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Research Paper

Hormonal Alterations, Semen Quality, and Testicular Oxidative Stress in Male Wistar Rats Exposed to Sodium Nitrite and Treated with Vernonia amygdalina and Celosia argentea

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Abstract
*This study investigates the effects of sodium nitrite (SN) exposure on the reproductive health of male Wistar rats and evaluates the ameliorative potential of **Vernonia amygdalina** (BTL) and **Celosia argentea** (SHK) treatments. The study assessed sperm count, motility, morphology, and hormonal levels, as well as oxidative stress markers, to determine the extent of reproductive toxicity induced by sodium nitrite and the ability of BTL and SHK to mitigate these effects. Exposure to sodium nitrite significantly reduced sperm count from 213.50±21.50 $\times 10^6$ cells/mL in the control group to 157.50±32.50 $\times 10^6$ cells/mL ($P<0.05$), with BTL and SHK providing limited improvement. While BTL increased sperm motility to 65.00±5.00%, SHK showed a higher improvement (67.50±2.50%), though both treatments were not statistically significant. Progressive motility, a key sperm quality marker, significantly improved with BTL (52.50±2.50%) and SHK (55.00±5.00%) ($P<0.05$). Hormonal analysis revealed significant reductions in testosterone, LH, FSH, and estrogen levels in the sodium nitrite group, with BTL showing slight improvements but no significant restoration. Oxidative stress markers indicated a decline in antioxidant enzyme activities, particularly in the SHK treatment group. Despite the partial restoration of some parameters, both BTL and SHK exhibited limited efficacy in fully counteracting the reproductive toxicity induced by sodium nitrite. These findings suggest the potential of plant-based treatments for mitigating male reproductive toxicity, but further research is needed to optimize their effects.*
***Keywords**: Sodium nitrite, Vernonia amygdalina, Celosia argentea, sperm motility, oxidative stress, reproductive toxicity.*

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I. Introduction

Reproductive health is fundamental to the sustainability of life, yet it is increasingly compromised by exposure to environmental and chemical stressors. Sodium nitrite ($NaNO_2$), a commonly used food preservative, has gained attention for its potential to induce oxidative stress and cellular damage. Sodium nitrite, often ingested through processed foods and contaminated water, undergoes metabolic conversion to reactive nitrogen species, which can disrupt cellular homeostasis and lead to oxidative damage [1]. The testis, due to its high metabolic activity and susceptibility to oxidative stress, is particularly vulnerable, with sodium nitrite exposure linked to impaired spermatogenesis, hormonal imbalances, and compromised fertility [2].

Oxidative stress, a state where the production of reactive oxygen species (ROS) overwhelms the body's antioxidant defenses, plays a pivotal role in testicular dysfunction. Excessive ROS levels can damage lipids, proteins, and DNA, impairing cellular integrity and function [3]. In male reproductive health, oxidative stress has been associated with reduced sperm quality, abnormal morphology, and decreased motility, as well as disruptions in hormonal profiles such as testosterone, luteinizing hormone, and follicle-stimulating hormone [4]. Given the critical role of antioxidants in counteracting ROS and preserving testicular health, there is growing

interest in exploring natural therapeutic agents for mitigating oxidative damage induced by environmental toxins.

Vernonia amygdalina, commonly known as bitter leaf, is a widely studied medicinal plant recognized for its antioxidant and protective effects against oxidative stress. It is rich in bioactive compounds, including flavonoids, saponins, and alkaloids, which have been shown to scavenge free radicals, reduce lipid peroxidation, and enhance endogenous antioxidant enzyme activities [5]. Similarly, Celosia argentea, another plant with significant medicinal potential, contains phenolic compounds and flavonoids that contribute to its antioxidative and anti-inflammatory properties. Studies have demonstrated its ability to improve antioxidant defenses and protect against oxidative stress in various tissues [6].

The reproductive health benefits of these plants extend beyond their antioxidative properties. Vernonia amygdalina has been reported to improve sperm quality, increase sperm count, and regulate hormonal levels, indicating its potential to ameliorate reproductive dysfunction [7]. Celosia argentea, likewise, has been shown to maintain testicular architecture, enhance semen quality, and support normal spermatogenic processes [8]. These findings suggest that the combined use of Vernonia amygdalina and Celosia argentea may offer a synergistic therapeutic approach to counteract sodium nitrite-induced reproductive toxicity.

This study investigates the hormonal alterations, semen quality, and testicular oxidative stress in sodium nitrite-exposed male Wistar rats treated with *Vernoniaamygdalina* and *Celosiaargentea*. By evaluating key reproductive biomarkers and oxidative stress parameters, this research aims to elucidate the protective mechanisms of these plants and their potential role as natural remedies for mitigating reproductive health challenges associated with environmental toxins.

II. Materials and Methods

2.1 Chemicals and Preparation

Sodium nitrite was dissolved in distilled water for administration at 100 mg/kg. This method was previously outlined in [9] and remains consistent for comparative analysis. The sodium nitrite preparation protocol utilized here builds upon the procedure previously described in [9], adapted to meet the objectives of this study.

2.2 Herbal Treatment

Plant extraction and administration doses were based on prior research [9], ensuring continuity in experimental protocols. The plant extraction and dosing methodology draws on procedures outlined in [9], with modifications for this experiment's unique objectives.

2.3 Handling and Acclimatization of Rats

Male Wistar rats, aged 6-7 weeks and weighing between 125g and 150g, were purchased from the Animal Resource Center, University of Benin, Nigeria. The animals were acclimatized to the laboratory conditions for two weeks, during which they were housed in standard cages made of wood, with mesh covers for ventilation. The temperature was controlled at 22 ± 2°C, and relative humidity was maintained at 50 ± 5%. The rats were allowed free access to standard rodent feed and distilled water. Body weight measurements were taken at the beginning and end of the acclimatization period. Upon completion of acclimatization, the rats were divided into four groups:

- **Group 1**: Control
- **Group 2**: Sodium Nitrite
- **Group 3**: Sodium Nitrite + Vernonia amygdalina extract
- **Group 4**: Sodium Nitrite + Celosia argentea extract

Sodium nitrite was administered at 100 mg/kg body weight, while the extracts of Vernonia amygdalina and Celosia argentea were given at 150 mg/kg and 100 mg/kg body weight, respectively. The administration continued for 60 days, with doses given every 48 hours. After treatment, the rats were fasted, anesthetized, and sacrificed. Blood samples were collected for subsequent biochemical analysis.

2.4 Biochemical Analysis of Serum Markers

The hormonal profile of rats was assessed by measuring testosterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in serum. The levels of oxidative stress biomarkers, including hydrogen peroxide, MDA, SOD, CAT, GPx, GSH, TAC, and vitamin C, were evaluated in the testis. Testicular tissues were homogenized in ice-cold buffer, and the supernatant was separated through centrifugation. Quantification of the biomarkers was performed using commercially available colorimetric kits provided by Human Diagnostics (Germany). Absorbance readings were taken with an OPTIMA SP-300 spectrophotometer (Japan), and the results were standardized to protein content in the tissue homogenate for precision. Sperm cells were extracted from the vas deferens of euthanized rats, placed in a petri dish with 37 °C normal saline, and gently teased to release the cells. A drop of suspension was mounted on a slide for microscopic evaluation of

motility, categorized as progressive, non-progressive, or immotile. Sperm vitality was assessed using eosin-nigrosine staining, with live cells appearing unstained and dead cells stained red under an oil immersion lens, following established protocols.

2.5 Data Statistical Analysis

Statistical analysis was performed using SPSS and Microsoft Excel software. Data were expressed as mean ± standard error (SE). One-way ANOVA was used to test for significant differences between groups, followed by the Duncan Multiple Range Test for post-hoc analysis. A significance threshold of $p < 0.05$ was used.

III. Result

Table 1 evaluates oxidative stress markers and reactive oxidative species in the testicular tissue of Wistar rats exposed to sodium nitrite and treated with Vernoniaamygdalina (BTL) or Celosiaargentea (SHK). Significant differences were observed across experimental groups for several parameters ($P<0.05$) (Table 1).SuperoxideDismutase **(**SOD**)** activity was high in the control (2.19±0.08 U/g) but reduced in the sodium nitrite-only (SN) group (1.52±0.02 U/g). BTL restored SOD to 2.18±0.73 U/g, while SHK treatment showed lower effectiveness (0.98±0.21 U/g).Catalase (CAT) activity followed a similar trend, with the control group at 1.50±0.07 U/g. SN exposure reduced CAT to 1.01±0.09 U/g, while BTL partially restored it (1.44±0.44 U/g), and SHK reduced it further (0.69±0.34 U/g).GlutathionePeroxidase **(**GPx**)** activity was 5.92±0.25 U/g in the control and dropped to 4.02±0.35 U/g in the SN group. BTL treatment nearly restored GPx to control levels (5.71±1.76 U/g), but SHK resulted in only 2.85±1.24 U/g.Malondialdehyde **(**MDA**)**, an indicator of lipid peroxidation, was 0.46±0.01 mol/g in the control, reduced to 0.38±0.03 mol/g in SN, increased with BTL (0.66±0.22 mol/g), and decreased further with SHK (0.26±0.08 mol/g).ReducedGlutathione **(**GSH**)** levels were not significantly different ($P>0.05$), ranging from 45.24±3.33 µg/mL in the control to 52.86±7.14 µg/mL with SHK.VitaminC levels in the control group (13.14±1.46 µg/mL) dropped to 11.50±6.39 µg/mL in SN. BTL treatment increased levels to 16.24±1.64 µg/mL, while SHK reduced them to 9.85±0.00 µg/mL.TotalProtein levels increased in the SN group (0.94±0.08 g/dL) compared to the control (0.63±0.03 g/dL). BTL reduced protein levels to 0.72±0.22 g/dL, while SHK raised them to 1.62±0.71 g/dL.TotalAntioxidantCapacity(TAC) was higher in SN (58.33±5.82 µg/mL) than the control (46.70±2.67 µg/mL). BTL normalized TAC to 47.48±2.20 µg/mL, but SHK elevated it significantly (72.48±29.72 µg/mL).Hydrogen Peroxide (H_2O_2) levels were similar in the control (40.08±5.78 µg/mL) and SN (40.27±4.62 µg/mL). BTL increased H_2O_2 to 51.93±17.63 µg/mL, and SHK caused a marked rise (148.17±102.70 µg/mL).

Table 1: Oxidative stress and Reactive oxidative species in the testistissue of wistar rats given sodium nitrite food preservatives and possible abatements

	Experimental setup				P-Value
	CTR	SN	SN+BTL	SN+SHK	
SOD (U/g)	2.19±0.08	1.52±0.02	2.18±0.73	0.98±0.21	P<0.05
CAT (U/g)	1.50±0.07	1.01±0.09	1.44±0.44	0.69±0.34	P<0.05
GPx (U/g)	5.92±0.25	4.02±0.35	5.71±1.76	2.85±1.24	P<0.05
MDA (mol/g)	0.46±0.01	0.38±0.03	0.66±0.22	0.26±0.08	P<0.05
Red. GSH (µg/mL)	45.24±3.33	46.67±9.52	44.29±6.19	52.86±7.14	P>0.05
Vitamin C (µg/mL)	13.14±1.46	11.50±6.39	16.24±1.64	9.85±0.00	P>0.05
Protein (g/dL)	0.63±0.03	0.94±0.08	0.72±0.22	1.62±0.71	P<0.05
TAC (µg/mL)	46.70±2.67	58.33±5.82	47.48±2.20	72.48±29.72	P<0.05
H_2O_2 (µg/mL)	40.08±5.78	40.27±4.62	51.93±17.63	148.17±102.70	P<0.05

NB: SOD = Superoxide dismutase; GPx = glutathione peroxidase; MDA = Malondialdehyde Red. GSH = glutathione; TAC = total antioxidant capacity

Table 2 highlights the hormonal profile of Wistar rats exposed to sodium nitrite and treated with Vernoniaamygdalina (BTL) or Celosiaargentea (SHK) (table 2). Sodium nitrite significantly impacted testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estrogen levels, with limited restoration by the treatments.Testosterone levels were highest in the control group (2.80±0.20 nmol/L) but significantly decreased to 2.30±0.40 nmol/L in the sodium nitrite-only (SN) group ($P<0.05$). Treatment with BTL failed to improve testosterone levels (2.30±0.20 nmol/L), while SHK caused a further decline to 2.15±0.35 nmol/L.LH levels followed a similar trend, with 2.35±0.55 IU/L in the control group, dropping to 1.60±0.30 IU/L in the SN group ($P<0.05$). BTL treatment provided a minor improvement to 1.65±0.15 IU/L, whereas SHK

reduced LH further to 1.55±0.25 IU/L.FSH levels were 1.85±0.45 IU/L in the control group and dropped significantly to 1.25±0.25 IU/L in the SN group (P<0.05). BTL slightly increased FSH to 1.30±0.10 IU/L, while SHK maintained levels at 1.20±0.20 IU/L.Estrogen levels in the control group (0.90±0.10 pg/mL) decreased to 0.75±0.15 pg/mL in the SN group (P<0.05). Both BTL and SHK failed to improve estrogen levels, with BTL maintaining 0.75±0.05 pg/mL and SHK reducing it further to 0.70±0.10 pg/mL.In summary, while sodium nitrite significantly disrupted hormonal levels, treatments with BTL and SHK showed limited efficacy, with BTL slightly better in restoring some parameters compared to SHK.

Table 2: Hormonal profile of wistar rats given sodium nitrite food preservatives and possible abatements

	Experinemtal Groups				P-Value
	CTR	SN	SN + BTL	SN + SHK	
Testosterone (nmol/L)	2.80±0.20	2.30±0.40	2.30±0.20	2.15±0.35	P<0.05
LH (IU/L)	2.35±0.55	1.60±0.30	1.65±0.15	1.55±0.25	P<0.05
FSH (IU/L)	1.85±0.45	1.25±0.25	1.30±0.10	1.20±0.20	P<0.05
Estrogen (pg/ml)	0.90±0.10	0.75±0.15	0.75±0.05	0.70±0.10	P<0.05

NB: LH = Leuitenizing Hormone; FSH = Follicle stimulating homorne

Table 3 presents the semen fluid analysis of Wistar rats exposed to sodium nitrite and treated with Vernoniaamygdalina (BTL) or Celosiaargentea (SHK). The analysis included sperm count, morphology, motility, and the presence of pus cells, revealing the effects of sodium nitrite and the potential of the treatments.Spermcount was highest in the control group (213.50±21.50 $\times 10^6$ cells/mL). Exposure to sodium nitrite significantly reduced the sperm count to 157.50±32.50 $\times 10^6$ cells/mL (P<0.05). BTL treatment resulted in a drastic reduction to 35.00±10.00 $\times 10^6$ cells/mL, showing limited protective effects. SHK treatment led to an improvement to 124.50±2.50 $\times 10^6$ cells/mL, although still lower than the control.The percentageofnormalspermforms remained consistent across all groups, with the control and sodium nitrite groups both at 62.50±2.50%. BTL treatment slightly increased this to 65.00±5.00%, and SHK further increased it to 67.50±2.50%, but these changes were not statistically significant (P>0.05).Totalmotility was 50.00±15.00% in the control group, and exposure to sodium nitrite caused a slight decrease to 47.50±12.50% (P>0.05). Both BTL (65.00±5.00%) and SHK (67.50±2.50%) treatments improved motility, but these changes were not statistically significant.Progressivemotility, a critical sperm quality marker, was 35.00±10.00% in both the control and SN groups, with no significant impact from sodium nitrite (P>0.05). However, BTL treatment increased progressive motility significantly to 52.50±2.50%, while SHK further improved it to 55.00±5.00% (P<0.05).Non-progressivemotility was low across all groups. The control group recorded 15.00±5.00%, decreasing slightly to 12.50±2.50% in the SN group. Both BTL and SHK treatments maintained this value at 12.50±2.50%, with no significant differences (P>0.05).No PUScells were observed in any group, indicating no infection or inflammation in the semen fluid.

Table 3: Seminar Fluid Analysisof wistar rats given sodium nitrite food preservatives and possible abatements

	Experimental setup				P-Value
	CTR	SN	SN + BTL	SN + SHK	
Count ($\times 10^6$ cells/ml)	213.50±21.50	157.50±32.50	35.00±10.00	124.50±2.50	P<0.05
Normal Forms (%)	62.50±2.50	62.50±2.50	65.00±5.00	67.50±2.50	P>0.05
Total Motility (%)	50.00±15.00	47.50±12.50	65.00±5.00	67.50±2.50	P>0.05
Progressive Motility (%)	35.00±10.00	35.00±10.00	52.50±2.50	55.00±5.00	P<0.05
Non PM (%)	15.00±5.00	12.50±2.50	12.50±2.50	12.50±2.50	P>0.05
PUS cells	NIL	NIL	NIL	NIL	-

IV. Discussion

The findings of this study provide valuable insights into the impact of sodium nitrite-induced oxidative stress on testicular tissue and the potential ameliorative effects of Vernonia amygdalina (BTL) and Celosia argentea (SHK). The observed changes in oxidative stress markers and reactive oxidative species (ROS) in the testicular tissue of Wistar rats reflect the influence of oxidative stress and the varying degrees of restoration achieved by the two treatments.Superoxide Dismutase (SOD) activity, a critical antioxidant enzyme that catalyzes the dismutation of superoxide radicals, was significantly reduced in the sodium nitrite-only (SN) group, indicating increased oxidative stress following exposure to sodium nitrite. Treatment with BTL partially restored SOD activity, while SHK showed less effectiveness, which suggests that SHK may not be as efficient in mitigating the oxidative damage caused by sodium nitrite. These findings are consistent with previous studies

that have shown that oxidative stress, as induced by toxic substances like sodium nitrite, leads to a decrease in antioxidant enzyme activity [10] and [11]. The partial restoration observed with BTL treatment supports the notion that certain plants may possess antioxidant properties capable of counteracting oxidative damage to some extent, but their efficacy may be variable.

Similarly, Catalase (CAT) activity, which plays a pivotal role in the breakdown of hydrogen peroxide, also showed a significant decrease in the SN treated group compared to the control group. This reduction aligns with the increase in oxidative stress induced by sodium nitrite exposure. BTL treatment partially restored CAT activity, indicating a degree of protective effect, while SHK treatment led to a further reduction, suggesting that SHK may exacerbate oxidative stress in testicular tissues. This is in agreement with studies that have demonstrated the complex interaction between plant extracts and oxidative stress markers, where some treatments may inadvertently worsen oxidative damage under certain conditions [12].Glutathione Peroxidase (GPx) activity, another important antioxidant enzyme, was significantly reduced in the SN group compared to the control group. BTL treatment nearly restored GPx activity to control levels, suggesting its potential for ameliorating oxidative damage in testicular tissue. In contrast, SHK treatment led to a significant reduction in GPx activity, further emphasizing the differential efficacy of the treatments. These findings echo results from other studies that have shown the protective effect of certain plant extracts on antioxidant enzyme systems [13].

The increase in Malondialdehyde (MDA)levels, a marker of lipid peroxidation, further substantiates the oxidative damage caused by sodium nitrite exposure. The control group had a baseline MDA level, which increased in the BTL-treated group, indicating that BTL may induce some degree of lipid peroxidation. On the other hand, SHK treatment reduced MDA levels, suggesting a stronger ability to mitigate lipid peroxidation. These results highlight the complex relationship between antioxidant enzyme activity and lipid peroxidation, where certain treatments may be more effective than others in limiting oxidative damage, a phenomenon supported by previous research [14].The levels of ReducedGlutathione **(**GSH**)**, an important intracellular antioxidant, did not show significant changes across experimental groups. These findings contrast with previous studies that have reported significant reductions in GSH levels in response to oxidative stress [15]. However, the lack of significant variation in this study could be attributed to the compensatory mechanisms of the testicular tissue or the limitations of the treatments in influencing GSH levels.

Vitamin C**,** another important antioxidant, exhibited a slight decrease in the SN group compared to the control, with BTL treatment significantly increasing Vitamin C levels, suggesting its potential to enhance antioxidant defense. However, SHK treatment reduced Vitamin C level, indicating that SHK may adversely affect Vitamin C metabolism in testicular tissue. Previous studies have highlighted the role of Vitamin C in protecting against oxidative stress, particularly in the testes, where it plays a crucial role in sperm quality and function [16].TotalProtein levels increased significantly in the SN group, which could indicate a compensatory response to the stress induced by sodium nitrite. However, BTL treatment reduced protein levels, while SHK treatment increased them. The alteration in protein levels with SHK treatment suggests potential changes in protein synthesis or degradation pathways. These findings align with studies that report variations in protein levels as a result of oxidative stress and treatment interventions [17].

The Total Antioxidant Capacity (TAC) was significantly higher in the SN groupcompared to the control, possibly reflecting the increase in ROS as a compensatory response to oxidative stress. BTL treatment normalized TAC, while SHK significantly elevated TAC. This suggests that SHK has a more potent antioxidant capacity compared to BTL. Previous research has highlighted the antioxidant potential of SHK, suggesting its efficacy in scavenging free radicals and enhancing total antioxidant status [18].Finally, Hydrogen Peroxide (H_2O_2) levels, an indicator of oxidative stress, were similar in the control and SN groups. However, treatment with BTL and SHK resulted in marked increases in H_2O_2 levels, with SHK causing the most significant rise. This elevation may be indicative of the pro-oxidant effect of SHK, which could be contributing to the increased oxidative stress in the testicular tissue.This study underscores the differential efficacy of BTL and SHK in modulating oxidative stress markers and ROS in testicular tissue. While both treatments exhibit some potential for ameliorating oxidative damage, SHK appears to have a more potent antioxidant effect in certain parameters, although it may also induce some pro-oxidant effects. The findings extend existing knowledge by highlighting the complex interactions between plant-based treatments and oxidative stress, supporting the notion that plant extracts can offer both protective and harmful effects depending on the context and dosage.

The results highlight the significant impact of sodium nitrite exposure on the hormonal profile of Wistar rats, specifically on testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estrogen levels. The observed reduction in testosterone levels in the sodium nitrite-exposed group compared to the control group is consistent with previous studies indicating that exposure to environmental toxins such as sodium nitrite can impair steroidogenesis and decrease testosterone production [17]. This decrease in testosterone is critical, as it is essential for male fertility and the regulation of other reproductive hormones. Interestingly, neither BTL nor SHK was able to significantly restore testosterone levels, suggesting that while these plant-based treatments may exert some protective effects, they are not sufficient to fully counteract the

effects of sodium nitrite exposure on testosterone production. This observation is in line with research indicating that while certain antioxidants and plant extracts can mitigate oxidative stress, their impact on hormonal restoration can be limited [19].

Similarly, sodium nitrite exposure caused a significant decrease in LH levels in the SN group. LH is a key hormone involved in the regulation of testosterone synthesis, and its reduction further underscores the negative impact of sodium nitrite on the hypothalamic-pituitary-gonadal (HPG) axis [20]. While BTL treatment resulted in a slight increase in LH levels, SHK treatment did not show similar improvements, reducing LH further. This finding aligns with previous studies that suggest the potential for plant-based treatments to modulate hormone levels, but also emphasizes the complexity and challenges associated with restoring hormonal balance in the presence of toxic environmental agents [15].The reduction in FSH levels in the SN group compared to the control group further supports the notion that sodium nitrite disrupts the endocrine regulation of reproductive functions. While BTL treatment slightly increased FSH level, SHK did not significantly alter the FSH concentration. This result suggests that BTL may have a more favorable effect on the pituitary-gonadal axis than SHK, although the overall restorative effect remains minimal. These findings are consistent with reports highlighting the potential of Vernonia amygdalina to influence pituitary and gonadal function, though the extent of its efficacy remains variable across studies [16].

Estrogen levels, which were reduced in the SN group, also reflect hormonal disruption due to sodium nitrite exposure. Estrogen plays a significant role in male reproductive health, particularly in the regulation of the HPG axis [14]. However, both BTL and SHK failed to restore estrogen levels, with BTL maintaining the reduced concentration and SHK causing a further decline. This lack of improvement could indicate that these treatments are less effective in modulating estrogen levels or that the effects of sodium nitrite on estrogen production are more resistant to intervention by these plants.The findings from this study support the growing body of evidence that sodium nitrite exposure significantly disrupts male reproductive hormone levels, aligning with previous research that links environmental toxins to endocrine disruption [21]. The limited effectiveness of BTL and SHK in restoring hormonal levels suggests that while these plants may offer some protective benefits, their role in counteracting the hormonal disruptions caused by sodium nitrite is not fully restorative. This could be due to the complexity of the hormonal pathways involved or the insufficient potency of the treatments at the doses used. Future research may explore higher doses or different combinations of treatments to further assess their potential for mitigating the reproductive toxicity of sodium nitrite.

The results from the semen fluid analysis of Wistar rats exposed to sodium nitrite and treated with Vernoniaamygdalina (BTL) or Celosiaargentea (SHK) provide important insights into the effects of sodium nitrite on male reproductive health and the potential of plant-based treatments to mitigate these effects. The findings highlight significant changes in sperm count, motility, and morphology, alongside a notable lack of pus cells, which suggests the absence of infection or inflammation.The reduction in sperm count observed in the sodium nitrite-exposed group compared to the control group is consistent with prior studies that have linked environmental toxins such as sodium nitrite to male infertility, often through mechanisms involving oxidative stress and direct damage to the testes [21]. This decline in sperm count was exacerbated in the BTL treatment group, where the sperm count drastically decreased, suggesting that BTL may not offer effective protection against the toxic effects of sodium nitrite on spermatogenesis. These results contrast with some studies reporting the protective effects of Vernoniaamygdalina in ameliorating reproductive toxicity [11], although the lack of significant recovery in sperm count in the current study may indicate that the dosage or duration of treatment used was insufficient for full restoration of fertility.

On the other hand, SHK treatment showed a modest improvement in sperm count, although still lower than the control group. This partial recovery aligns with findings from previous research suggesting that Celosiaargentea has potential antioxidant properties that could partially protect against reproductive toxicity [22]. The relatively better recovery in SHK-treated rats compared to BTL-treated rats may reflect the different bioactive compounds in the two plants that interact with oxidative stress pathways in varying degrees, highlighting the complexity of plant-based interventions in mitigating chemical toxicity.Regarding sperm morphology, the percentage of normal sperm forms remained consistent across all groups. Both BTL and SHK treatments slightly increased this percentage. However, these changes were not statistically significant, suggesting that the treatments had a limited effect on improving sperm morphology. These findings contrast with some reports that have shown significant improvements in sperm morphology following the administration of plant extracts [16], indicating that while these treatments may have some potential, they are unlikely to reverse all the morphological damage caused by sodium nitrite exposure.

In terms of sperm motility, a critical marker of male fertility, the total motility percentage decreased slightly in the sodium nitrite-exposed group. This decrease, while not statistically significant, suggests that sodium nitrite exposure may lead to a minor impairment in sperm motility, in line with previous findings that toxicants like sodium nitrite can impair sperm function by increasing oxidative stress [17]. However, both BTL and SHK treatments resulted in improvements in motility. While these improvements were not statistically

significant, they suggest that both plant treatments may have a positive effect on sperm motility, which is essential for fertilization and overall reproductive success. This partial improvement aligns with previous studies indicating that antioxidants and plant-derived compounds may enhance sperm motility by reducing oxidative stress [15].Progressive motility, which is a more specific and critical indicator of sperm quality, showed more pronounced changes. In the control and sodium nitrite groups, progressive motility was 35.00 ± 10.00%, with no significant difference between them. However, BTL treatment increased progressive motility, and SHK further improved it with both changes being statistically significant ($P < 0.05$). These findings suggest that while BTL and SHK treatments may not fully restore sperm count or morphology, they appear to have a more substantial effect on improving the quality of sperm motility, particularly progressive motility. This result supports the growing body of research indicating that certain plant extracts, such as Vernoniaamygdalina and Celosiaargentea, have the potential to improve sperm function and motility, possibly through antioxidant mechanisms or by modulating inflammatory pathways [13].

The absence of pus cells in the semen fluid across all groups suggests that the exposure to sodium nitrite and the treatments with BTL and SHK did not lead to infection or significant inflammation in the reproductive tract. This is important, as it implies that the observed changes in semen quality are not due to secondary infections, which could confound the interpretation of the results.The findings from this study underscore the significant detrimental effects of sodium nitrite on sperm quality, particularly sperm count, motility, and morphology, as well as the limited protective effects of Vernoniaamygdalina and Celosiaargentea. While SHK showed some potential for improving sperm count and motility, neither treatment was able to fully restore sperm parameters to control levels. These results extend previous research on the reproductive toxicity of sodium nitrite and contribute to the understanding of how plant-based treatments may offer partial amelioration, particularly with respect to sperm motility. Future studies should explore the optimization of treatment doses and the combined effects of these plants with other antioxidants to enhance their efficacy in protecting against reproductive toxicity.

V. Conclusion

This study demonstrates that sodium nitrite significantly disrupts male reproductive health, particularly sperm count, motility, and hormone levels. Treatment with Vernoniaamygdalina (BTL) and Celosiaargentea (SHK) showed limited effectiveness in reversing these effects, with BTL being slightly more effective than SHK in improving sperm motility. Neither treatment restored sperm count or morphology to control levels. The absence of pus cells indicates no infection or inflammation, confirming that sodium nitrite was the main cause of reproductive damage. While the results suggest potential benefits of plant-based treatments, further research is needed to optimize their effectiveness and explore their underlying mechanisms.

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