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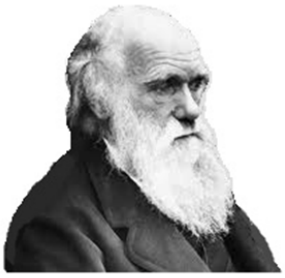

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Structural and molecular study of the adrenal gland of trauma-induced *Rattus norveginus* (Berkenhout, 1769)

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ABSTRACT

Trauma is an emotional and physiological response to an overwhelming event such as an accident, sexual violence, or natural disaster; immediately after such an event, shock can overwhelm an individual's ability to cope, and prolonged exposure produces significant physiological disruption in endocrine organs, particularly the adrenal gland (Pai, Suris & North, 2017; Shalev, Cho & Marmar, 2024). This study investigated the structural and molecular characteristics of the adrenal gland following trauma-like induction in *Rattus norvegicus*. Wistar rats weighing between 100 g and 150 g were divided into three experimental groups: female socially induced trauma, male socially induced trauma, and male physically induced trauma. Each group was further subdivided into four subgroups: trauma only, trauma plus treatment, treatment only, and control. Garlic was administered to the female social group, Cellgevity to the male social group, and Amaranthus cruentus extract to the male physical group. At the conclusion of the experiment, the adrenal glands were harvested, fixed in formal saline, and processed histologically using routine Haematoxylin and Eosin staining. Chromaffin cell counts were determined using Fiji ImageJ software. Behavioural assessments were conducted using the open field test and the Y-maze spontaneous alternation test. The results demonstrated that trauma significantly altered the structural and molecular characteristics of the adrenal gland. Chromaffin cells, the principal catecholamine-secreting cells of the adrenal medulla (Berends et al., 2019), were markedly reduced in trauma-exposed animals compared with controls. These findings indicate that trauma exerts a destructive effect on the structural and functional integrity of the adrenal gland in mammalian models, consistent with reports of phenotype-associated adrenal hypofunction in other rodent models of post-traumatic stress (Tseilikman et al., 2021).

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***Keywords*:** Trauma, Adrenal gland, Chromaffin cells, Rattus norvegicus, Stress hormones

1. INTRODUCTION

Trauma is an emotional and physiological response to a terrible event such as an accident, rape, or natural disaster. Immediately after the event, shock can overwhelm an individual's ability to cope, leading to longer-term reactions including unpredictable emotions, flashbacks, strained relationships, and physical symptoms such as headaches or nausea. It results from exposure to an incident or series of events that are emotionally disturbing or life-threatening, with lasting adverse effects on the individual's functioning and mental, physical, social, and emotional well-being. Experiences that may be traumatic include physical and emotional abuse, sudden unexplained separation from a loved one, violence, discrimination, and oppression. Traumatic events are generally characterised by three components: an external cause, a sense of violation or intrusion into the individual's personal space, and a loss of control that leaves the person feeling overwhelmed and helpless.

Acute stress disorder (ASD) can develop following exposure to one or more traumatic events, with symptoms lasting from three days to one month. When symptoms persist beyond one month and seriously impair functioning, the diagnosis changes to post-traumatic stress disorder (PTSD). In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, PTSD was relocated from the anxiety-disorders category into a new „Trauma and Stressor-Related Disorders" category, the subjective-response requirement was removed from the trauma criterion, and the symptom criteria were reorganised into four clusters: intrusion, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity (Pai, Suris & North, 2017). Contemporary overviews of PTSD neurobiology describe disruption across genetic, molecular and neural-circuit domains, including exaggerated noradrenergic signalling and impaired top-down regulation of amygdala reactivity by frontal-limbic circuitry, alongside continuing advances in evidence-based pharmacological and neuromodulatory treatment (Shalev, Cho & Marmar, 2024). Social anxiety disorder (SAD), a specific form of trauma response, makes individuals fearful of social situations involving humiliation and rejection and increases the risk of major depressive disorder. Social isolation, a paradigm frequently used in animal studies, disrupts normal social hierarchies and reliably produces anxiety- and depression-like behaviour together with endocrine changes, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and altered catecholamine and glucocorticoid release (Mumtaz, Khan, Zubair & Dehpour, 2018).

Physically induced trauma, particularly traumatic brain injury (TBI), is brain damage caused by an external mechanical force that may result in temporary or permanent impairment of cognitive, physical, and psychosocial functioning. The initial injury involves immediate mechanical disruption of brain tissue and the release of chemical mediators that cause further cell death. Neuropsychiatric sequelae including anxiety disorders can result from TBI, with the amygdala, hippocampus and prefrontal cortex notably implicated as stress- and fear-relevant circuitry (Fesharaki-Zadeh & Datta, 2024). Rodent models that combine physical head injury with a concurrent psychosocial stressor such as social defeat show that the two insults interact, producing more pronounced anxiety-like behaviour and greater disturbance of limbic monoamine systems than either insult alone (Davies et al., 2016), underscoring the

physiological overlap between physically and socially induced trauma that the present study sought to compare directly.

The adrenal glands, also known as suprarenal glands, are paired endocrine glands located above the kidneys (Figure 1). They produce a variety of hormones including adrenaline, noradrenaline, aldosterone, and cortisol (Iacobellis, Rossi, Castinetti & Letizia, 2015).

The adrenal cortex is divided into three functional zones: the zona glomerulosa, which produces mineralocorticoids such as aldosterone; the zona fasciculata, which produces glucocorticoids such as cortisol; and the zona reticularis, which secretes androgens (Iacobellis, Rossi, Castinetti & Letizia, 2015). The adrenal medulla, located at the centre of each adrenal gland, is the primary source of catecholamines. Chromaffin cells are the principal neuroendocrine cells of the adrenal medulla; they synthesise and secrete dopamine, norepinephrine and epinephrine through a tightly regulated, stimulus-coupled biosynthetic and secretory pathway, and also serve as key mediators of the wider stress response (Berends et al., 2019). The normal histological architecture of the adrenal gland is illustrated in Figure 2.

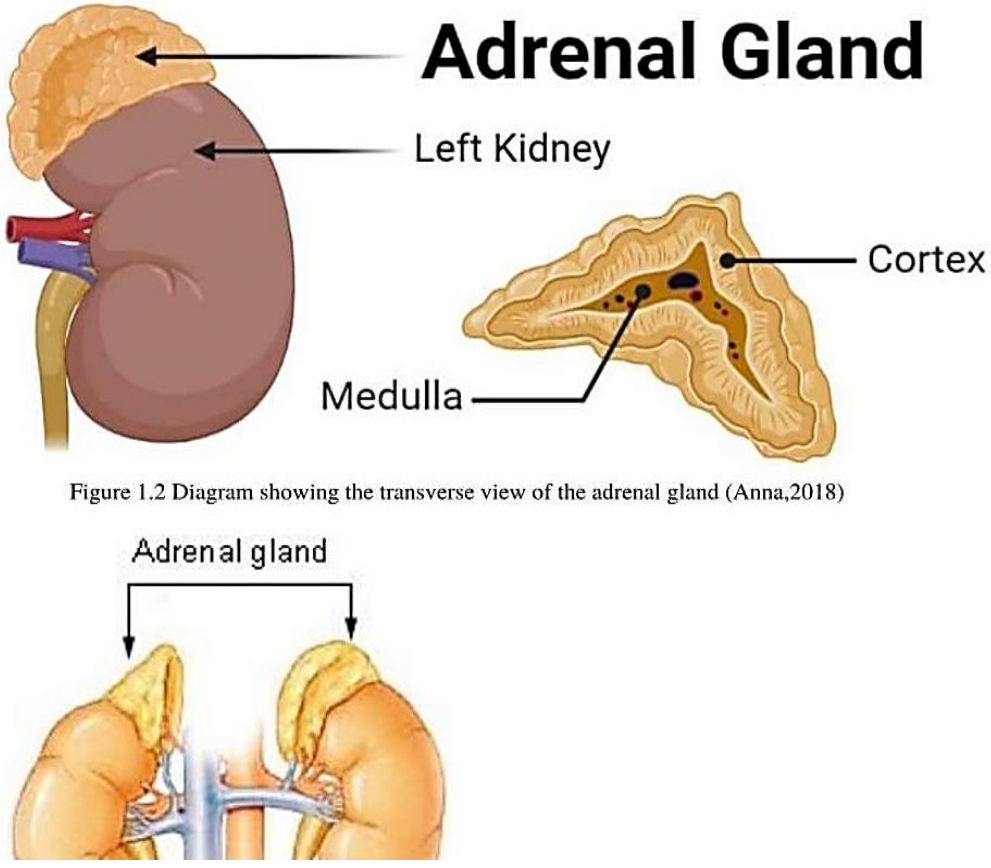

Figure 1.2 Diagram showing the transverse view of the adrenal gland (Anna,2018)

Figure 1. Diagrams showing the transverse and anterior views of the adrenal gland.

When the body is exposed to trauma, the hypothalamic-pituitary-adrenal (HPA) axis is activated. The hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotropin hormone (ACTH), which in turn stimulates the adrenal glands to produce cortisol. Cortisol helps control the body's use of fats,

proteins, and carbohydrates; suppresses inflammation; regulates blood pressure; and increases blood sugar levels, while the adrenal medulla simultaneously releases epinephrine and norepinephrine to prepare the body for the fight-or-flight response. Prolonged activation of these pathways, as seen in traumatic stress, produces subregion-specific structural change in the adrenal gland, including hyperplasia of the outer zona fasciculata and hypertrophy of the inner zona fasciculata and medulla, together with a reduction in zona glomerulosa cell size (Ulrich-Lai et al., 2006).

Persistent HPA-axis dysregulation of this kind has also been linked to neuroinflammatory and oxidative changes in stress-responsive brain regions such as the hippocampus (Lei et al., 2025) and, more broadly, to immune imbalance and elevated risk of autoimmune disease (Nunez, Rabelo, Subotic, Caruso & Knezevic, 2025). Disorders of adrenal function — including Addison's disease, Cushing's syndrome and congenital adrenal hyperplasia — further illustrate the wide-ranging metabolic, immune, cardiovascular and stress-response consequences of adrenal dysfunction (Iacobellis, Rossi, Castinetti & Letizia, 2015).

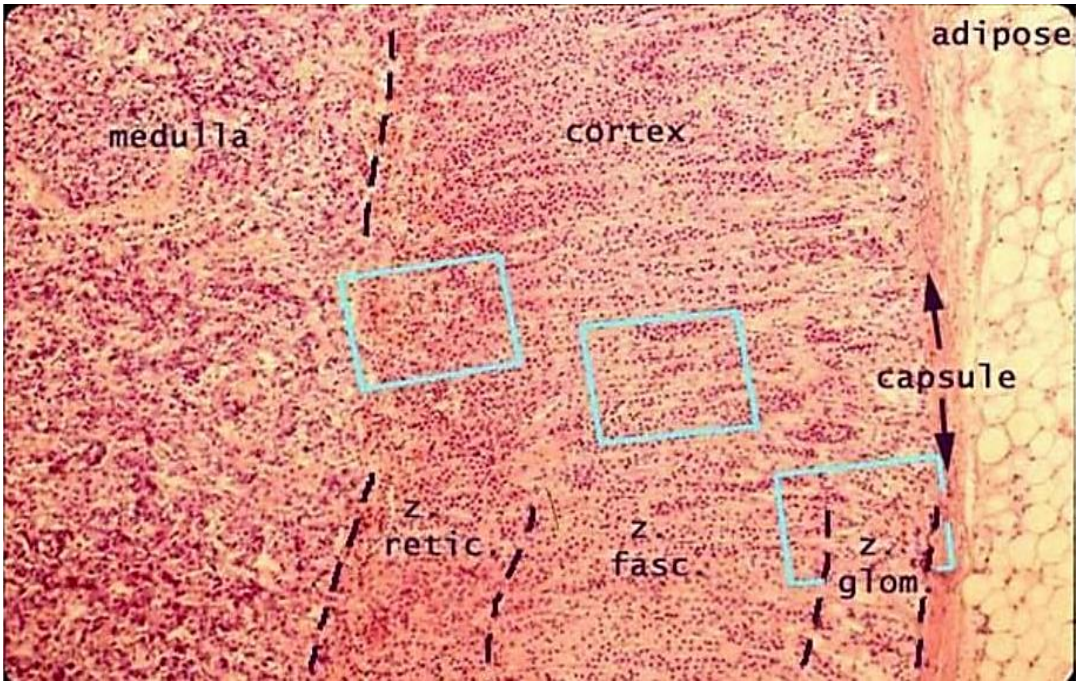

Figure 2. Histological diagram of adrenal gland showing the adrenal cortex and adrenal medulla.

Rodent models exposed to chronic predator-based psychosocial stress — closely paralleling the cat-exposure paradigm used in the present study — have been shown to develop phenotype-associated morphological changes and hypofunction of the adrenal gland, with high- and low-anxiety phenotypes differing in cortical and medullary histoarchitecture and corticosterone output (Tseilikman et al., 2021), and altered glucocorticoid responses to dexamethasone challenge that persist for weeks after the stressor (Zoladz et al., 2021). Early-life and chronic-stress paradigms similarly produce measurable HPA-axis dysfunction on pharmacological challenge testing in both clinical and experimental settings (Barroca et al.,

2021). The present study was designed to investigate the structural and molecular characteristics of the adrenal gland of trauma-induced Rattus norvegicus, with specific focus on histopathological changes and chromaffin cell counts across different trauma paradigms and treatment conditions.

2. MATERIALS AND METHODS

2. 1. Experimental Animals

White laboratory Wistar rats were obtained and housed at the Animal Experimental House, College of Health Sciences, Bowen University. They were kept in plastic cages at room temperature with a 12-hour light/dark cycle and were allowed to acclimatize to their new housing conditions. The rats were fed a rodent pellet diet and given water ad libitum throughout the acclimatization period. All animals were maintained and treated in accordance with the guidelines for animal care established by the National Institutes of Health, and using protocols approved by the Binghamton University Institutional Animal Care and Use Committee (IACUC). In each experiment, no more than one male and one female animal from a given litter was placed into a particular experimental group.

2. 2. Animal Grouping

The rats were divided into three experimental groups. The first group comprised female rats in which social trauma was induced; the second comprised male rats in which social trauma was induced; and the third comprised male rats in which physical trauma was induced. Each group was further subdivided into four subgroups. For the female social group: Group 1 received trauma by cat exposure; Group 2 received trauma plus garlic treatment; Group 3 received garlic treatment only; and Group 4 served as the control. For the male social group: Group 1 received Cellgevity only; Group 2 received trauma plus Cellgevity; Group 3 received trauma only; and Group 4 served as the negative control. For the male physical group: Group 1 received trauma by weight drop; Group 2 received trauma plus Amaranthus cruentus extract; Group 3 received Amaranthus cruentus extract only; and Group 4 served as the control.

2. 3. Induction of Trauma-Like Conditions

Social trauma was induced in rats by exposing them to a live cat. In addition, audio recordings of angry cat sounds were played on a speaker for three hours on alternating days. After each exposure session, the rats were fed and drugs were administered. Housing instability was introduced on the third day of the experiment to supplement the cat exposure paradigm, as cat exposure alone was found insufficient to reliably induce trauma-like conditions. This combination of predator exposure with chronic social instability follows an established preclinical paradigm for modelling PTSD-like states, which has been shown to produce reliable and lasting anxiety-like behaviour and altered glucocorticoid regulation in exposed animals (Zoladz et al., 2021).

Physical trauma was induced using a modified weight-drop device adapted from the technique of Farran et al. (2014) and previously used by Owoeye et al. (2017). Rats were anaesthetized, and the hair over the skull was shaved and cleaned with methylated spirit. Each rat was placed on the levered table of the modified weight-drop apparatus. A metallic ball fashioned to the end of a Steinmann's pin was used as the impacting object. The levered table

was adjusted to a traumatic distance of 2.5 mm. A metallic weight of 425 g was dropped from a uniform height of 5 cm onto the skull to induce TBI. The apparatus is shown in Figure 3. Weight-drop devices of this kind remain among the most widely used preclinical TBI models because they reproduce the primary mechanical injury and the secondary neurochemical and inflammatory cascade of human closed-head injury without requiring craniotomy (Fesharaki-Zadeh & Datta, 2024), building on classic weight-drop protocols that first established reproducible clinical, pathophysiological and neurologic outcome measures in rats (Shapira et al., 1988).

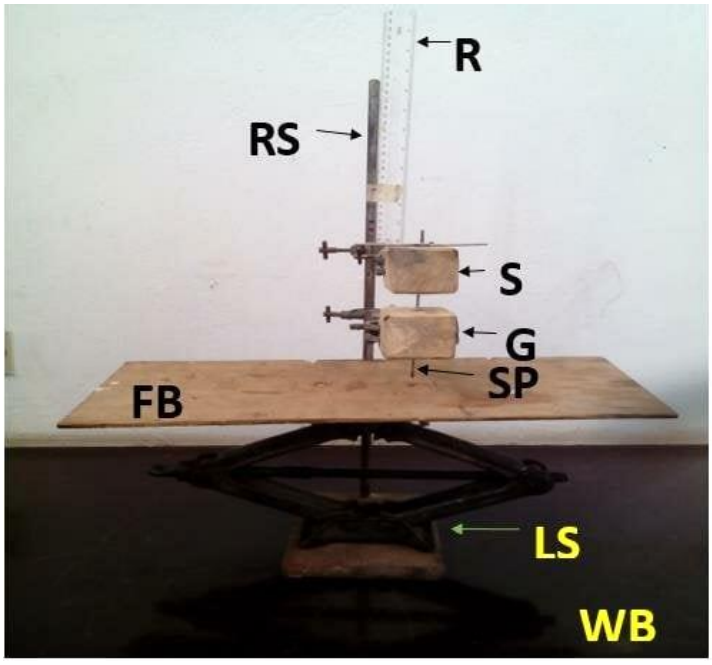

Figure 3. The modified weight drop apparatus after Farran et al. (2014). R – Ruler, RS – Retort Stand, LS – Lever Stand, WB – Work Bench, FB – Flat Board, SP – Steinmann's Pin, G – Guide, S – Stopper.

2. 4. Sample Collection

Following the behavioural studies, rats were sacrificed by euthanasia using chloroform inhalation. The adrenal glands were carefully harvested; one gland was stored on ice for molecular analysis, while the other was placed in formal saline for histological processing.

2. 5. Tissue Processing and Histopathology

The harvested adrenal glands were fixed in 10% formalin, paraffin-embedded, cut into 5 µm sections, and stained with Haematoxylin and Eosin (H&E). Tissue processing was performed automatically using a Leica TP 1020 tissue processor over 12 hours. Tissues passed through formal saline, ascending grades of alcohol (70% – absolute), two changes of xylene for clearing, and three paraffin wax baths for infiltration, spending 1 hour at each station. Embedding was performed using a semi-automatic tissue embedding centre. Blocks were trimmed at 6 µm and ribbons sectioned at 4 µm, floated on a 55 °C water bath, picked onto

labelled slides, and dried at 60 °C for 1 hour. For H&E staining, sections were dewaxed in xylene for 15 minutes, hydrated through graded alcohols, stained in Harris haematoxylin for 5 minutes, differentiated in 1% acid alcohol, blued under running tap water for 10 minutes, counterstained with 1% aqueous eosin for 2 minutes, dehydrated, cleared in xylene, and mounted in DPX. Stained slides were examined at ×400 magnification by a pathologist.

2. 6. Behavioural Tests

The open field test (OFT) was used to measure locomotion and anxiety-like behaviour using a square box (100 cm × 100 cm × 100 cm). Each rat was placed at the centre of the box and observed for 5 minutes under natural light in a quiet room. The number of droppings, time spent in the central zone, and number of line crossings were recorded. The OFT remains one of the most widely used platforms for assessing general locomotor ability and anxiety-like emotionality in rodents (Seibenhener & Wooten, 2015); however, its outcomes can diverge from those of other anxiety assays such as the elevated plus maze depending on which anxiogenic variable is dominant in a given paradigm, which is a relevant consideration when interpreting concordant and discordant behavioural findings across trauma groups (Cerqueira et al., 2023). The Y-maze spontaneous alternation test was used to measure exploratory activity and spatial working memory. Three opaque arms were arranged at 120-degree angles. Each rat was placed at the centre and allowed to freely explore all three arms, with arm entries and alternation sequences recorded to calculate the percentage of spontaneous alternation. Because the precision of this test depends on how consistently an arm visit is defined, quantitative approaches that standardise the arm-entry threshold have been shown to sharpen detection of genuine stress-induced impairments in spatial working memory, as distinct from confounding differences in locomotion (Kim et al., 2023).

2. 7. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7.0. Statistical significance was set at $p < 0.05$. Two-sample t-tests and paired t-tests were used to analyse differences between two groups. One-way ANOVA was used to compare means across multiple groups, followed by Dunnett's multiple comparison post-test. Chromaffin cell counts were determined from histological sections using Fiji ImageJ software.

3. RESULTS AND DISCUSSION

3. 1. Body Weight Changes

The body weight data for socially induced trauma Wistar rats are presented in Table 1 and Figures 4 and 5. There was a statistically significant difference ($p < 0.05$) in mean body weight across all groups both before and after administration. The mean body weight before administration (BW1) was 169.0 ± 4.68 g (control), 147.5 ± 7.91 g (control + garlic), 156.2 ± 6.55 g (PTSD only), and 164.4 ± 1.04 g (PTSD + garlic), with an overall p-value of 0.0416. After administration (BW2), the values were 172.2 ± 5.40 g, 136.5 ± 5.67 g, 154.6 ± 6.38 g, and 159.5 ± 8.80 g respectively ($p = 0.0060$). The decrease in weight in the treatment groups was highly significant and is attributable to reduced food and water intake following administration of garlic, Amaranthus cruentus, and Cellgevity, which appeared to suppress appetite and reduce activity levels in the treated animals.

Table 1. The body weight data for socially induced trauma Wistar rats.

Parameter	Control	Control + Garlic	PTSD Only	PTSD + Garlic
BW1 (g)	169.0 ± 4.68	147.5 ± 7.91	156.2 ± 6.55	164.4 ± 1.04
BW2 (g)	172.2 ± 5.40	136.5 ± 5.67	154.6 ± 6.38	159.5 ± 8.80
p-value	BW1: 0.0416			
p-value	BW2: 0.0060			

BW1 = Body weight before administration; BW2 = Body weight after administration; SEM = Standard error of mean; $p < 0.05$.

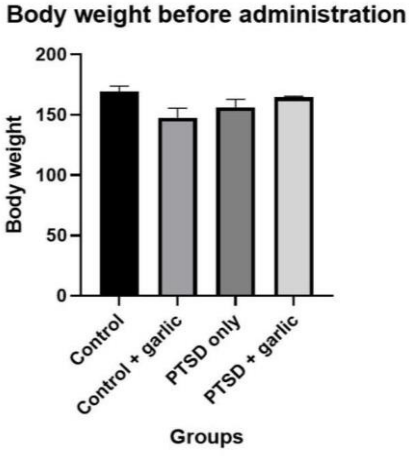

Figure 4. Chart showing body weight of rats before administration ($p < 0.05$).

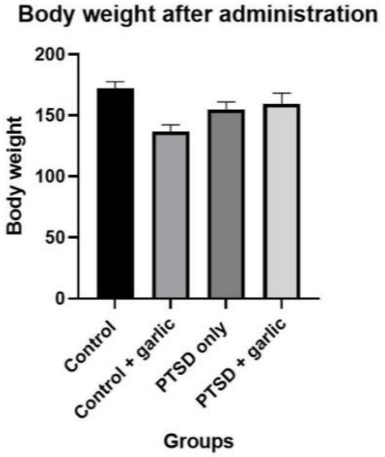

Figure 5. Chart showing body weight of rats after administration ($p < 0.05$).

3. 2. Behavioural Test Results

The Y-maze spontaneous alternation results are shown in Figure 6. The PTSD plus garlic group recorded the lowest percentage of alternation among the female social groups, suggesting that garlic did not significantly improve spatial memory in trauma-exposed animals, though the differences were not statistically significant ($p > 0.05$). Similarly, there was a significant reduction in exploratory activity in trauma-induced male social and male physical animals compared to controls.

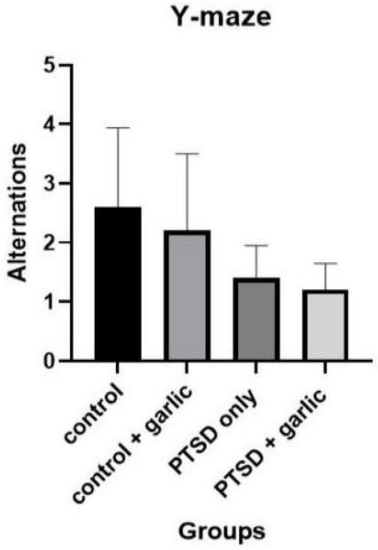

Figure 6. Y-maze spontaneous alternation results across the female social groups ($p > 0.05$).

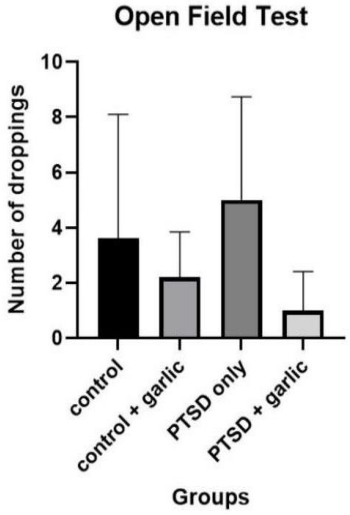

Figure 7. Open field test — number of droppings in the female social group ($p > 0.05$).

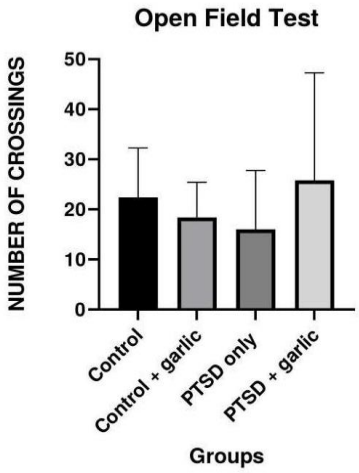

Figure 8. Open field test — number of line crossings in the female social group ($p > 0.05$).

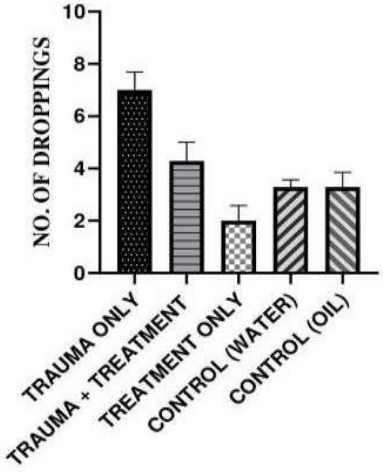

Figure 9. Open field test — number of droppings in the male physical group ($p < 0.05$).

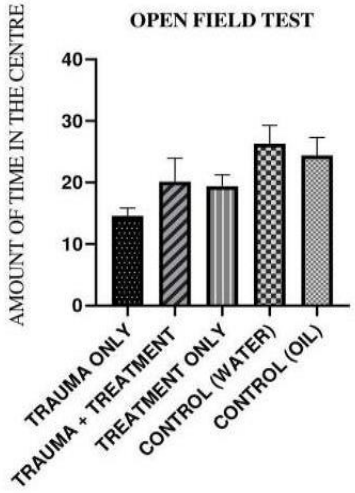

Figure 10. Open field test — time spent in centre of chamber, male physical group ($p < 0.05$).

The open field test results for the female social groups are shown in Figures 7 and 8. Animals administered garlic recorded the lowest number of droppings, suggesting garlic helped mitigate anxiety-like responses, though differences did not reach statistical significance ($p > 0.05$). In the male physical trauma group (Figures 9–11), the trauma only group had the highest number of droppings and spent significantly less time in the centre of the open field chamber ($p < 0.05$), indicating heightened anxiety.

The trauma plus Amaranthus cruentus group had the highest number of line crossings ($p < 0.05$), demonstrating that the extract improved locomotor exploration. These divergent patterns across droppings, centre-time and line-crossing measures illustrate why open-field outcomes should be interpreted as a multidimensional profile rather than a single anxiety index, since different components of open-field behaviour can respond differently to the same experimental variable (Cerqueira et al., 2023).

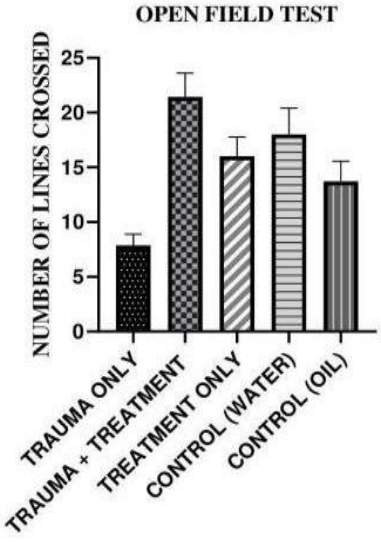

Figure 11. Open field test — number of lines crossed, male physical group ($p < 0.05$).

3. 3. Histopathology of the Adrenal Gland

Histopathological examination of the adrenal glands using H&E staining revealed distinct structural changes across the experimental groups. In the female social trauma group (Plate 1), the adrenal cortex showed a normal pattern of zonation with clusters of cells with stainable cytoplasm in the zona glomerulosa, columns of cells with clear cytoplasm in the zona fasciculata, and cells with acidophilic cytoplasm in the zona reticularis. The medullary layer appeared normal.

In the female social trauma plus treatment group (Plate 2), normal cortical zonation was maintained and the medullary layer also appeared normal. The female social treatment only group (Plate 3) showed a focal area of cortical necrosis alongside poor cortical zonation with pale cytoplasm in the zona glomerulosa and moderate medullary congestion. The female social control (Plate 4) displayed moderately normal cortical zonation but severe vascular dilatation and congestion in the medullary layer.

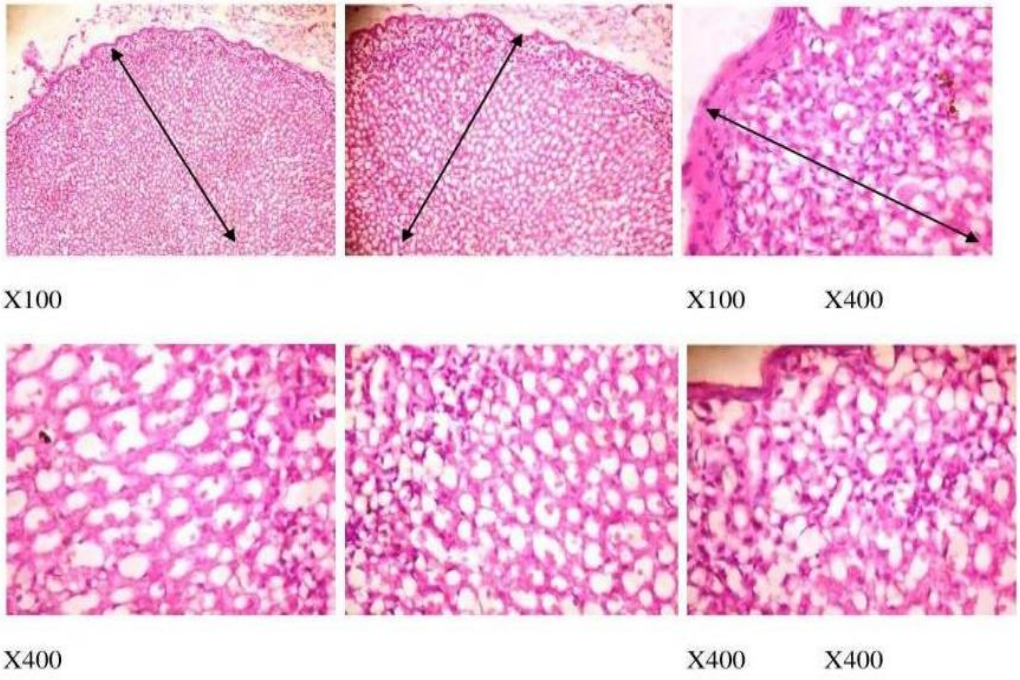

Plate 1. Adrenal gland from female social trauma group (H&E stain).
Normal cortical zonation pattern; normal medullary layer.

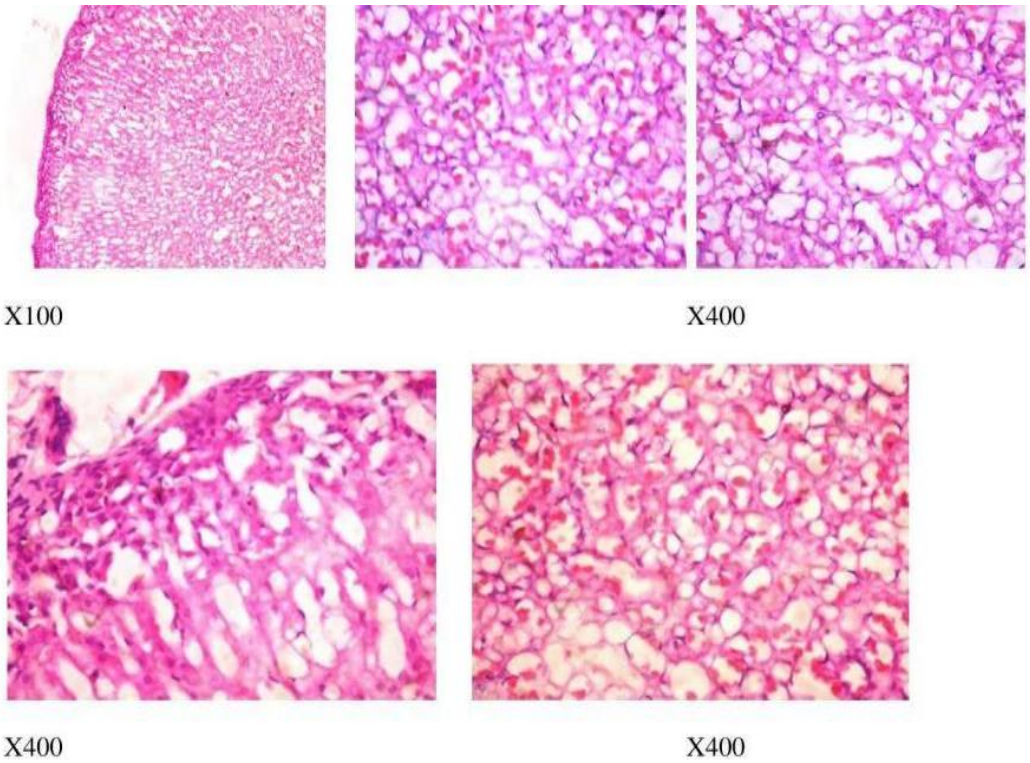

Plate 2. Adrenal gland from female social trauma + treatment group (H&E stain).
Normal cortical zonation; normal medullary layer.

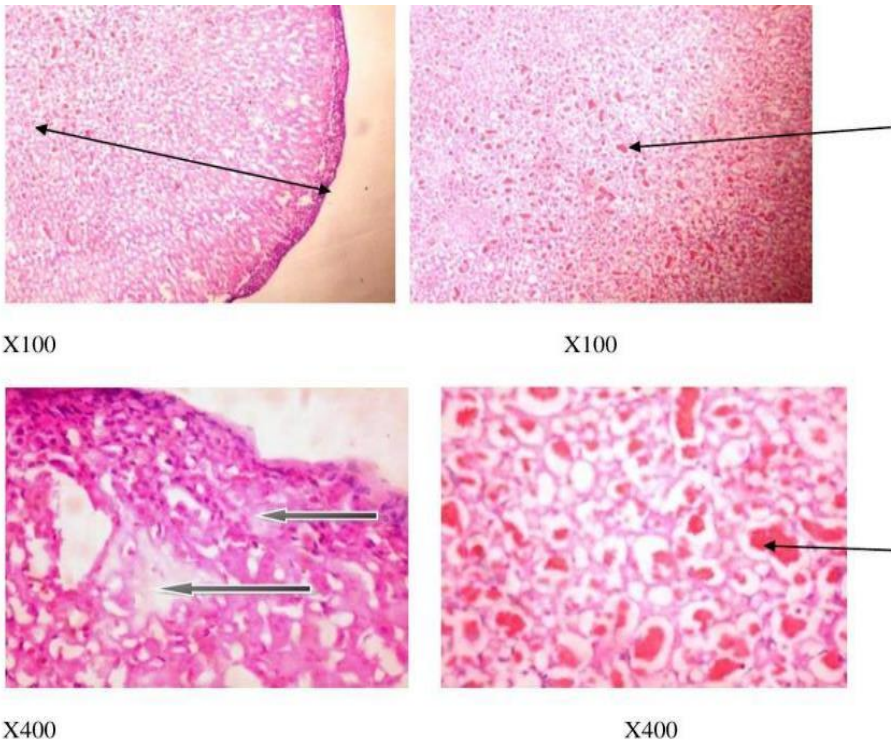

Plate 3. Adrenal gland from female social treatment only group (H&E stain). Focal cortical necrosis; moderate medullary congestion.

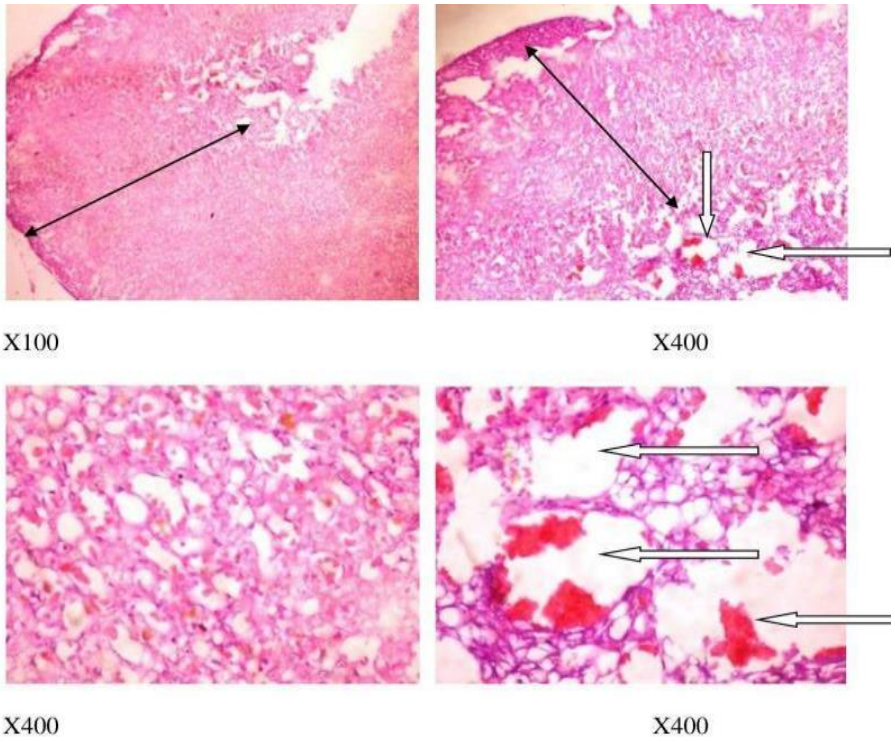

Plate 4. Adrenal gland from female social control group (H&E stain). Moderately normal cortical zonation; severe medullary vascular dilatation and congestion.

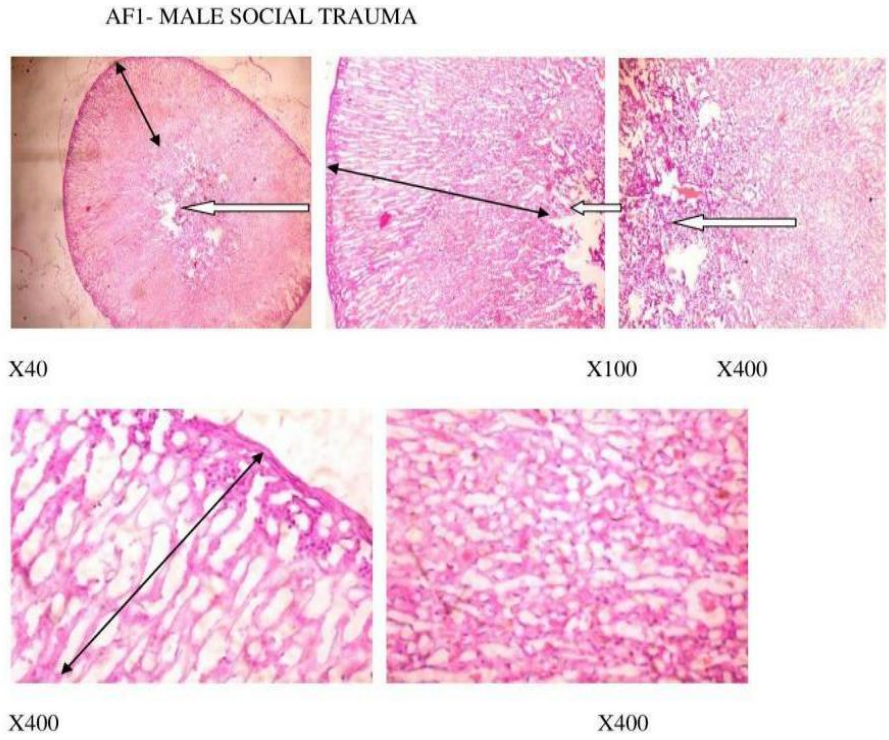

Plate 5. Adrenal gland from male social trauma group (H&E stain). Poor cortical zonation; poorly stained cytoplasm in zona glomerulosa.

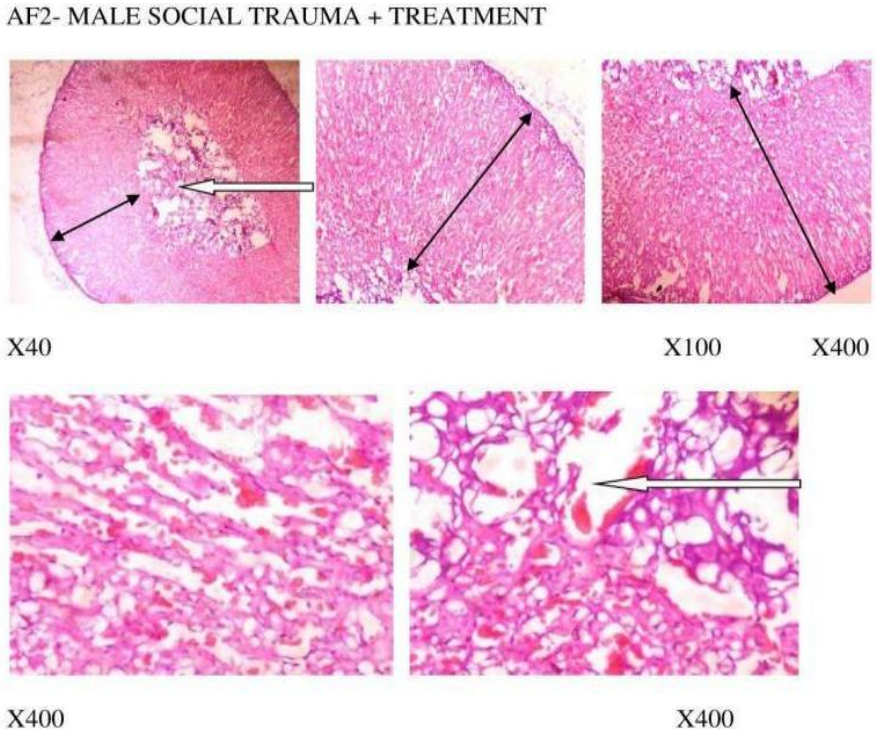

Plate 6. Adrenal gland from male social trauma + treatment group (H&E stain). Normal cortical zonation; mild medullary vascular dilation.

In the male social trauma group (Plate 5), the cortex showed poor zonation with poorly stained cytoplasm in the zona glomerulosa, while the medullary layer appeared normal. The male social trauma plus treatment group (Plate 6) showed restoration of normal cortical zonation with mild medullary vascular dilation and congestion.

The male social treatment only group (Plate 7) showed poor zonation with pale and ballooned cytoplasm in the zona glomerulosa and swollen medullary cells. The male social control (Plate 8) displayed moderate cortical zonation with a normal medullary appearance. This pattern — near-normal architecture in the trauma-plus-treatment subgroup set against poor zonation in the trauma-only and treatment-only subgroups — mirrors the subregion-specific hyperplasia and hypertrophy that chronic HPA-axis activation is known to produce in the outer and inner zona fasciculata and medulla (Ulrich-Lai et al., 2006).

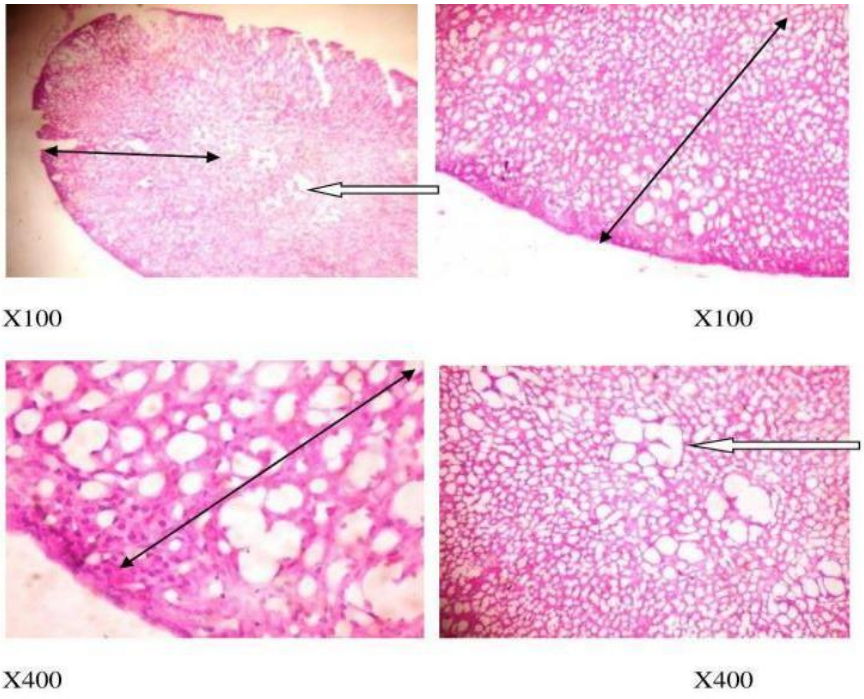

Plate 7. Adrenal gland from male social treatment only group (H&E stain). Poor cortical zonation; swollen medullary cells.

In the male physical trauma group (Plate 9), a moderate pattern of cortical zonation was observed with pale and swollen cells in the zona glomerulosa and swollen medullary cells. The male physical trauma plus treatment group (Plate 10) displayed normal cortical and medullary patterns, indicating the protective effect of Amaranthus cruentus extract. The male physical treatment only group (Plate 11) showed poorly preserved cortical zonation and mild haemorrhage in the parenchyma. The male physical control (Plate 12) showed moderately normal cortical zonation with severe medullary vascular dilatation and congestion. These findings are consistent with prior reports of stress-induced adrenal remodelling — cortical cell hypertrophy, altered cytoplasmic lipid content, and medullary vascular congestion reflecting heightened hormonal demand — in other rodent models of chronic and post-traumatic stress (Ulrich-Lai et al., 2006; Tseilikman et al., 2021).

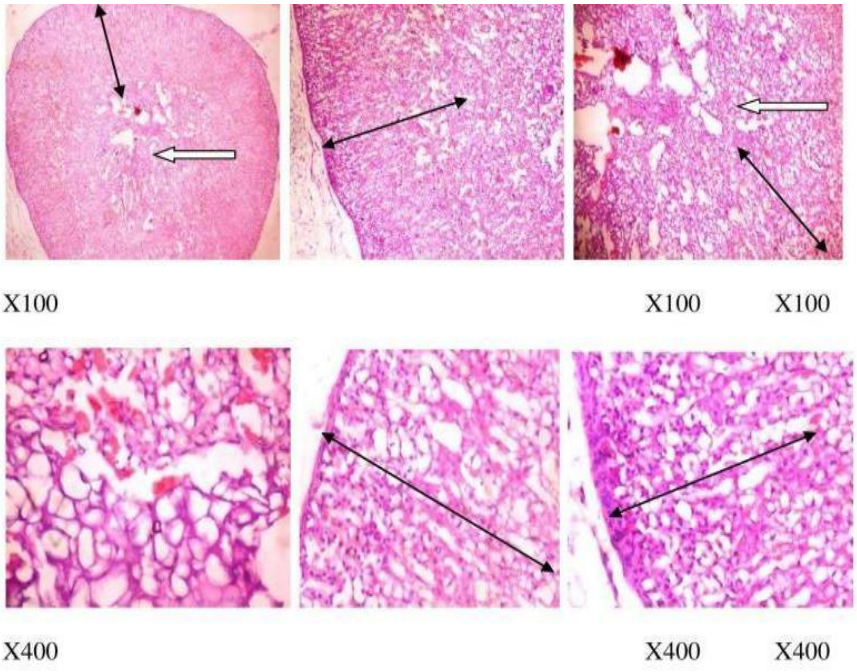

Plate 8. Adrenal gland from male social control group (H&E stain). Moderate cortical zonation; normal medullary layer.

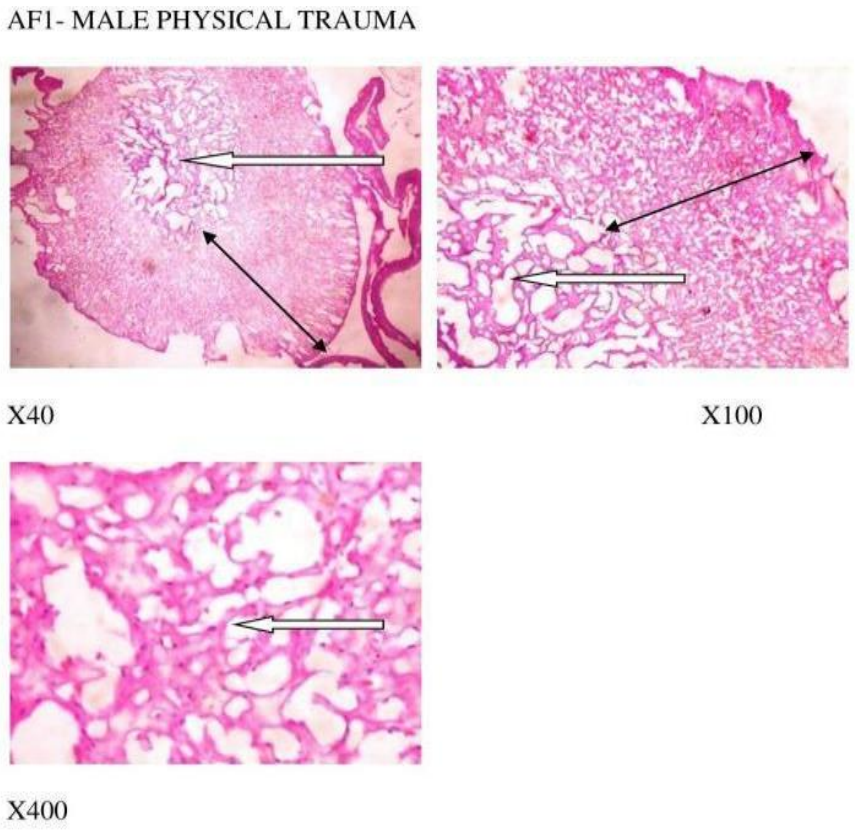

Plate 9. Adrenal gland from male physical trauma group (H&E stain). Moderate cortical zonation; swollen medullary cells.

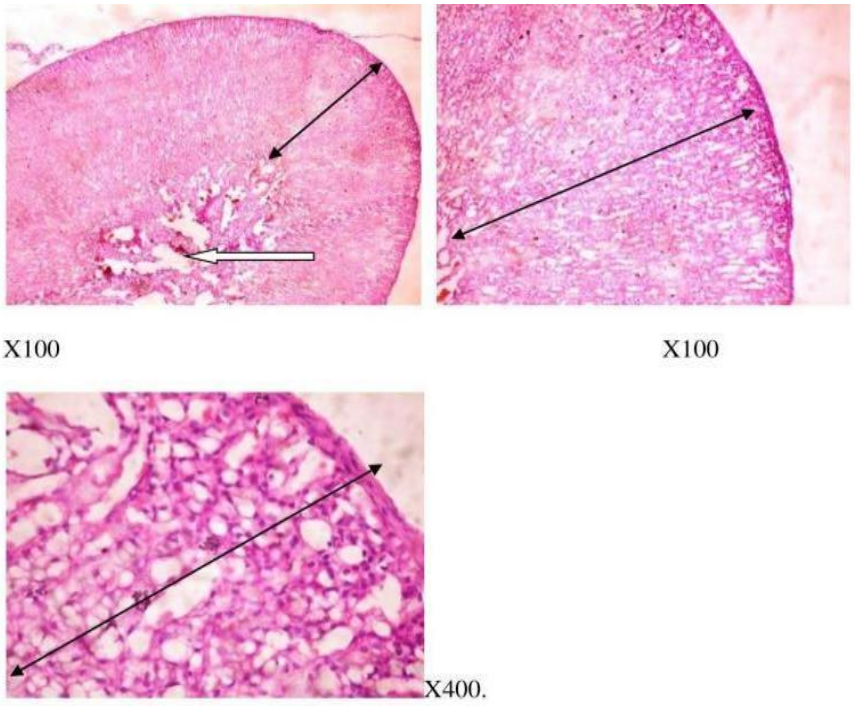

Plate 10. Adrenal gland from male physical trauma + treatment group (H&E stain). Normal cortical zonation; normal medullary layer.

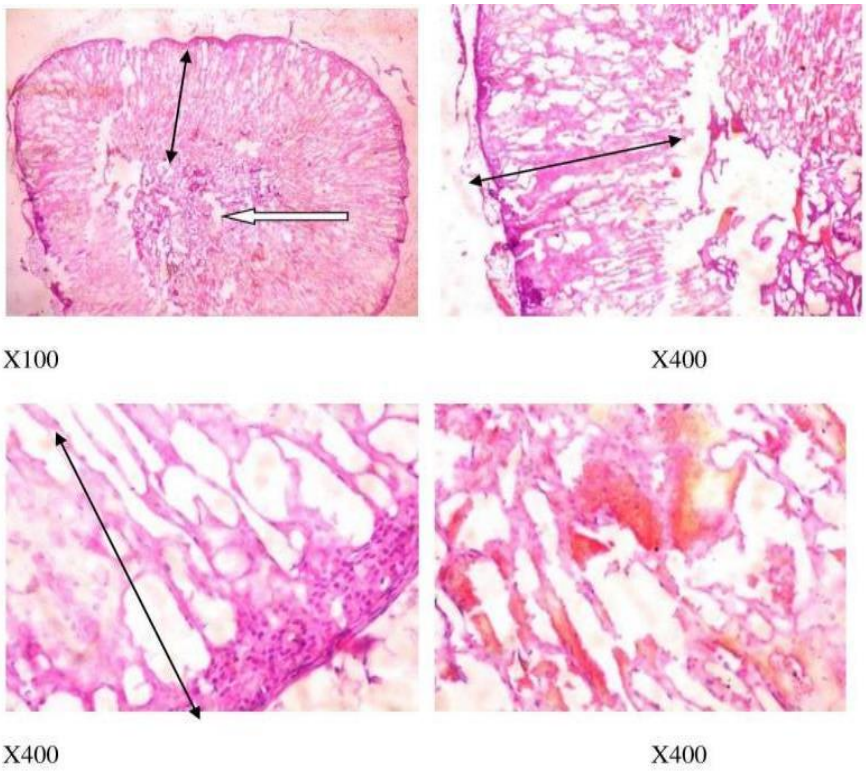

Plate 11. Adrenal gland from male physical treatment only group (H&E stain). Poorly preserved cortical zonation; mild haemorrhage.

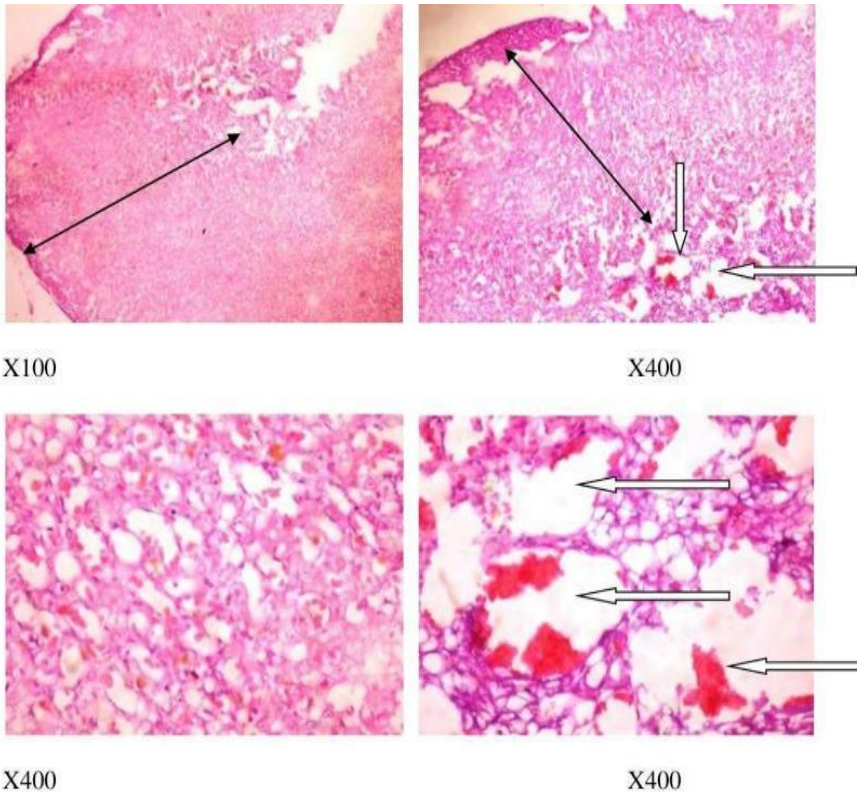

Plate 12. Adrenal gland from male physical control group (H&E stain). Moderately normal cortical zonation; severe medullary vascular dilatation and congestion.

3. 4. Chromaffin Cell Count

Chromaffin cell counts showed a consistent pattern of reduction in trauma-exposed groups compared to controls across all three experimental paradigms (Figures 12–14). In the female social group (Figure 12), the trauma only group had the lowest chromaffin cell count compared to the control, while the treatment only group had the highest count, suggesting that garlic treatment alone may promote chromaffin cell preservation. In the male social group (Figure 13), the trauma only group again showed the lowest count compared to the control, with the treatment only group recording the highest. The most striking reduction was observed in the male physical group (Figure 14), where the trauma only group had a markedly depleted chromaffin cell count compared to the control, with partial recovery in the trauma plus treatment group.

The depletion of chromaffin cells following trauma reflects sustained demand on the adrenal medulla to secrete epinephrine and norepinephrine in response to persistent stress stimuli, potentially leading to cellular exhaustion and structural damage. Because chromaffin cells rely on a tightly coupled, stimulus-driven biosynthetic and secretory machinery to sustain catecholamine output, prolonged or repeated activation of this pathway under chronic stress can plausibly exceed the cells' capacity for compensatory turnover (Berends et al., 2019). The more pronounced depletion in the physically induced trauma group may be attributed to the more acute and severe nature of TBI compared to social stressors, which tends to produce a more intense initial catecholamine surge.

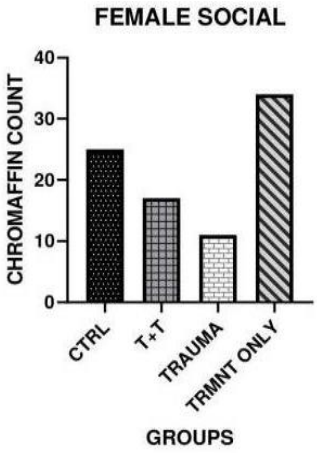

Figure 12. Chromaffin cell count in the female social group. Trauma reduces chromaffin cells compared to control.

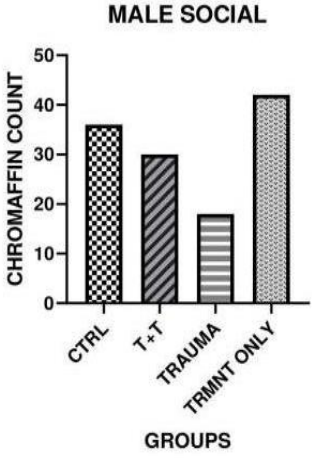

Figure 13. Chromaffin cell count in the male social group. Trauma reduces chromaffin cells compared to control.

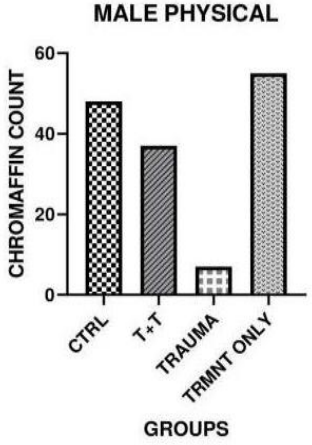

Figure 14. Chromaffin cell count in the male physical group. Dramatic depletion in trauma only group.

These findings are consistent with reports of phenotype-associated morphological change and adrenal hypofunction in rats exposed to chronic predator-based psychosocial stress (Tseilikman et al., 2021) and with the altered, persistently blunted glucocorticoid responses documented in the same predator-stress PTSD model (Zoladz et al., 2021), together supporting the broader body of evidence linking trauma to impaired adrenal function and neuroendocrine dysregulation.

The partial protective effects observed with the natural interventions used in this study are consistent with their documented antioxidant and anti-inflammatory actions. Garlic's principal organosulfur compound, allicin, exerts antioxidant and neuroprotective effects by down-regulating NADPH-oxidase-mediated reactive oxygen species production and supporting redox-sensitive cytoprotective pathways (Nadeem, Kazmi, Ullah, Muhammad & Anwar, 2021); allicin has additionally been shown to attenuate depressive-like behaviour and hippocampal neuroinflammation in a chronic social defeat stress model specifically by suppressing NLRP3 inflammasome activity (Gao et al., 2019), a mechanism plausibly relevant to the partial chromaffin-cell preservation observed in the garlic-treated female social groups of the present study. Cellgevity, administered to the male social group, contains the glutathione precursor Riboceine (D-ribose-L-cysteine), which is designed to raise intracellular glutathione availability and thereby buffer stress-induced oxidative damage, consistent with the relatively preserved chromaffin cell counts seen in the male social treatment-only subgroup. Similarly, *Amaranthus cruentus* extracts are rich in phenolic and flavonoid compounds that exhibit substantial free-radical-scavenging activity and induce Nrf2-mediated antioxidant enzyme expression across multiple models of oxidative injury (Park, Sharma & Lee, 2020), consistent with the improved locomotor exploration and partial chromaffin cell recovery observed in the Amaranthus-treated male physical group. Future studies should explore the molecular mechanisms underlying chromaffin cell loss, including apoptotic and necrotic pathways, and the potential neuroprotective effects of these natural compounds on adrenal tissue integrity in greater molecular depth.

4. CONCLUSIONS

The findings of this study demonstrate that both socially and physically induced trauma significantly alter the structural and molecular characteristics of the adrenal gland in Rattus norvegicus. Histopathological examination revealed changes in cortical zonation patterns, focal necrosis, medullary congestion, and cellular swelling in trauma-exposed animals compared to controls. Chromaffin cells, which serve as the primary mediators of the stress hormone response, were markedly reduced following trauma induction across all experimental groups, with the most severe depletion observed in physically traumatized male rats. Behavioural assessments confirmed significant increases in anxiety-like behaviour and reduced exploratory activity in trauma-exposed animals. The administration of natural extracts including garlic, Cellgevity, and Amaranthus cruentus showed partial protective effects on adrenal structure and behaviour. These results underscore the destructive impact of trauma on adrenal gland integrity and function and are consistent with the wider literature linking chronic HPA-axis dysregulation to immune imbalance and systemic disease risk (Nunez et al., 2025), highlighting the potential of natural interventions in mitigating trauma-induced adrenal dysfunction in mammalian models.

References

[1] Barroca, N. C. B., Baes, C. V. W., Martins-Monteverde, C. M. S., Bosaipo, N. B., da Silva Umeoka, M. S., Tejada, J., Antunes-Rodrigues, J., de Castro, M., Juruena, M. F., Garcia-Cairasco, N., & de Lima Umeoka, E. H. (2021). Evaluation of the HPA axis' response to pharmacological challenges in experimental and clinical early-life stress-associated depression. *eNeuro,* 8(1), https://doi.org/10.1523/ENEURO.0222-20.2020

[2] Berends, A. M. A., Eisenhofer, G., Fishbein, L., van der Horst-Schrivers, A. N. A., Kema, I. P., Links, T. P., Lenders, J. W. M., & Kerstens, M. N. (2019). Intricacies of the molecular machinery of catecholamine biosynthesis and secretion by chromaffin cells of the normal adrenal medulla and in pheochromocytoma and paraganglioma. *Cancers,* 11(8), 1121

[3] Cerqueira, M. M. F., Castro, M. M. L., Vieira, A. A., Kurosawa, J. A. A., Amaral Junior, F. L., Mendes, F. C. C. S., & Sosthenes, M. C. K. (2023). Comparative analysis between Open Field and Elevated Plus Maze tests as a method for evaluating anxiety-like behavior in mice. *Heliyon,* 9(4), e14522

[4] Davies, D. R., Olson, D., Meyer, D. L., Scholl, J. L., Watt, M. J., Manzerra, P., Renner, K. J., & Forster, G. L. (2016). Mild traumatic brain injury with social defeat stress alters anxiety, contextual fear extinction, and limbic monoamines in adult rats. *Frontiers in Behavioral Neuroscience*, 10, 71

[5] Fesharaki-Zadeh, A., & Datta, D. (2024). An overview of preclinical models of traumatic brain injury (TBI): Relevance to pathophysiological mechanisms. *Frontiers in Cellular Neuroscience,* 18, 1371213

[6] Gao, W., Wang, W., Liu, G., Zhang, J., Yang, J., & Deng, Z. (2019). Allicin attenuated chronic social defeat stress induced depressive-like behaviors through suppression of NLRP3 inflammasome. *Metabolic Brain Disease,* 34(1), 319-329

[7] Iacobellis, G., Rossi, G. P., Castinetti, F., & Letizia, C. (2015). Diseases of adrenal glands. *International Journal of Endocrinology,* 2015, 403521

[8] Kim, J., Kang, H., Lee, Y. B., Lee, B., & Lee, D. (2023). A quantitative analysis of spontaneous alternation behaviors on a Y-maze reveals adverse effects of acute social isolation on spatial working memory. *Scientific Reports,* 13, 14722

[9] Lei, A. A., Phang, V. W. X., Lee, Y. Z., Kow, A. S. F., Tham, C. L., Ho, Y. C., & Lee, M. T. (2025). Chronic stress-associated depressive disorders: The impact of HPA axis dysregulation and neuroinflammation on the hippocampus — A mini review. *International Journal of Molecular Sciences,* 26(7), 2940

[10] Mumtaz, F., Khan, M. I., Zubair, M., & Dehpour, A. R. (2018). Neurobiology and consequences of social isolation stress in animal model — A comprehensive review. *Biomedicine & Pharmacotherapy*, 105, 1205-1222

[11] Nadeem, M. S., Kazmi, I., Ullah, I., Muhammad, K., & Anwar, F. (2021). Allicin, an antioxidant and neuroprotective agent, ameliorates cognitive impairment. *Antioxidants,* 11(1), 87

[12] Nunez, S. G., Rabelo, S. P., Subotic, N., Caruso, J. W., & Knezevic, N. N. (2025). Chronic stress and autoimmunity: The role of HPA axis and cortisol dysregulation. *International Journal of Molecular Sciences,* 26(20), 9994

[13] Pai, A., Suris, A. M., & North, C. S. (2017). Posttraumatic stress disorder in the DSM-5: Controversy, change, and conceptual considerations. *Behavioral Sciences,* 7(1), 7

[14] Park, S. J., Sharma, A., & Lee, H. J. (2020). A review of recent studies on the antioxidant activities of a third-millennium food: Amaranthus spp. *Antioxidants,* 9(12), 1236

[15] Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments,* (96), e52434

[16] Shalev, A., Cho, D., & Marmar, C. R. (2024). Neurobiology and treatment of posttraumatic stress disorder. *American Journal of Psychiatry,* 181(8), 705-719

[17] Shapira, Y., Shohami, E., Sidi, A., Soffer, D., Freeman, S., & Cotev, S. (1988). Experimental closed head injury in rats: Mechanical, pathophysiologic, and neurologic properties. *Critical Care Medicine,* 16(3), 258-265

[18] Tseilikman, V., Komelkova, M., Kondashevskaya, M. V., Manukhina, E., Downey, H. F., Chereshnev, V., Chereshneva, M., Platkovskii, P., Goryacheva, A., Pashkov, A., Fedotova, J., Tseilikman, O., Maltseva, N., Cherkasova, O., Steenblock, C., Bornstein, S. R., Ettrich, B., Chrousos, G. P., & Ullmann, E. (2021). A rat model of post-traumatic stress syndrome causes phenotype-associated morphological changes and hypofunction of the adrenal gland. *International Journal of Molecular Sciences,* 22(24), 13235

[19] Ulrich-Lai, Y. M., Figueiredo, H. F., Ostrander, M. M., Choi, D. C., Engeland, W. C., & Herman, J. P. (2006). Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. *American Journal of Physiology-Endocrinology and Metabolism,* 291(5), E965-E973

[20] Zoladz, P. R., Del Valle, C. R., Smith, E. A., Goodman, C. S., Dodson, J. L., Elmouhawesse, K. M., Kasler, C. D., & Rorabaugh, B. R. (2021). Glucocorticoid abnormalities in female rats exposed to a predator-based psychosocial stress model of PTSD. *Frontiers in Behavioral Neuroscience,* 15, 675206