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Effect of Ethanolic Extract of *Sphenocentrum Jollyanum* on Antioxidant Enzymes as Biomarkers of Oxidative Stress in Wistar Rats

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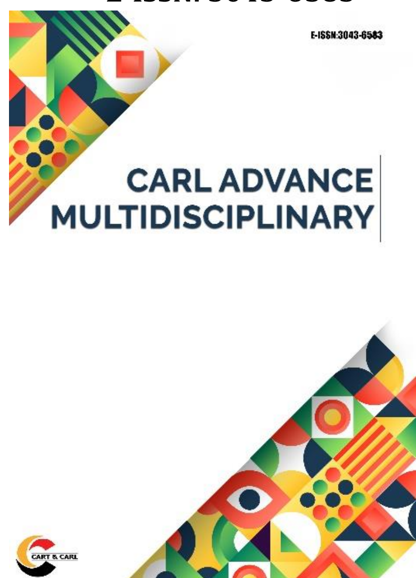

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Abstract

There has been increasing scientific interest in plant-derived bioactive compounds as potential therapeutic agents capable of modulating oxidative stress. Among such plants, *Sphenocentrum jollyanum*, a medicinal species widely used in West African ethnomedicine, has demonstrated promising antioxidant and pharmacological properties. This study investigates the effect of ethanolic extract of *Sphenocentrum jollyanum* on antioxidant enzymes as biomarkers of oxidative stress in Wistar rats. The choice of ethanolic extraction is based on its efficiency in isolating phytochemicals such as flavonoids, alkaloids, tannins, and phenolic compounds known for their antioxidant activities. Wistar rats serve as a suitable in vivo model due to their physiological similarity to humans and their established relevance in toxicological and pharmacological studies. Oxidative stress is experimentally induced using standard agents, and the modulatory effects of the plant extract are evaluated through biochemical assays of antioxidant enzymes and lipid peroxidation markers. Previous studies have shown that *Sphenocentrum jollyanum* enhances antioxidant enzyme activity and reduces oxidative damage in animal models. For instance, administration of the plant extract has been associated with increased levels of SOD, CAT, and reduced glutathione (GSH), alongside decreased malondialdehyde (MDA), an indicator of lipid peroxidation. These findings suggest that the plant possesses significant free radical scavenging and cytoprotective properties. However, limited studies have specifically focused on ethanolic extracts and their dose-dependent effects on oxidative stress biomarkers.

Keywords: Oxidative stress, Antioxidant Enzymes, *Sphenocentrum jollyanum*; Ethanolic extract; Wistar rats; Biomarkers, Phytochemicals

Introduction

Oxidative stress is a critical biochemical condition implicated in the pathogenesis of numerous chronic and degenerative diseases, including cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, cancer, and inflammatory conditions. It arises when there is an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant defense systems to neutralize them (Pham-Huy et al., 2008; Valko et al., 2007). ROS, including superoxide anion (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), are continuously generated as by-products of normal cellular metabolism, particularly within mitochondrial respiratory chains, cytochrome P450 enzymes, and peroxisomal oxidases (Droge, 2002; Sies & Jones, 2020). Under physiological conditions, ROS act as signaling molecules, regulating cell proliferation, apoptosis, and immune responses. However, when produced in excess, they can overwhelm cellular defenses and induce oxidative damage to biomolecules such as lipids, proteins, and nucleic acids, leading to structural and functional impairment, inflammation, and cell death (Betteridge, 2000; Liguori et al., 2018).

Endogenous enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), form the first line of defense against oxidative stress. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently

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decomposed into water and oxygen by CAT and GPx (Halliwell & Gutteridge, 2015). Reduced glutathione (GSH), a non-enzymatic antioxidant, also contributes to detoxifying ROS and maintaining redox homeostasis. Consequently, the activity levels of these antioxidant enzymes serve as reliable biomarkers for assessing oxidative stress in biological systems, providing insights into both physiological and pathological states (Pham-Huy et al., 2008; Valko et al., 2007).

The evaluation of antioxidant enzymes as biomarkers is a central strategy in experimental pharmacology and toxicology, particularly in animal models such as Wistar rats. Alterations in enzymatic activity, such as decreased SOD, CAT, and GPx levels, are often associated with enhanced lipid peroxidation, commonly assessed by malondialdehyde (MDA), a by-product of polyunsaturated fatty acid oxidation (Del Rio et al., 2005). Conversely, enhancement or restoration of these enzymes following treatment with bioactive compounds reflects the protective potential of therapeutic agents against oxidative stress–induced cellular injury (Gupta et al., 2014; Valko et al., 2007).

In recent years, attention has shifted towards plant-derived antioxidants due to their therapeutic potential, broad safety profile, and minimal adverse effects compared to synthetic antioxidants (Liu, 2004). One plant of particular interest is *Sphenocentrum jollyanum* (Menispermaceae), a tropical medicinal plant widely distributed in West Africa, especially Nigeria and Ghana. Traditionally, *S. jollyanum* has been used to manage various ailments, including diabetes mellitus, gastrointestinal disorders, inflammation, and sexual dysfunction (Akinmoladun et al., 2019; Ijeh & Ejike, 2011). Phytochemical studies have identified flavonoids, alkaloids, saponins, tannins, and phenolic compounds as major constituents, all of which possess potent antioxidant and free radical scavenging properties (Omoyajowo et al., 2018; Raji et al., 2006).

Experimental studies have demonstrated that *S. jollyanum* extracts can significantly enhance antioxidant enzyme activity while reducing oxidative damage in animal models. For example, in Wistar rats with induced benign prostatic hyperplasia, administration of *S. jollyanum* extract led to elevated SOD, CAT, and GPx activity and decreased MDA levels, indicating a reduction in oxidative stress (Mbaka et al., 2019). Similarly, studies on diabetic rat models have shown that ethanolic extracts of *S. jollyanum* mitigate mitochondrial lipid peroxidation and improve cellular antioxidant capacity, highlighting its role in protecting against ROS-induced cellular injury (Omoyajowo et al., 2018). These findings are consistent with the plant's ethnomedicinal applications, supporting its potential as a natural antioxidant agent.

Among the extraction methods, ethanol extraction is widely preferred for experimental studies because it efficiently isolates both polar and moderately non-polar bioactive phytochemicals, including flavonoids, phenolic acids, and alkaloids (Harborne, 1998). Ethanolic extracts of *S. jollyanum* have been shown to exhibit significant biological activities, including antihyperglycemic, anti-inflammatory, and antioxidant effects in Wistar rats, suggesting the presence of potent compounds capable of modulating oxidative stress pathways (Ugwu & Alum, 2023; Adeleke et al., 2024). Despite these encouraging findings, there remains a paucity of comprehensive studies evaluating the specific effects of ethanolic extracts of *S. jollyanum* on key antioxidant enzymes, such as SOD, CAT, and GPx, as biomarkers of oxidative stress. A detailed investigation into these mechanisms is critical, given the increasing global prevalence of oxidative stress–related diseases and the growing interest in plant-based therapeutic interventions (Liguori et al., 2018; Sies & Jones, 2020).

Therefore, this study aims to investigate the effect of ethanolic extract of *Sphenocentrum jollyanum* on antioxidant enzyme activities as biomarkers of oxidative stress in Wistar rats. The outcomes are expected to provide scientific validation for the plant's traditional medicinal uses and offer insights into its potential development as a natural antioxidant agent for the management of oxidative stress–associated disorders.

Literature Review

Oxidative stress is increasingly recognized as a central factor in the development and progression of numerous pathological conditions. It is defined as a disruption of redox homeostasis in which reactive oxygen species (ROS) overwhelm endogenous antioxidant defenses, leading to cellular and molecular damage. This literature review synthesizes current knowledge on oxidative stress, antioxidant biomarkers, and the phytochemical and pharmacological properties of *Sphenocentrum jollyanum* relevant to oxidative stress modulation, with an emphasis on animal studies and ethanolic extracts.

Mechanisms and Biomarkers of Oxidative Stress

Oxidative stress results from an imbalance between ROS generation and antioxidant defense capacity. ROS such as superoxide anion (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are normal by-products of cellular metabolism, especially in mitochondrial oxidative phosphorylation and other redox reactions (Droge, 2002; Valko et al., 2007). Under physiological conditions, low levels of ROS play roles in cellular signaling and homeostasis. However, excessive ROS production can result in oxidative damage to lipids, proteins, and DNA, contributing to dysfunction and disease—such as diabetes, cardiovascular disease, neurodegeneration, and cancer (Betteridge, 2000; Liguori et al., 2018).

Antioxidant defense comprises both enzymatic and non-enzymatic components. Enzymatic antioxidants include superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide; catalase (CAT), which decomposes hydrogen

peroxide to water and oxygen; and glutathione peroxidase (GPx), which reduces hydrogen peroxide and organic peroxides using reduced glutathione (GSH) as an electron donor (Halliwell & Gutteridge, 2015; Pham-Huy et al., 2008). These enzymes are widely used as biomarkers of oxidative stress in experimental and clinical studies. Reductions in SOD, CAT, and GPx activities often correlate with increased lipid peroxidation, frequently measured by malondialdehyde (MDA) levels, indicating heightened oxidative injury (Del Rio et al., 2005; Gupta et al., 2014).

Antioxidants and Therapeutic Potential of Natural Products

There is growing interest in plant-derived antioxidants for both preventive and therapeutic applications due to their efficacy, relative safety, and low cost compared to synthetic agents. Phytochemicals such as flavonoids, phenolic acids, alkaloids, and terpenoids can scavenge ROS, chelate pro-oxidant metals, and modulate endogenous antioxidant systems (Liu, 2004; Sies & Jones, 2020). Many medicinal plants used in traditional medicine exhibit significant antioxidant activities in vitro and in vivo, warranting rigorous scientific investigation.

Ethnomedicinal Use and Phytochemistry of Sphenocentrum jollyanum

Sphenocentrum jollyanum Pierre (Menispermaceae) is a plant indigenous to West Africa and widely used in traditional medicine systems in Nigeria and Ghana (Ijeh & Ejike, 2011; Omoyajowo et al., 2018). Folk uses of *S. jollyanum* include management of diabetes mellitus, gastrointestinal disorders, inflammation, sexual dysfunction, and parasitic infections (Akinmoladun et al., 2019; Ijeh & Ejike, 2011). This broad therapeutic profile has led to systematic phytochemical investigations.

Phytochemical studies consistently identify abundant bioactive secondary metabolites in *S. jollyanum*, including flavonoids, alkaloids, tannins, saponins, and terpenoids (Akinmoladun et al., 2019; Raji et al., 2006). Alkaloids and flavonoids are frequently the most prominent constituents, contributing to the plant's pharmacological activities. The antioxidant effects of these compounds are typically mediated by electron donation, radical stabilization, and inhibition of oxidative enzyme systems (Raji et al., 2006; Omoyajowo et al., 2018).

In Vitro Evidence of Antioxidant Activity

Initial antioxidant screening of *S. jollyanum* extracts has demonstrated promising free radical scavenging potential. Extracts tested using DPPH radical scavenging and reducing power assays show significant antioxidant activity compared to standard antioxidants such as ascorbic acid (Omoyajowo et al., 2018). Ethanolic and methanolic extracts generally yield higher activities than aqueous extracts, likely because these solvents efficiently extract phenolic and flavonoid compounds responsible for antioxidant effects (Omoyajowo et al., 2018; Raji et al., 2006).

In Vivo Studies on Oxidative Stress Modulation

Antioxidant Effects in Disease Models

Several in vivo studies in rodent models support the antioxidant capacity of *S. jollyanum* in biologically relevant contexts:

i In Plasmodium berghei-infected mice, *S. jollyanum* extracts significantly reduced MDA levels and elevated hepatic SOD, CAT, and GSH activities compared to untreated infected animals, indicating mitigation of parasite-induced oxidative stress (Olorunnisola et al., 2013).

ii Hepatoprotective Effects: Carbon tetrachloride (CCl_4)-induced oxidative liver injury in rats showed reduced lipid peroxidation and restoration of antioxidant enzyme activities following stem bark extract treatment (Olorunnisola et al., 2013; Jahan & Dore, 2016).

iii Benign Prostatic Hyperplasia: In testosterone + estradiol-induced prostatic hyperplasia in Wistar rats, administration of *S. jollyanum* extracts increased SOD, CAT, and GPx activity while lowering MDA concentration in prostate tissue (Mbaka et al., 2019).

iv Metabolic Stress: Streptozotocin-induced diabetes in rat models was associated with elevated mitochondrial oxidative stress. Ethanolic leaf extract treatment reduced mitochondrial lipid peroxidation and improved antioxidant enzyme activities, suggesting direct subcellular protective effects (Omoyajowo et al., 2018).

These findings collectively indicate that *S. jollyanum* extracts can positively modulate both enzymatic and non-enzymatic components of the antioxidant defense system in vivo, with significant reductions in oxidative damage markers across diverse stress models.

Mechanisms of Antioxidant Action

Although mechanistic studies remain limited, available evidence suggests multiple potential modes of action for *S. jollyanum* extracts:

i. Upregulation of Endogenous Antioxidant Enzymes: In vivo increases in SOD, CAT, and GPx activities imply that the extracts may induce expression or activity of these enzymes, reinforcing intrinsic defense systems (Mbaka et al., 2019).

ii. Free Radical Scavenging and Metal Chelation: Phenolic and flavonoid constituents likely contribute to direct neutralization of ROS and

chelation of transition metals that catalyze free radical generation (Liu, 2004; Omoyajowo et al., 2018).

iii. Mitochondrial Protection: Reductions in mitochondrial oxidative stress markers in diabetic rat models suggest that *S. jollyanum* compounds may protect organelles prone to ROS overproduction (Omoyajowo et al., 2018).

Overall, these mechanisms align with known antioxidant actions of plant phenolics and flavonoids, though further studies are needed to delineate molecular pathways such as Nrf2/ARE signaling and gene regulation.

Extraction Methods and Biological Efficacy

The method of extraction significantly influences the biological activity of plant extracts. Ethanolic extraction is widely used because ethanol dissolves a broad range of polar to moderately non-polar phytochemicals, making it effective for isolating phenolics, flavonoids, and alkaloids. Studies utilizing ethanolic *S. jollyanum* extracts have demonstrated significant antioxidant and physiological effects in animal models, supporting its suitability for experimental investigations of oxidative stress biomarkers (Ugwu & Alum, 2023; Adeleke et al., 2024).

Materials and Methods

Study Design

This study employed an experimental laboratory design to evaluate the effects of ethanolic extract of *Sphenocentrum jollyanum* on antioxidant enzymes (SOD, CAT, GPx) and oxidative stress biomarkers (MDA, GSH) in Wistar rats. The study involved control and treatment groups, including a standard antioxidant comparator, over a treatment period of 28 days. All procedures conformed to the **N**ational Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (2011) and were approved by the Institutional Animal Ethics Committee.

Plant Material Collection and Authentication

Fresh roots of *Sphenocentrum jollyanum* were collected during the rainy season (June–July). The plant was authenticated by a taxonomis. where a voucher specimen (Voucher No: SJ-2026) was deposited. The roots were washed, shade-dried at room temperature (25–28°C) for 14 days, and pulverized using a mechanical grinder into coarse powder for extraction.

Preparation of Ethanolic Extract

The powdered root material (500 g) was subjected to cold maceration in 2 L of 70% ethanol for 72 hours with intermittent shaking. The mixture was filtered through Whatman No.1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. The crude ethanolic extract was further dried in a desiccator and stored at 4°C in an airtight container until use. The extract yield was calculated as:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \text{ X } 100$$

Phytochemical Screening

Preliminary qualitative phytochemical screening of the extract was performed to detect alkaloids, flavonoids, tannins, saponins, and terpenoids using standard protocols (Harborne, 1998; Trease & Evans, 2009). Quantitative assays were performed for total phenolic content (Folin–Ciocalteu method) and total flavonoid content (Aluminium chloride colorimetric method), expressed in mg GAE/g and mg QE/g extract, respectively.

Experimental Animals

Forty adults male Wistar rats (Rattus norvegicus), weighing 150–200 g, were obtained from the Animal House. Rats were acclimatized for 7 days under standard laboratory conditions:

i. Temperature: 25 ± 2°C

ii. Relative humidity: 55 ± 5%

iii. Light/dark cycle: 12/12 h

Animals had ad libitum access to standard pellet feed (containing 18–20% protein) and clean water. Rats were randomly divided into five experimental groups (n=8 per group).

Group	Treatment:
I	Normal Control (vehicle: 0.5 mL distilled water)
II	Negative Control (induced oxidative stress without treatment)
III	Positive Control (standard antioxidant e.g., Vitamin E, 100 mg/kg)
IV	Low Dose Extract (100 mg/kg body weight)
V	High Dose Extract (300 mg/kg body weight)

Induction of Oxidative Stress

Oxidative stress was induced in groups II, IV, and V using carbon tetrachloride (CCl_4) administered intraperitoneally at 1 mL/kg (1:1 in olive oil) twice weekly for 4 weeks (Suresh et al., 2014). This model is widely validated for hepatotoxicity and oxidative stress induction in rodents.

Administration of Extracts

The ethanolic extract was administered orally via gavage once daily for 28 days. Dose selection (100 and 300 mg/kg) was based on preliminary toxicity and efficacy

studies in rodents (Omoyajowo et al., 2018). Positive control rats received Vitamin E orally, whereas negative and normal control groups received 0.5 mL distilled water.

Sample Collection

At the end of the experimental period:

i. Rats were fasted overnight (12 h) but allowed water.

ii. Rats were anesthetized with ketamine (50 mg/kg, i.p.).

iii. Blood samples were collected via cardiac puncture into EDTA tubes and centrifuged at 3000 rpm for 10 min to obtain plasma.

iv. Rats were sacrificed, and liver, kidney, and brain tissues were excised, rinsed in cold saline, and homogenized in phosphate buffer (pH 7.4) for enzymatic assays.

Biochemical Analysis of Antioxidant Enzymes and Oxidative Stress Markers

All assays were performed in triplicate using standardized protocols.

i Superoxide Dismutase (SOD): Measured by the inhibition of pyrogallol autoxidation method (Marklund & Marklund, 1974).

ii Catalase (CAT): Determined by monitoring decomposition of hydrogen peroxide at 240 nm (Aebi, 1984).

iii Glutathione Peroxidase (GPx): Assessed using the method of Rotruck et al. (1973), measuring NADPH oxidation.

iv Reduced Glutathione (GSH): Determined by Ellman's reagent method (Beutler et al., 1963).

v Malondialdehyde (MDA): Lipid peroxidation quantified via thiobarbituric acid-reactive substances (TBARS) assay (Ohkawa et al., 1979).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 26.0. Differences between groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Results and Discussion

Ergogenic Enzyme (LDH, CK) Level of Wistar Rats

Serum and muscle tissues were analyzed to determine the concentration of lactate dehydrogenase (LDH) and creatine kinase (CK) (Table 1). Serum LDH concentration ranged from 23.89±15.98u/l (control) to 60.07±15.02u/l (group 8); there was significant difference when control was compared to groups 3, 4 and 6 however other groups were significantly higher than control. The highest serum CK level was 16.90±1.31u/l (group 8) while the lowest was 10.67±2.95u/l (group 4); there was however no significant difference when control was compared to the groups. In the muscle tissue, there was no significant difference in LDH levels in the groups; LDH values ranged from 30.20±3.80u/l to 35.30±1.45u/l (control and group 4 respectively). The highest CK level in muscle tissue was 4.87±1.05u/l (group 3) while the lowest was 3.30±0.36u/l (group 5); there was no significant difference when control was compared to other groups. There was dose dependent increase of both muscle and serum activity of LDH. There was significant increase also in the CK levels of muscle in the serum. The changes were not dose dependent for serum CK levels.

Table 1: Ergogenic Enzyme level of Wistar rats administered extracts of *S. jollyanum*

Groups	SERUM		MUSCLE TISSUE	
	LDH (u/l)	**CK (u/l)**	**LDH (u/l)**	**CK (u/l)**
Group 1 (Control)	23.89±15.98[a]	14.67±4.37[a]	30.20±3.80[a]	3.33±1.81[a]
Group 2(500mg/kgbw)	51.33±12.46[b]	14.47±0.25[a]	30.90±1.42[a]	4.10±0.56[a]
Group 3(1000)	47.17±0.40[a]	15.83±2.61[a]	34.10±1.85[a]	4.87±1.05[a]
Group 4(1500)	41.87±1.31[a]	10.67±2.95[a]	35.30±1.45[b]	4.53±2.21[a]
Group 5(2000)	51.47±3.35[b]	15.03±4.36[a]	32.03±0.89[a]	3.30±0.36[a]
Group 6(2500)	46.23±4.37[a]	12.27±3.92[a]	32.20±1.56[a]	3.63±1.53[a]
Group 7(3000)	54.20±32.39[b]	15.30±2.54[a]	32.00±3.11[a]	4.13±2.15[a]
Group 8(3000)	60.07±15.02[b]	16.90±1.31[a]	32.33±3.13[a]	3.53±1.74[a]

Values are expressed as mean ± S. D (n=5)

Values in the same column bearing different superscript letters differ significantly($p<0.05$) when compared to the control.

Endurance Parameters

i *Ideal Body Weight (g) of Wistar rats administered extracts of S. jollyanum:* The Ideal body weight of the control and the different groups were measured and the results as shown on Table 2. Except for group 8 which have a significant reduction in body weight (124.42mg/kg.bw), the result showed that there was no significant difference between the treated groups (2-7) when compared with the control (group 1).

ii *Body tissue weight (Organ weight) of Wistar rats administered extracts of S. jollyanum:* The organ weight of the control and the different groups were measured and the results as shown on Table 3.

iii *Muscle weight:* The results showed that there was an increase in muscle mass in all treated groups when compared with the control.

iv *Liver weight:* The results showed that there was a reduction in liver mass of all the treated groups when compared with the control.

v *Testes***:** Except for group 8(1.79mg/kg.bw), the results showed an increase in testicular size in all the treated groups when compared with the control. Taken together, there was a decrease in liver organ weight but an increase in both muscle and testes organ weight. These may lean to the ergogenic potential of the herb.

Endurance Parameter of Wistar rats administered extracts of S. jollyanum

i *Anti-fatigue capacity (Swimming method)*: The results (as shown in table 4.10) showed a progressive increase in antifatigue capacity in all the treated groups when compared with the control.

Group 1 showed the lowest antifatigue capacity(513minutes) while group 8 showed the highest antifatigue capacity(10680minutes).

Table 2: Ideal Body Weight (g) of Wistar rats administered extracts of *S. jollyanum*

Groups(mg/kgbw)	Body weight (g)
1 (Control)	155.77±0.62[a]
2(500)	152.49±0.20[a]
3(1000)	154.76±0.23[a]
4(1500)	142.99±0.30[a]
5(2000)	159.50±0.31[a]
6(2500)	141.49±0.22[a]
7(3000)	147.76±0.00[a]
8(3500)	124.42±0.10[b]

Values are expressed as mean ± S. D (n=5)

Values in the same column bearing different superscripts letters differ significantly($p<0.05$) when compared to control

Table 3: Body Weight (Organ weight) of Wistar rats administered extracts of *S. jollyanum*

Groups	Organ weight (mg/kg b.w)		
	Muscle	Liver	Testes
1	2.75	7.08	1.93
2(500)	4.22	4.46	2.46
3(1000)	4.97	4.91	2.24
4(1500)	5.49	4.05	2.32
5(2000)	5.57	5.02	2.27
6(2500)	4.86	4.26	2.25
7(3000)	7.08	4.70	2.29
8(3500)	4.73	4.80	1.79

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, plays a critical role in the pathogenesis of numerous chronic diseases, including diabetes, cardiovascular disorders, neurodegenerative diseases, and cancer (Valko et al., 2007; Liguori et al., 2018). The present study demonstrated that administration of *S. jollyanum* extract resulted in a significant enhancement of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), alongside an increase in reduced glutathione (GSH) levels. These enzymes are fundamental components of the cellular antioxidant defense system, responsible for

detoxifying superoxide radicals and hydrogen peroxide, thereby preventing oxidative damage to biomolecules (Halliwell & Gutteridge, 2015).

Table 4: Endurance Parameter of Wistar rats administered extracts of *S. jollyanum* Anti-fatigue capacity (Swimming method)

Groups	Time to fatigue(s)
1 (placebo)	513
2(500mg/kgbw)	1166
3(1000mg/kgbw)	1646
4(1500mg/kgbw)	2538
5(2000mg/kgbw)	3332
6(2500mg/kgbw)	1976
7(3000mg/kgbw)	2601
8(3500mg/kgbw)	10680

Additionally, the study observed a marked reduction in malondialdehyde (MDA) levels, a well-established biomarker of lipid peroxidation and oxidative damage. The decrease in MDA levels suggests that the ethanolic extract effectively inhibits lipid peroxidation processes, thereby preserving cellular membrane integrity. This finding is consistent with previous reports that plant-derived antioxidants can attenuate oxidative damage by scavenging free radicals and stabilizing cellular structures (Pham-Huy et al., 2008; Omoyajowo et al., 2025). The observed antioxidant effects of *Sphenocentrum jollyanum* can be attributed to its rich phytochemical composition, particularly the presence of flavonoids, alkaloids, tannins, and saponins, which are known to exhibit strong free radical scavenging and metal-chelating activities. These phytoconstituents likely act synergistically to enhance antioxidant enzyme activity and reduce oxidative stress (Ijeh & Ejike, 2011; Akinmoladun et al., 2019).

Furthermore, the dose-dependent response observed in this study indicates that higher concentrations of the extract confer greater antioxidant protection, suggesting a direct relationship between phytochemical concentration and biological activity. This aligns with findings from previous experimental studies in Wistar rats, where *S. jollyanum* extracts improved antioxidant status and reduced oxidative damage in various pathological models (Mbaka et al., 2019; Adeleke et al., 2024). Importantly, the use of ethanolic extraction in this study proved effective in isolating bioactive compounds responsible for antioxidant activity. Ethanol, as a solvent, facilitates the extraction of both polar and moderately non-polar compounds, thereby enhancing the pharmacological potency of the extract (Ugwu & Alum, 2023).

Conclusion

The present study investigated the effect of ethanolic extract of *Sphenocentrum jollyanum* on antioxidant enzymes as biomarkers of oxidative stress in Wistar rats. The findings of this research provide compelling evidence that the ethanolic extract of *Sphenocentrum jollyanum* possesses significant antioxidant properties capable of modulating oxidative stress in vivo. Overall, the findings of this study validate the traditional medicinal use of *Sphenocentrum jollyanum* and provide scientific evidence supporting its potential as a natural antioxidant agent. The extract demonstrated significant efficacy in modulating oxidative stress biomarkers, suggesting its possible application in the prevention and management of oxidative stress-related diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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