61.18^{A}	4.33 ± 1.23^{BA}
0.2	54.33 ± 4.33^{C}	219.00 ± 19.00^{B}	9.00 ± 4.93^{A}	6.66 ± 3.33^{BA}	4.00 ± 2.00^{BA}	238.66 ± 24.12^{B}	7.78 ± 4.14^{BA}
0.4	60.00 ± 5.77^{C}	201.00 ± 16.46^{B}	4.00 ± 4.00^{A}	1.00 ± 1.00^{A}	0.00 ± 0.00^{C}	206.00 ± 21.00^{B}	2.04 ± 2.04^{B}
0.6	62.33 ± 12.33^{C}	141.33 ± 82.31^{B}	21.66 ± 13.69^{A}	0.33 ± 0.33^{A}	1.66 ± 1.66^{BC}	165.00 ± 97.43^{B}	10.24 ± 5.13^{A}
0.8	207.66 ± .22^{B}	224.33 ± 13.73^{B}	19.33 ± 3.28^{A}	2.33 ± 1.85^{BC}	2.66 ± 1.76^{BA}	248.66 ± 13.86^{B}	9.84 ± 0.57^{A}
1.0	246.33 ± 14.89^{A}	246.66 ± 4.91^{B}	22.00 ± 6.02^{A}	2.00 ± 0.57^{BC}	2.33 ± 0.88^{BA}	273.00 ± 4.93^{B}	9.58 ± 2.56^{A}

Note: Values are expressed as Mean ± S.E. (n = 3). Means within the same column with different superscript letters are significantly different at $p < 0.05$.

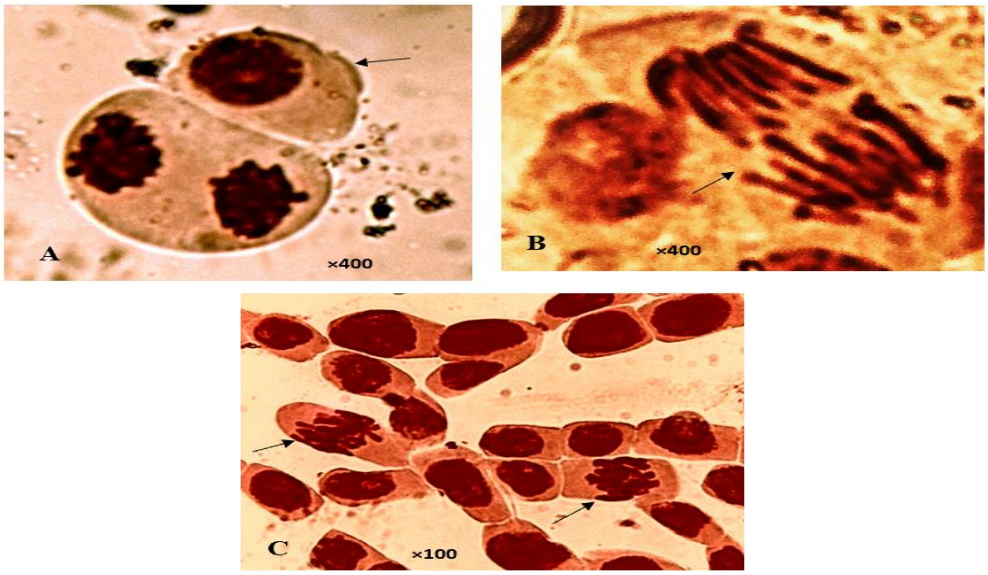

Figure 1: Mitotic cells of *Allium cepa* studied after treatment with *C. lutea* leaf extract. A: Interphase (arrowed) and telophase (below); B: Anaphase (arrowed); C: Prophase and metaphase (arrowed).

Effect of C. lutea on oxidative stress parameters (GSH, MDA, NO, ROS, RNS, total thiols)

The inner cell ROS species and Reactive nitrogen species (RNS) in the brain cells were quantified via a flow cytometer with DHE and HPF fluorescent probes, as depicted in Figure 2B and C. The reserpine-induced group showed a considerable increase in superoxide anion and NO concentrations (140 ± 30.1 DHE a.u. and 0.0056 ± 0.0001 µmol/mg protein, $P < 0.05$), relative to the untreated group (80 ± 10.1 DHE a.u. and 0.0023 ± 0.0002 µmol/mg protein, Figure 2). We did not observe any significant changes in peroxynitrite/hydroxy radical concentrations ($P > 0.05$) after treatment with *C. lutea* at all dosage levels. The generated NO and superoxide anion consistently decreased in the *C. lutea* treated groups (60 ± 20.1 DHE a.u. and 0.0026 ± 0.00019 µmol/mg protein), relative to the reserpine group (140 ± 30.1 DHE a.u. and 0.0056 ± 0.0001 µmol/mg protein, $P < 0.05$), respectively. On the contrary, the levels of $ONOO^-$ and ·OH determined by HPF remained unchanged across the groups ($P > 0.05$) (Figure 2A).

Furthermore (Figure 2D and E), reserpine significantly affected ($P < 0.05$) the oxidative stress parameters as seen in the elevated lipid peroxidation and decreased glutathione concentration levels compared to the corresponding control values. The upsurge was reversed after daily treatment with 100, 200 and 400 mg/kg of *C. lutea*; these were compared with the reserpine-induced group and standard group treated with moclobemide ($P < 0.05$).

The results obtained for Total Thiol levels (Figure 2F) indicated that reserpine administration resulted in a slight drop in thiol content (0.0019 ± 0.00031 µmol/mg protein) relative to the control group (0.0025 ± 0.0007 µmol/mg protein), but this difference is not statistically significant ($P > 0.05$). Treatment with *C. lutea* at higher concentrations (200 and 400 mg/kg) markedly elevated Total Thiol levels (0.0037 ± 0.0002 and 0.0048 ± 0.0001 µmol/mg protein, respectively; $P < 0.05$) in comparison to the reserpine-only group (0.0019 ± 0.00031 µmol/mg protein). Also, the group administered with *C. lutea* (400 mg/kg) only demonstrated the highest thiol levels (0.0059 ± 0.0004 µmol/mg protein), indicating a strong intrinsic antioxidant-boosting power of the extract.

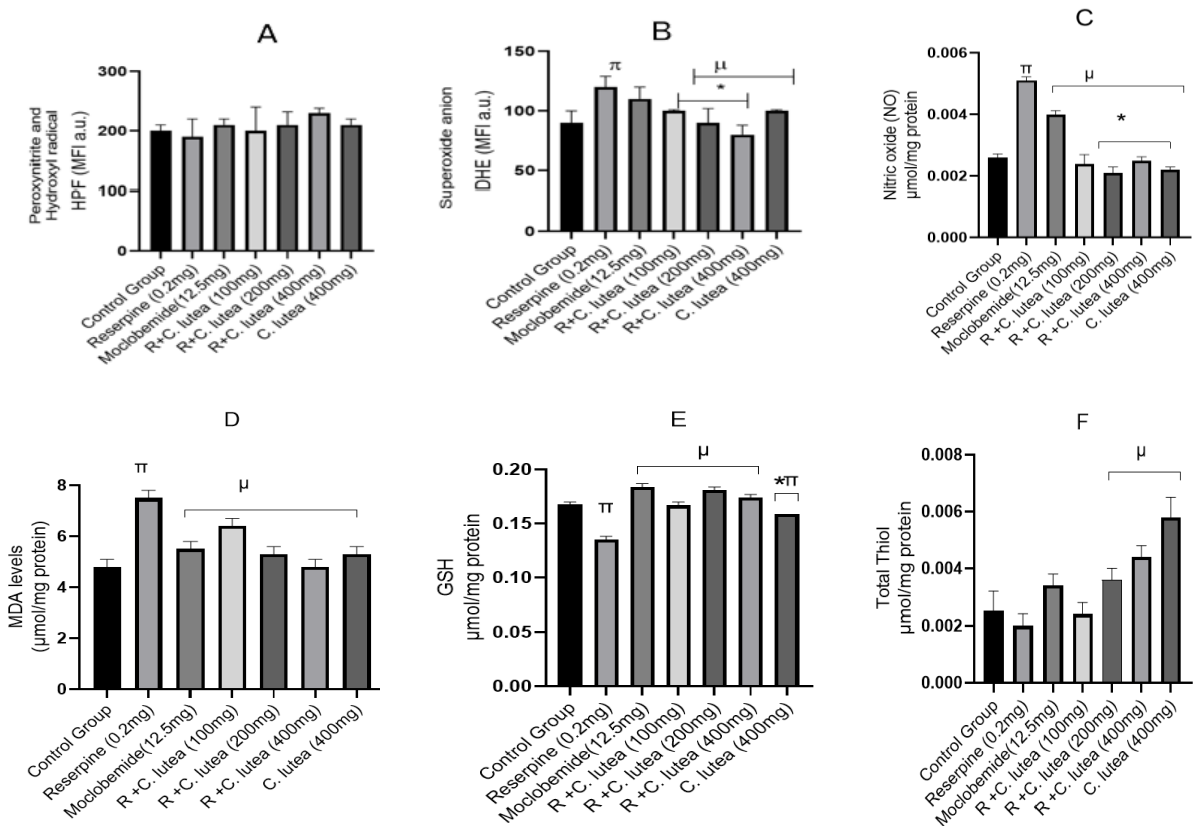

Figure 2: Effect of *C. lutea* on oxidative stress biomarkers. The observed oxidative indicators in the mouse model of depression include **A**: Hydroxyl/peroxynitrite radical; **B**: Superoxide anion; **C**: Nitric oxide; **D**: Lipid peroxidation; **E**: Glutathione, and **F**: total thiol protein. Data are expressed as mean ± SEM (n = 5). Statistical significance ($P < 0.05$) is indicated as follows: π vs. Control; µ vs. Reserpine-induced group; * vs. Standard drug group. Experimental Groups: (1) Control; (2) Reserpine (0.2 mg/kg); (3) Standard drug (12.5 mg/kg) + Reserpine; (4 - 6) *C. lutea* (100, 200, 400 mg/kg, respectively) + Reserpine; (7) *C. lutea* (400 mg/kg) alone.

DISCUSSION

The present study investigated the antioxidant and cytotoxic effects of *C. lutea* in a reserpine-induced mouse model of depression. The current results indicate that reserpine administration considerably heightened the intracellular oxidative and nitrosative stress in neuronal cells; this is evidenced by an increase in brain superoxide anion, decreased reduced GSH levels, and elevated MDA and NO contents. This pattern aligns with reported studies on reserpine that established that reserpine could disturb monoaminergic homeostasis and induce redox imbalance in the brain, leading to excessive formation of reactive oxygen and nitrogen species and compromising the antioxidant defence system, which leads to neuronal dysfunction seen in depressive-like mental disorders (Hritcu *et al*., 2017; Czarny *et al*., 2018). Additionally, oxidative stress might be the cause of the neurodegeneration seen in depression and could account for the hippocampal reduction in volume (Kuwar et a., 2016). Moreover, elevated inducible nitric oxide synthase (iNOS), which has been previously shown in rats with depression (Samad *et al*., 2021), may explain the increase in NO observed in this study.

The reserpine-induced increases in superoxide, NO and MDA were consistently reduced after treatment with *C. lutea*, indicating that the extract presumably improves radical scavenging or decreases upstream radical formation. This result is in line with earlier findings, which showed that the in vivo administration of *C. lutea* led to an increase in antioxidant activity (Abiodun and Oshinloye, 2017). In addition, Nwidu *et al*. (2014) and Akinola *et al*. (2022) previously

demonstrated that the plant could improve mobility in animal models of depression by altering the levels of oxidative stress markers. The antioxidant properties present in these plants may be contributing favourably to the increase of these activities. The protective benefits of *C. lutea* noted on oxidative stress may be ascribed to the presence of flavonoids and polyphenols in the extract that alleviate oxidative damage and regulate monoaminergic pathways to restore ant-oxidant defence system (Hritcu *et al*., 2017; Abiodun and Oshinloye, 2017; Akinola *et al*., 2022). Collectively, these findings suggest that CL-E demonstrates significant antioxidant properties in vivo and may safeguard brain tissue from redox-related damage linked to reserpine exposure. The restoration of glutathione indicates the improvement in intracellular antioxidant activity and a stronger response to oxidative stress, given its crucial role in peroxide detoxification and cellular redox buffering.

Although reserpine markedly increasing intracellular $O_2\cdot^-$ and NO in our study, the levels of $ONOO^-$ and ·OH determined by HPF remained unchanged across the groups. Several factors could account for this disparity. $O_2\cdot^-$ and NO radicals are highly reactive and ephemeral; hence, their concentrations may be low and unstable, complicating their detection by fluorescence probes using HPF (Khelfi, 2024; Wu *et al*., 2026). Furthermore, the formation of $ONOO^-$ largely relies on the presence of $O_2\cdot^-$ and NO (Han *et al*., 2025). compartmentalized production or rapid local detoxification in subcellular microdomains could limit bulk-suspension detection (Han *et al*., 2025). Furthermore, natural antioxidant defences, such as glutathione and enzymatic scavengers, can rapidly neutralise $ONOO^-$ and ·OH, thus preventing the accumulation of $ONOO^-$ and ·OH to be detectable by fluorescence probes even when their precursor radicals are highly present (Apak *et al*., 2022). Treatment with CL E consistently reinstated brain GSH and mitigated lipid peroxidation, indicating effective antioxidant buffering that may have mitigated downstream $ONOO^-$ and ·OH buildup. The degree of sensitivity and specificity of the probe must be but into consideration: HPF were able to detect both $ONOO^-$ and ·OH, however, it may be insufficiently sensitive to resolve minor or highly localised changes (Khelfi, 2024; Wu *et al*., 2026). Further studies using alternative approaches such as Electron paramagnetic resonance (EPR), spin trapping, 3-Nitrotyrosine (3-NT) immunodetection, selective scavenger inhibitor experiments or targeted subcellular assays could help in elucidate $ONOO^-$ and ·OH transient and formation and its functional significance in this model (Petre, 2026).

The *A. cepa* meristematic root cell assay has been used for testing cytotoxicity and/or genotoxicity of various substances in many parts of the world (Tedesco and Laughinghouse, 2012). It has been considered by many researchers (Bolaji *et al*., 2019; Camilo-Cotrim *et al*., 2022; Giri *et al*., 2024; Duta-Cornescu *et al.*, 2025) to be one of the most efficient and cost-effective approaches to determine the toxic potential of plant extracts and other substances in the environment. It has also been noted to have high sensitivity and good correlation with the results of mammalian test systems (Herrero *et al.*, 2012; Camilo-Cotrim *et al*., 2022).

The cytological evaluation of this study provides an insight into the safety profile and anti-proliferative ability of *Carpolobia lutea*. The findings indicated an insignificant change in the mitotic index of the meristematic root cells after treatment with the extracts at lower concentrations from 0.4 mg/ml and below; while at higher concentrations (from 0.6 mg/ml and above) there is possibility of the extract to induce cell proliferation due to significant increase in the mitotic index of the cells. The significant increase in mitotic index noted in this study at higher concentrations of the leaf extract of *C. lutea* imply that the leaf extracts of *C. lutea* have the ability to induce cell growth or cell division in the meristematic tissues at high concentrations but are safer at lower concentrations, since they have no significant efect on the cell growth at lower concentrations. It is also worthy of note that no mutagenic effect was observed across the concentrations investigated, although higher concentrations could be further investigated. Also, the ability of *C.*

lutea to induce cell division could be further explored by the pharmaceutical industries in sourcing for alternative medicine with ability to induce cell growth/division.

CONCLUSION

In conclusion, our investigation reveals that *C. lutea* concurrently alleviating reserpine-induced oxidative stress. The extract successfully reinstated the brain's redox equilibrium by alleviating superoxide and nitric oxide levels, lowering lipid peroxidation, and replenishing glutathione levels, thus safeguarding neuronal cells from oxidative damage. Furthermore, the cytological evaluation confirmed that *C. lutea* exhibits dose-dependent mitodepressive activity without inducing chromosomal abnormalities or mutagenic effects, highlighting its cellular safety, especially at low concentrations. These findings indicate that *C. lutea* is a promising candidate for managing oxidative and genomic aspects linked with depression.

ABBREVIATIONS

BSA, bovine serum albumin; CL-E, *Carpolobia lutea* ethanol extract; C. lutea, *Carpolobia lutea*; DHE, dihydroethidium; DNA, deoxyribonucleic acid; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid), GSH, reduced glutathione; HPF, hydroxyphenyl fluorescein; HPLC-UV, high-performance liquid chromatography–ultraviolet; iNOS, inducible nitric oxide synthase; MAO, monoamine oxidase; MDA, malondialdehyde; MFI, median fluorescence intensity; MI, mitotic NO, nitric oxide; PBS, phosphate-buffered saline; RNS, reactive nitrogen species; ROS, reactive oxygen species; SEM, standard error of the mean.

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