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Protective Effects of Chromolaena odorata on Oxidative Stress Biomarkers and Penile Histoarchitecture in Paroxetine-Induced Erectile Dysfunction

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ABSTRACT

Paroxetine is the most serotonergically active SSRI, and is linked to the most severe degree of sexual dysfunction, such as erectile dysfunction (ED) occurring in 30–70% of male patients. It works through inhibition of neuronal and endothelial nitric oxide synthase (nNOS/eNOS), inhibition of NO-cGMP signalling pathway, serotonin-dopamine antagonism, hormonal dysregulations and inducing oxidative stress. Chromolaena odorata (L.) The medicinal plant R.M. King & H. Robinson (Asteraceae) has been well documented with antioxidants, anti-inflammatory, vasorelaxant and cytoprotective properties due to its flavonoids and phenolic acids. The protective effects against paroxetine-induced penile oxidative stress and histoarchitectural injury have not been studied. The protective effect of ethanolic leaf extract of C. odorata on oxidative stress biomarkers malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and penile histoarchitecture was therefore evaluated in an ED model caused by paroxetine in adult male Wistar rats. Twenty rats (180–220 g) were randomly divided into four groups (n = 4), namely: Control (distilled water), Paroxetine only (10 mg/kg), Paroxetine + low-dose of C. odorata (100 mg/kg), and Paroxetine + high-dose of C. odorata (200 mg/kg). All the treatments were given for 28 days orally continuously. Paroxetine administration reduced SOD activity (0.0757 ± 0.0048 U/mg vs. 0.1011 ± 0.0106 U/mg in controls) and CAT activity (1.433 ± 0.0923 vs. 2.331 ± 0.4225 U/mg), and elevated MDA levels (0.7830 ± 0.0380 µM vs. 0.6483 ± 0.0041 µM). The co-administration of low doses of C. odorata significantly increased GSH (0.6343 ± 0.0657 mM) when compared to all the other groups ($p < 0.05$). The highest was observed in the low dose group (1.139 ± 0.2090 µM), potentially due to pro-oxidant activity of polyphenolic compounds with concentration. Cavernosal disorganisation, eosinophilic fibrosis, and compression of the sinusoidal spaces in penile tissue were the most significant histopathologic findings in penile tissue following paroxetine treatment, whereas neuropil vacuolation and pyknotic neuronal nuclei were the most significant neuropathologic findings in brain tissue. Both doses of C. odorata extract reduced these alterations, with the highest dose showing most complete structural restoration, in the form of well-defined deeply folded mucosal architecture, relaxed and organised smooth muscle stroma, and consolidated neuropil with good neuronal morphology. The present findings suggest a dose-dependent cytoprotective activity against paroxetine-induced penile and neural tissue injury of C. odorata which underscores its potential further investigation as a candidate herbal therapeutic agent for SSRI-associated erectile dysfunction, mainly at the histological level.

Keywords: *Chromolaena odorata, erectile dysfunction, oxidative stress, paroxetine, penile histoarchitecture, malondialdehyde, glutathione, superoxide dismutase, catalase, Wistar rats, SSRI, neuroprotection.*

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Introduction

Erectile dysfunction (ED) is the persistent failure to obtain and/or maintain an erection that is adequate for satisfactory sexual function (NIH Consensus Conference, 1993) and is a significant reproductive health problem in the world, estimated to affect 322 million men by 2025 (Ayta et al., 1999). ED in sub-Saharan Africa including Nigeria is increasingly being acknowledged

as an important public health issue with significant psychosocial implications; however, access to specialist urological health care is limited (Shamloul & Ghanem, 2013). The condition is aetiologically heterogeneous (vasculogenic, neurogenic, hormonal, psychogenic and drug-induced causes) with oxidative stress being a common pathophysiological process across all aetiologies and increasingly being recognized as a common mechanism (Agarwal et al., 2006).

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressant medications in the world, with more than 200 million prescriptions written annually (Edinoff et al., 2021). Sexual dysfunction is the most frequently reported side effect of SSRI treatment ranging from 25.8% to 80.3% of patients on different assessment measures (Serretti & Chiesa, 2009). Montejo et al. (2001) performed a large prospective, multicentre study, which showed that paroxetine had the highest incidence of sexual dysfunction (58.1%) compared to the other three SSRIs used, sertraline (42.6%), fluoxetine (37.4%) and citalopram (24.4%). This high incidence of sexual side effects is a significant factor in medication non-adherence with 50–70% of antidepressant users reporting non-adherence due to sexual dysfunction (Bet et al., 2013).

Multiple mechanisms exist by which paroxetine impairs erectile function: serotonin-dopamine antagonism leading to decreased dopaminergic tone in sexually relevant brain regions (Hull et al., 2004); inhibition of neuronal nitric oxide synthase (nNOS) activity, which decreases NO-cGMP smooth muscle relaxation in penile tissue (Finberg & Gillman, 2011); hormonal dysregulation manifested by elevated levels of prolactin and decreased levels of testosterone (Safarinejad, 2008); and induction of oxidative stress by auto-oxidation of serotonin, mitochondrial dysfunction, and depletion of antioxidant enzymes in penile tissue (Bilici et al., 2001). In animal studies, chronic paroxetine treatment leads to an increase in MDA level, while it decreases SOD, CAT, and GSH activities in penile tissue and induces smooth muscle atrophy and collagen deposition (Ateş et al., 2021; Öztürk et al., 2020).

There are limited current pharmacological treatments for paroxetine-induced ed. the first-line therapy is the phosphodiesterase type-5 (pde5) inhibitors, which are contraindicated in the use of nitrates and have systemic side effects and high costs – which significantly reduces the accessibility in low and middle-income settings (lue, 2000). thus, there is a pressing need to develop safe, effective and readily available therapeutic options, especially from the rich african pharmacopoeia.

Chromolaena odorata (l.) r.m. king & h. robinson (asteraceae), also referred as siam weed, is a pantropical medicinal shrub that is widely used in the traditional medicine of west africa for wound healing, anti-inflammatory conditions and in male sexual dysfunctions, with the leaves decocted and consumed as medicine to improve erectile function and libido (owolabi et al., 2007). it has several phytochemicals that together provide strong antioxidant activity, such as quercetin, kaempferol, luteolin, chlorogenic acid and caffeic acid, which have direct free radical scavenging activity, activation of endogenous antioxidant enzymes (sod, cat, gpx, gsh), activation of nrf2/are pathway and inhibition of pro-inflammatory mediators (procházková et al., 2011; vital & afolayan, 2015). interestingly, owolabi et al. (2007) found that c. odorata aqueous extract produced a dose-dependent, endothelium-dependent vasorelaxation of rat aortic smooth muscle, which is directly relevant to the relaxation of the cavernosal smooth muscle of the penis during an erection.

Although this is a good mechanistic argument and ethnopharmacological evidence, there is no published study which investigated the protective effect of *C. odorata* against the oxidation stress and histoarchitectural changes caused by paroxetine in penile tissues. thus, the present study was designed to fill this critical gap by examining the effects of ethanolic leaf extract of *C. odorata* on the key oxidative stress biomarkers and penile and brain histoarchitecture in a validated model of erectile dysfunction developed by using paroxetine in rats.

2. Materials And Methods

2.1 Ethical Approval and Animal Management

The Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, gave the ethical approval for this study. Ethical approval for the study was obtained under Ethical Approval No. DRERC/ASN/ANA/2026/047. Twenty Wistar rats weighing between 180–220 g were kept in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. Animals were kept in standardised conditions, room temperature (25 ± 2°C), 12 h light/dark photoperiod and wire-gauze cages with double-cross ventilation. Commercial pelletised grower feed (15% crude protein, 7% fat and 10% crude fibre) (Ogo-Oluwa Livestock and Aqua Feed Enterprises, Ilorin) was fed to rats, while they were given drinking water ad libitum. Animals were acclimatised for two weeks before the start of the experiment.

2.2 Collection, Identification and Preparation of Plant Extract

Chromolaena odorata fresh leaves were picked from an appropriate site in Nigeria and identified by a taxonomist expert in the Department of Botany. A voucher specimen was assigned a number and deposited in the departmental herbarium for future reference. Leaves were collected and washed several times with clean water and air-dried at room temperature. Leaves were dried and then finely ground in an electric blender. Maceration with ethanol for 72 hours with shaking was used for ethanolic extraction. The crude ethanolic extract was obtained by filtering the extracts on Whatman filter paper, concentrating with a rotary evaporator and drying. The extract was preserved at 4°C in an airtight container until it was used. The crude extract was dissolved in distilled water to obtain working concentrations of 100 mg/kg (low dose) and 200 mg/kg (high dose) for use in

treatment.

2.3 Experimental Design and Drug Administration

Animals were randomly assigned to four experimental groups (n = 4 per group) as follows:

Group A (Paroxetine only): Oral paroxetine 10 mg/kg body weight daily.

Group C (Low-dose treatment): Oral paroxetine 10 mg/kg + *C. odorata* extract 100 mg/kg daily.

Group D (High-dose treatment): Oral paroxetine 10 mg/kg + *C. odorata* extract 200 mg/kg daily.

Group B (Normal control): Distilled water only.

Note: A fifth group originally designated for paroxetine + sildenafil citrate (positive control) was excluded from analysis following an inconclusive pre-experimental phase; data from this group were not included. All treatments were administered orally by orogastric gavage for 28 consecutive days. Paroxetine tablets (20 mg) were dissolved in 10 mL distilled water to obtain a stock concentration of 2 mg/mL. Doses were calculated as mg/kg body weight and adjusted weekly based on individual body weight measurements using the formula: Volume (mL) = Dose (mg) ÷ Stock concentration (mg/mL).

2.4 Animal Sacrifice and Tissue Collection

At the end of the experimental period, animals were sacrificed by cervical dislocation. Penile, testicular, and brain tissues were carefully dissected, rinsed in normal saline to remove blood and tissue debris, and divided into portions for biochemical and histological analyses respectively.

2.5 Biochemical Assays

Tissue portions designated for biochemical analysis were homogenised in appropriate buffer solution and centrifuged to obtain clear tissue supernatants. The following parameters were assessed using validated spectrophotometric methods:

Superoxide Dismutase (SOD): Determined by measurement of enzyme-mediated inhibition of epinephrine autoxidation at 480 nm.

Catalase (CAT): Determined spectrophotometrically by measuring the rate of hydrogen peroxide decomposition at 240 nm.

Reduced Glutathione (GSH): Estimated using Ellman's reagent (DTNB) method based on formation of a yellow-coloured chromogen at 412 nm.

Malondialdehyde (MDA): Determined as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay at 532 nm. Results were expressed as mean ± standard error of mean (SEM).

2.6 Histological Analysis

Tissue portions fixed in 10% buffered formalin were processed through standard histological procedures: graded ethanol dehydration, xylene clearing, paraffin wax embedding, and sectioning at 5 µm using a rotary microtome. Sections were stained with haematoxylin and eosin (H&E) and examined under a light microscope for histomorphological evaluation of penile and brain tissue architecture.

2.7 Statistical Analysis

Data were expressed as mean ± SEM and analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results

Body Weight Parameters

Initial body weights were comparable across all experimental groups at baseline, confirming successful randomisation prior to treatment. The relative weight gain was highest in the control group (21.88 ± 1.74%) and lowest in the paroxetine-only group (2.18 ± 1.74%). No overt signs of systemic toxicity (mortality, piloerection, or significant weight loss) were observed across any experimental group throughout the 28-day treatment period (Table 1).

Table 1. Mean body weight and relative weight gain of experimental groups (Mean ± SEM; n = 4).

Group	IBW (g)	FBW (g)	RWG (%)
Control	207.00 ± 19.49	252.75 ± 25.62	21.88 ± 1.74
Paroxetine	265.67 ± 6.89	271.67 ± 11.29	2.18 ± 1.74
PXT + *C. odorata* LD	269.67 ± 15.06	282.33 ± 16.13	4.68 ± 0.24

PXT + *C. odorata* HD	213.00 ± 8.96	229.33 ± 13.78	7.52 ± 2.17

IBW: Initial body weight; FBW: Final body weight; RWG: Relative weight gain (%); PXT: Paroxetine; LD: Low dose (100 mg/kg); HD: High dose (200 mg/kg)

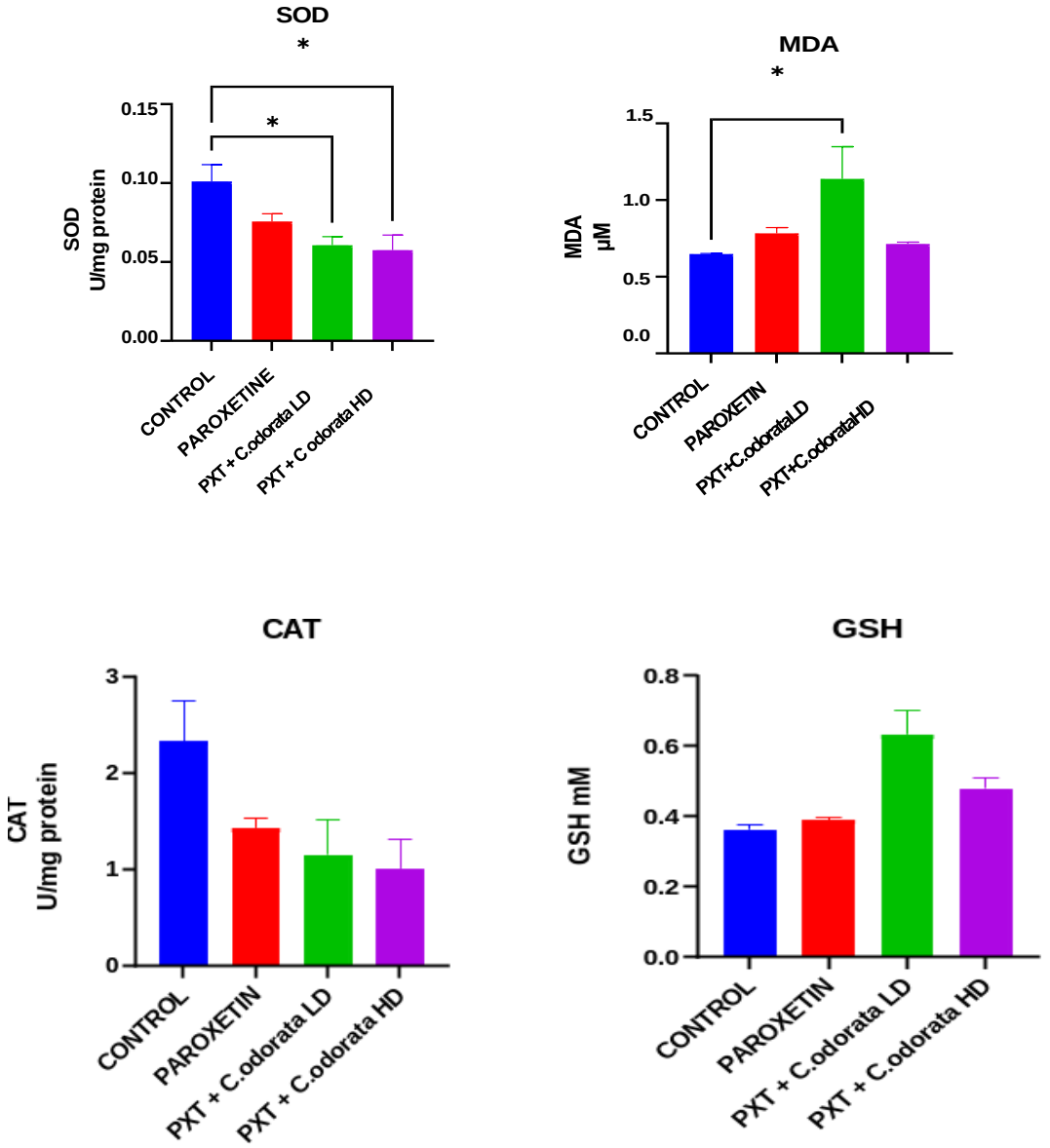

Figure 1: The bar graph shows the effect of paroxetine and Chromolaena odorata on oxidative stress markers.

Superoxide Dismutase (SOD) Activity

SOD activity in penile tissue was highest in the normal control group (0.1011 ± 0.0106 U/mg) and declined progressively through the paroxetine-only (0.0757 ± 0.0048 U/mg), low-dose *C. odorata* (0.0606 ± 0.0055 U/mg), and high-dose *C. odorata* (0.0576 ± 0.0094 U/mg) groups. The overall difference in the SOD activity among the experimental groups was statistically significant as determined by One-way ANOVA. The post hoc Tukey test showed that the control group was significantly different from both *C. odorata*-treated groups ($p < 0.05$). No statistically significant difference was seen between the paroxetine group and either extract-treated group, or the control group and the paroxetine group. The results of these are shown in Table 2 and Figure 1.

Table 2. Effect of Chromolaena odorata on SOD activity in penile tissue (Mean ± SEM; n = 4).

Group	SOD (U/mg) Mean ± SEM
Control	0.1011 ± 0.0106
Paroxetine	0.0757 ± 0.0048

PXT + C. odorata LD	0.0606 ± 0.0055*
PXT + C. odorata HD	0.0576 ± 0.0094*

**p < 0.05 vs. control group (Tukey's post hoc test).*

Malondialdehyde (MDA) Levels

Normal control group showed a lower concentration of MDA (0.6483 ± 0.0041 µM) and the paroxetine-only group showed a higher concentration of MDA (0.7830 ± 0.0380 µM). The group exposed to low dose *C. odorata* had the highest concentration of MDA (1.139 ± 0.2090 µM) and was statistically significantly different from control group ($p < 0.05$). The high-dose *C. odorata* group had an MDA value (0.7145 ± 0.0116 µM) that was not significantly different from the control value, but was lower than the paroxetine-only group. All other group comparisons had non-significant differences. The results are shown in Table 3 and Figure 1.

Table 3. Effect of *Chromolaena odorata* on MDA levels in penile tissue (Mean ± SEM; n = 4).

Group	MDA (µM) Mean ± SEM
Control	0.6483 ± 0.0041
Paroxetine	0.7830 ± 0.0380
PXT + *C. odorata* LD	1.139 ± 0.2090*
PXT + *C. odorata* HD	0.7145 ± 0.0116

**p < 0.05 vs. control group (Tukey's post hoc test).*

Catalase (CAT) Activity

Catalase activity followed a decreasing trend across experimental groups: Control (2.331 ± 0.4225 U/mg) > Paroxetine (1.433 ± 0.0923 U/mg) > Low dose *C. odorata* (1.155 ± 0.3660 U/mg) > High dose *C. odorata* (1.012 ± 0.3075 U/mg). One-way ANOVA did not reveal any statistical significant differences in CAT activity among any of the experimental groups ($p > 0.05$). The observed numerical trend was therefore not statistically confirmed at the current sample size (Table 4; Figure 1).

Table 4. Effect of Chromolaena odorata on CAT activity in penile tissue (Mean ± SEM; n = 4).

Group	CAT (U/mg) Mean ± SEM
Control	2.331 ± 0.4225
-Paroxetine	1.433 ± 0.0923
PXT + C. odorata LD	1.155 ± 0.3660
PXT + C. odorata HD	1.012 ± 0.3075

No statistically significant differences observed among groups ($p > 0.05$).

Reduced Glutathione (GSH) Levels

Reduced glutathione levels were comparable between the control group (0.3638 ± 0.0111 mM) and the paroxetine-only group (0.3902 ± 0.0061 mM), with no statistical significant difference between them. The low-dose *C. odorata* group exhibited a substantially and significantly elevated GSH level (0.6343 ± 0.0657 mM), which was significantly higher than the control group, the paroxetine-only group, and the high-dose extract group ($p < 0.05$). The high-dose group recorded an intermediate GSH level (0.4807 ± 0.0280 mM), higher than the control and paroxetine groups but not reaching statistical significance in comparison to either. Results are presented in Table 5 and Figure 1.

Table 5. Effect of Chromolaena odorata on GSH levels in penile tissue (Mean ± SEM; n = 4).

Group	GSH (mM) Mean ± SEM
Control	0.3638 ± 0.0111
Paroxetine	0.3902 ± 0.0061
PXT + C. odorata LD	0.6343 ± 0.0657*
PXT + C. odorata HD	0.4807 ± 0.0280

**p < 0.05 vs. control, paroxetine, and high-dose groups (Tukey's post hoc test).*

Penile Histoarchitecture

Histological examination of the penile tissue section stained with H&E showed obvious differences in structure between each experimental group (Fig. 5).

The paroxetine-only group had severe structural disorganisation of the cavernosal architecture, and prominent compression of cavernous spaces and eosinophilic histoarchitecture were key features of fibrous tissue replacement. These changes are similar to those observed in paroxetine-induced cavernosal smooth muscle atrophy and progressive fibrosis, which are mediated by ROS activation of the TGF-β1 and proliferation of fibroblasts. Structurally these spaces are compressed, which indicates impaired veno-occlusive capacity.

In the low dose C. odorata group there was a partial recovery in the histology, with a decrease in fibrosis compared to the paroxetine-only group, and progressive reorganisation of the underlying connective tissue, with the appearance of a stratified epithelial lining around the tissue periphery. Within the 28-day window of treatment, however, there was still not complete recovery at the lower dose, as evidenced by the presence of mild structural distortion, dense stromal areas, and limited vascular spaces were found.

The group exposed to the high dose of C. odorata demonstrated the greatest structural remission, of all the paroxetine exposed groups. A clear, deeply incised lumen and mucosal shape was observed. The surrounding smooth muscle and stromal organization were relaxed and well organized, and the dense sinusoidal congestion found in the paroxetine-only group was significantly decreased indicating a marked dose-dependent structural protection by C. odorata at the higher dose.

Fig. 2. Photomicrographs of H&E-stained penile tissue sections (Magnification ×100). Control: Normal cavernosal architecture with patent sinusoidal spaces. PXT (Paroxetine only): Marked structural disorganisation, compressed cavernous spaces, and eosinophilic fibrosis (black arrows). PXT + C. odorata LD (Low dose, 100 mg/kg): Partial structural recovery with stratified epithelial lining (white arrows) and early connective tissue reorganisation (blue arrows). PXT + C. odorata HD (High dose, 200 mg/kg): Most profound structural restoration with well-preserved deeply folded mucosal architecture and relaxed smooth muscle stroma (green arrows). Stain: Haematoxylin & Eosin.

Brain Histoarchitecture

The systemic effects of C. odorata extract on penile tissue was further substantiated by the evaluation of brain tissue by histological examination (Fig. 6).

A diffuse vacuolation of the neuropil (clear white areas throughout the tissue) and dark, shrunken, pyknotic (apoptosis or cellular stress) neuronal nuclei, and a highly reticulate (loose, mesh-like) neuropil texture were noted in the paroxetine-only group. These changes mirror those previously reported in serotonin-mediated excitotoxic neuronal injury and oxidative neurodegeneration in SSRIs rodent models.

A low-dose of C. odorata was found to provide only partial neuroprotection, with a small number of intensely pyknotic neuronal nuclei compared with the paroxetine-only group, but many micro-vacuoles were present, and there was mild tissue oedema, whereas the neuropil remained reticulate.

The group with high dose of C. odorata showed the highest level of neuroprotection. The neuropil was very condensed, regularly consolidated, with very prominent eosinophilic matrix. The other groups showed little change with abundant clear vacuolations and enlarged perineuronal spaces, while for the other groups there was a decrease in clear vacuolations, greater numbers of glia and more even distribution of neuronal cell bodies with healthier nuclear and cytoplasmic morphology, indicating a significant dose-dependent neuroprotection.

Fig. 3. Photomicrographs of H&E-stained brain tissue sections (Magnification ×100). Control: Normal neuropil with healthy neuronal architecture. PXT (Paroxetine only): Diffuse neuropil vacuolation (red arrows), dark pyknotic nuclei (white circles) indicative of apoptosis, and loose reticulate neuropil. PXT + C. odorata LD (Low dose): Partial neuroprotection with reduced pyknotic nuclei (white arrow) but persistent microvacuolation and mild tissue oedema (circle). PXT + C. odorata HD (High dose): Marked neuroprotection with dense consolidated neuropil, reduced vacuolations, and uniformly distributed healthy neuronal morphology (black arrows). Stain: Haematoxylin & Eosin.

4. Discussion

This study investigated the protective effects of ethanolic leaf extract of *C. odorata* against paroxetine-induced oxidative stress and histopathological injury in penile and brain tissues of adult male Wistar rats. The findings present a nuanced biochemical and histological picture, with clear dose-dependent protection at the structural level and differential, sometimes unexpected, effects across the four oxidative stress biomarkers assessed.

Body Weight and Systemic Toxicity

The absence of significant weight loss or clinical signs of toxicity across all experimental groups confirms that paroxetine at 10

mg/kg and *C. odorata* extract at 100 and 200 mg/kg were well tolerated over 28 days. The modest reduction in relative weight gain in extract-treated groups most pronounced in the paroxetine-only group (2.18 ± 1.74%) is consistent with changes observed in other rodent studies involving co-administration of plant extracts with pharmacological agents, attributable to increased metabolic demand associated with extract biotransformation, possible mild appetite suppression, or physiological remodelling (Vital & Afolayan, 2015; Ateş *et al.*, 2021). This finding is in keeping with the documented low acute toxicity of *C. odorata* leaf extract, with LD_{50} values exceeding 5000 mg/kg in rodents (Vital & Afolayan, 2015).

Superoxide Dismutase Activity

The decline in SOD activity from control through paroxetine to the extract-treated groups was an unexpected pattern given the well-established antioxidant properties of *C. odorata*. Paroxetine-induced reduction of SOD is consistent with the established capacity of SSRIs to suppress first-line enzymatic antioxidant defences through ROS overproduction, particularly through auto-oxidation of serotonin following SERT inhibition, which generates superoxide radicals that overwhelm and progressively deplete endogenous SOD (Bilici *et al.*, 2001; Atmaca *et al.*, 2015; Akintunde *et al.*, 2021).

The further reduction in SOD activity in *C. odorata*-treated groups may be explained by several non-mutually exclusive mechanisms. The abundant flavonoids and polyphenolics in *C. odorata* are potent direct superoxide scavengers that may have directly neutralised superoxide radicals in penile tissue, thereby reducing the substrate available for endogenous SOD and precipitating compensatory downregulation of SOD synthesis a phenomenon documented for flavonoid-rich plant extracts administered in vivo (Halliwell & Gutteridge, 2015; Procházková *et al.*, 2011). Additionally, high exogenous antioxidant load may signal reduced demand for endogenous enzymatic defence through transcriptional downregulation, as observed in animal tissues supplemented with high doses of dietary phytochemical antioxidants. The small sample size ($n = 4$) markedly limits statistical power, and the observed pattern may not be representative of the true population-level response; a study with $n \geq 8$ per group would be required to reliably characterise the SOD dose-response.

Malondialdehyde Levels

Paroxetine-induced elevation of MDA in penile tissue is consistent with previous reports of SSRI-mediated lipid peroxidation through mitochondrial dysfunction and serotonergic hyperactivity-driven ROS overproduction (Bilici *et al.*, 2001; Ateş *et al.*, 2021). The paradoxically highest MDA concentration in the low-dose *C. odorata* group (1.139 ± 0.2090 µM) is the most counterintuitive finding of the biochemical assessment and warrants careful interpretation.

At sub-optimal concentrations, polyphenolic compounds can exhibit pro-oxidant behaviour: phenolics may reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which participates in Fenton chemistry to generate the highly reactive hydroxyl radical, paradoxically enhancing lipid peroxidation (Procházková *et al.*, 2011; Halliwell & Gutteridge, 2015). This pro-oxidant/antioxidant duality is concentration-dependent, with antioxidant behaviour predominating at higher concentrations consistent with the relatively attenuated MDA observed in the high-dose group. The wide SEM (± 0.2090 µM) in the low-dose group indicates substantial inter-individual variability; with $n = 4$, a single outlier can substantially shift the group mean. Additionally, the low dose may have been insufficient to mount a meaningful antioxidant response against the paroxetine-induced oxidative load, whereas the higher dose provided partial, though statistically non-significant, attenuation.

Catalase Activity

The decreasing trend in CAT activity across experimental groups highest in controls and lowest in the high-dose *C. odorata* group mirrors the SOD pattern, though without reaching statistical significance. The lack of significance, despite the apparent numerical trend, most likely reflects the study's limited statistical power at $n = 4$ per group, and should not be interpreted as evidence of a true null effect.

The mechanism underlying CAT reduction in *C. odorata*-treated groups likely parallels that proposed for SOD: direct H_2O_2 scavenging by plant phenolics may reduce the substrate available for catalase, leading to indirect reduction in measured catalase activity even in the absence of direct CAT inhibition. As SOD and CAT operate sequentially (SOD converts superoxide to H_2O_2, which CAT then decomposes), a reduction in SOD-mediated H_2O_2 production would reduce catalase substrate flux, indirectly lowering measured activity (Halliwell & Gutteridge, 2015). This mechanistic coupling may explain the parallel trends observed for both enzymes across treatment groups.

Reduced Glutathione Levels

The GSH findings provided the most statistically robust and biochemically coherent evidence of *C. odorata* antioxidant activity. The comparable GSH levels between control and paroxetine groups suggests that, at the dose and duration studied, paroxetine did not significantly deplete non-enzymatic glutathione reserves in penile tissue at this timepoint, possibly reflecting a compensatory upregulation of glutathione synthesis in response to the oxidative challenge, as described by Lu (2013).

The significantly elevated GSH in the low-dose *C. odorata* group (0.6343 ± 0.0657 mM; $p < 0.05$ vs. all other groups) is consistent with the well-established capacity of *C. odorata* flavonoids particularly quercetin and its glycosides to upregulate

gamma-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in glutathione biosynthesis, through Nrf2/ARE pathway activation (Procházková *et al.*, 2011). The co-occurrence of elevated MDA and GSH in the same low-dose group is consistent with a hormetic adaptive response: the elevated oxidative burden (high MDA) serves as a potent stimulus for compensatory GSH synthesis, while the phytochemical constituents of *C. odorata* provide an additional, direct Nrf2-mediated GSH induction stimulus (Lu, 2013). The apparently paradoxical dose-response wherein the low dose elevates GSH more than the high dose may reflect hormetic dose-response kinetics, wherein low-to-moderate phytochemical concentrations optimally stimulate adaptive antioxidant signalling, while higher doses may partially counteract GSH induction through pro-oxidant effects (Procházková *et al.*, 2011).

Penile Histoarchitectural Findings

Cytoprotective effect of C. odorata against paroxetine-induced penile tissue injury was consistently and meaningfully supported by its histological data. The cavernosal disorganisation, sinus compression and eosinophils fibrosis seen with paroxetine are all characteristic of the activation of TGF-β1 and subsequent fibroblast proliferation and collagen deposition of the cavernosal tissue previously observed in ED rodent models of SSRI (Ateş et al., 2021; Öztürk et al., 2020). The compressed cavernous spaces are structurally similar to impaired veno-occlusive capacity, a mechanism for failure to sustain intracavernosal pressure for an erection.

The dose-dependent structural recovery observed in C. odorata treated groups, partial at lower dose and most at higher dose is in accordance with its documented anti-fibrotic, wound healing, and vasorelaxant effects, such as modulation of collagen synthesis and matrix metalloproteinase (MMP) activity in connective tissue (Phan et al., 2001; Thang et al., 2001). The highly folded mucosal architecture and loose smooth muscle stroma of the high dose group are surrogates of successful cavernosal tissue restoration which should correlate with functional evaluation of erectile capacity if such could be done.

More importantly, the biochemical and histological results are not totally congruent, suggesting that the anti-oxidant defence in biological tissues is complicated; structural cytoprotection involves anti-inflammatory, anti-fibrotic and vasorelaxative action, which are partly independent of the enzymatic antioxidant pathways that were measured in this study (Owolabi et al., 2007; Musicki & Burnett, 2020). Although helpful, the four oxidative stress markers do not reflect the range of cytoprotective mechanisms that C. odorata may exhibit.

Brain Histoarchitectural Findings

The neuropathological changes seen in the paroxetine group are characterized by diffuse neuropil vacuolation, pyknotic neuronal nuclei and a loose reticulate neuropil, which have previously been reported in ssri-induced rodent models (atmaca et al., 2015). the most pronounced neuroprotection in the groups treated with c. odorata in a dose-dependent fashion, with the highest dose group exhibiting the best neuropil consolidation and healthiest neuronal morphology, aligns with the antioxidant, anti-inflammatory and antiapoptotic potential of flavonoids and phenolic acids found in c. odorata (akinmoladun et al., 2007; procházková et al., 2011). the results of these secondary endpoints are preliminary neuroprotective findings that are of scientific interest and deserve dedicated mechanistic studies, especially in light of the increasing number of reports of central nervous system toxicity associated with SSRI

5. Conclusion

Sub-chronic oral administration of paroxetine at 10 mg/kg body weight for 28 days induced measurable oxidative stress and histopathological injury in the penile and brain tissues of adult male Wistar rats, confirming the utility of this model for studying SSRI-induced erectile tissue toxicity. Ethanolic leaf extract of *C. odorata* produced variable, dose-dependent effects on oxidative stress biomarkers: while SOD and CAT activities were not restored by extract treatment possibly reflecting compensatory enzymatic downregulation secondary to direct phytochemical antioxidant activity reduced glutathione was significantly elevated at the low dose ($p < 0.05$), indicating modulation of the non-enzymatic antioxidant compartment. The paradoxical elevation of MDA in the low-dose group likely reflects concentration-dependent pro-oxidant activity of polyphenolic constituents and warrants further investigation.

Histological outcomes were more consistently and robustly protective: both extract doses attenuated paroxetine-induced structural injury in penile and brain tissue in a dose-dependent manner, with the high dose producing the most significant structural restoration of cavernosal architecture and neuropil morphology. The null hypothesis that *C. odorata* leaf extract has no significant protective effect on oxidative stress biomarkers and penile histoarchitecture in paroxetine-induced erectile dysfunction is partially rejected: specifically with respect to GSH levels, penile histoarchitecture, and brain histoarchitecture, where statistically significant or clearly demonstrable protective effects were observed. These preliminary findings support further investigation of *C. odorata* as a herbal cytoprotective candidate against SSRI-induced reproductive and neural tissue toxicity, warranting studies with larger sample sizes, additional experimental groups, complementary mechanistic endpoints (NO assay, eNOS/nNOS immunohistochemistry, reproductive hormone profiling), and phytochemical standardisation of the extract.

Declarations

Ethical Approval

This study was approved by the University of Ilorin Ethics Review Committee, and all procedures involving animals conformed to institutional guidelines for the care and use of laboratory animals.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

This research was conducted by Onoja, B.A, with designed methods by Onoja, B.A and Oyewopo, A.O.
Data entry and Analysis were conducted by Onoja, B.A. and Abdulfatai, H.A., Manuscript writing by Olowolayemo, K.O and reviewed by Onoja, B.A and Oyewopo, A.O.

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