

## Comparative analysis of plant-derived antioxidants and their impact on endocrine function in male Wistar Rats

EJENADIA Humphrey and IBEZUTE Albert Chukwuemeka \*

Department of Environmental Management and Toxicology, College of Sciences, Federal University of Petroleum Resources, P.M.B. 1221, Effurun, Delta State, Nigeria.

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### Abstract

This study evaluated the impact of aqueous leaf extracts from five vegetables—*Solanum aethiopicum*, *Amaranthus hybridus*, *Pterocarpus mildraedii*, *Ocimum gratissimum*, and *Telfairia occidentalis*—on oxidative stress and endocrine function in male Wistar rats. Fresh leaves were collected from Effurun, Delta State, and processed by air-drying at  $30 \pm 2^\circ\text{C}$  for two weeks, followed by aqueous extraction (25 g in 250 mL water over 48 h) and freeze-drying. Male rats (125–150 g) were acclimatized for two weeks and then randomly allocated into a control group and five treatment groups. Each treatment group received one extract at three dose levels (100, 200, or 300 mg/kg body weight) via oral gavage every 48 h for 60 days. In control animals, malondialdehyde (MDA) levels averaged  $26.55 \pm 0.02$ , while administration of *S. aethiopicum* reduced MDA to  $23.49 \pm 0.00$  at the low dose, with similar reductions observed for *P. mildraedii* ( $23.70 \pm 0.01$ ) and *T. occidentalis* ( $23.60 \pm 0.04$ ). Superoxide dismutase (SOD) activity increased from  $3.91 \pm 0.03$  in controls to  $5.85 \pm 0.01$  with *S. aethiopicum* at low dose, and catalase (CAT) activity rose from  $155.71 \pm 0.04$  to  $164.36 \pm 0.02$ . Glutathione peroxidase (GPx) activity increased from  $104.31 \pm 0.02$  to  $120.94 \pm 0.10$ . Hormonal assays revealed that follicle-stimulating hormone (FSH) increased from  $2.30 \pm 0.02$  to  $3.10 \pm 0.02$  and luteinizing hormone (LH) from  $0.26 \pm 0.04$  to  $0.43 \pm 0.02$ ; notably, *T. occidentalis* and *O. gratissimum* elicited the most pronounced endocrine responses. Testosterone levels nearly doubled from  $3.87 \pm 0.05$  to  $7.42 \pm 0.03$  with *S. aethiopicum*. Statistical analysis confirmed that both dose and extract type significantly influenced these biomarkers ( $p < 0.05$ ). These findings support the potential use of these plant extracts as natural therapeutic agents in managing oxidative stress and endocrine disorders.

**Keywords:** Aqueous Leaf Extract; Oxidative Stress; Endocrine Function; Wistar Rats; Malondialdehyde; Antioxidant Enzymes; Reproductive Hormones

### 1. Introduction

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses of the body, is increasingly recognized as a central factor in the development and progression of various pathological conditions, including those that affect the endocrine system (Valko *et al.*, 2007). Lipid peroxidation, a process in which ROS damage cell membranes, can be reliably assessed by measuring malondialdehyde (MDA) levels, a biomarker that has been extensively used to gauge oxidative damage (Lobo *et al.*, 2010). Elevated MDA levels are indicative of cellular stress and have been linked to various diseases, emphasizing the importance of effective antioxidant defenses. In recent decades, considerable attention has turned to plant-derived antioxidants, owing to their rich content of bioactive compounds such as polyphenols and flavonoids. These compounds are known to scavenge free radicals and boost the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Rice-Evans *et al.*, 1997; Pandey & Rizvi, 2009). Numerous studies have

\* Corresponding author: IBEZUTE Albert Chukwuemeka

demonstrated that these natural antioxidants can mitigate oxidative damage, thereby contributing to improved cellular function and overall health.

Concurrently, emerging research has begun to elucidate the intricate relationship between oxidative stress and endocrine function. Specifically, oxidative stress has been shown to impact the hypothalamic-pituitary-gonadal axis, thereby modulating the secretion of key reproductive hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (Agarwal *et al.*, 2012). Disruptions in these hormonal pathways can lead to compromised reproductive health and may contribute to the development of endocrine disorders. The present study, titled "Comparative Analysis of Plant-Derived Antioxidants and Their Impact on Endocrine Function in Male Wistar Rats," aims to bridge these two domains by exploring the effects of various aqueous leaf extracts on both oxidative stress and reproductive hormone profiles in male Wistar rats. Male Wistar rats serve as an ideal model due to their well-characterized physiology and extensive use in toxicological and pharmacological research. In this investigation, the effects of different doses of several plant extracts were evaluated by monitoring a panel of biomarkers. These include MDA for oxidative stress; SOD, CAT, and GPx as indicators of antioxidant enzyme activity; and FSH, LH, and testosterone as measures of endocrine function.

## 2. Materials and Methods

### 2.1. Collection, Identification, and Preparation of Plant Extracts

Fresh leaves of *Solanum aethiopicum* (SA), *Amaranthus hybridus* (AH), *Pterocarpus mildraedii* (PM), *Ocimum gratissimum* (OG), and *Telfaria occidentalis* (TO) were procured from a local market in Effurun, Delta State. The taxonomic identities of these plants were verified by the Department of Environmental Management and Toxicology at the College of Sciences, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria. Phytochemical analyses of the leaf powders were conducted according to standard protocols described by Sofowora (1982), Trease and Evans (1989), Kokate *et al.* (2008), and Harborne (1988).

For this study, only the leaf extracts were used. Fresh leaves were dried in the laboratory at  $30 \pm 2^\circ\text{C}$  until crisp—a process that took approximately two weeks. The dried leaves were first coarsely ground with a pestle and mortar and subsequently pulverized into a fine powder using a Viking Exclusive Joncod machine (Model: YLH2M2-4). Twenty-five grams of powdered leaves from each plant were extracted with 250 mL of water over a 48-hour period. The resulting extract was filtered through sterile Whatman No. 1 filter paper and then dried into a solid form using a freeze dryer. The dried extract was reconstituted in distilled water to obtain the desired concentrations for the study.

Previous research (Olayemi & Adeleke, 2009; Dhellot *et al.*, 2006; Okokon *et al.*, 2007; Njoku *et al.*, 2011; Imosemi, 2018) has reported that the estimated LD<sub>50</sub> values for the extracts of SA, AH, PM, OG, and TO in Wistar rats exceed 5000 mg/kg body weight, suggesting that these extracts exhibit low acute toxicity. Based on these findings, the concentrations used in this study were chosen to be within a safe range.

### 2.2. Collection and Acclimatization of Experimental Rats

Male Wistar rats, aged 6–7 weeks and weighing between 125 and 150 g, were obtained from the Anatomy Department of the University of Benin, Nigeria. The rats were acclimatized for two weeks under standard laboratory conditions ( $22 \pm 2^\circ\text{C}$ , 12-hour light/dark cycle) until they reached 8–9 weeks of age, at which point their weights were recorded. Animals were housed separately by gender in wooden cages with wire mesh covers and provided with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo State, Nigeria) and distilled water ad libitum.

Following acclimatization, the rats were randomly allocated into six groups: one control group (Group A) and five treatment groups corresponding to the five plant extracts—SA (Group B), AH (Group C), PM (Group D), OG (Group E), and TO (Group F). Each treatment group was further subdivided into three dosing subgroups: low (100 mg/kg body weight), medium (200 mg/kg body weight), and high (300 mg/kg body weight). The extract was administered via oral gavage once every 48 hours. Prior to each administration, the extract solution was thoroughly shaken to ensure uniform distribution of particles.

The rats were maintained under standard laboratory conditions with free access to food and water for 60 days. At the end of the treatment period, the animals were fasted overnight and then sacrificed under light anesthesia. Blood samples were collected from a large vein using a sterile syringe and transferred into plain tubes. After clotting, the blood was centrifuged to separate the serum, which was stored at  $-80^\circ\text{C}$  until further analysis.

### 2.3. Laboratory Analysis

Serum biochemical markers were measured to assess various health indicators. Hormonal levels—including testosterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH)—were determined to establish hormonal profiles. Additionally, hydrogen peroxide levels were measured to assess reactive oxygen species. Markers of oxidative stress, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), total antioxidant capacity (TAC), vitamin C, and protein levels, were evaluated. All biomarkers were determined using standard ready-to-use assay kits, following the manufacturers' instructions, with absorbance readings taken using a spectrophotometer (OPTIMA SP-300, Japan).

### 2.4. Data Analysis

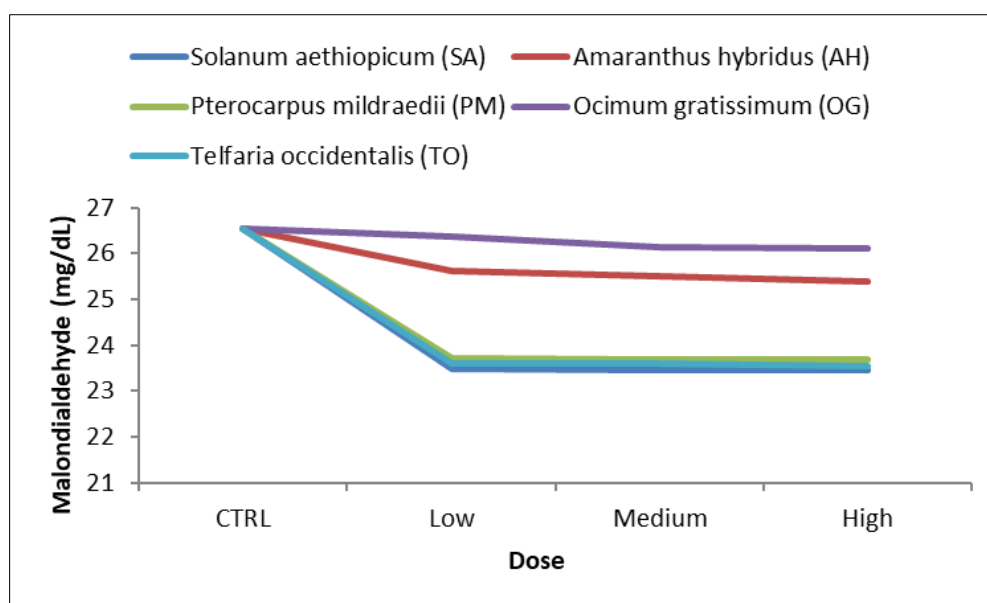
Statistical analyses were performed using SPSS and Microsoft Excel. Data are presented as mean  $\pm$  standard error (SE). Two-way ANOVA was used to compare differences between groups. A p-value of less than 0.05 was considered statistically significant.

## 3. Result

This study comprehensively investigated the impact of various aqueous leaf extracts on both oxidative stress parameters and endocrine function in male Wistar rats. The investigation evaluated several biomarkers—including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) for oxidative stress, as well as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone for reproductive function—using a range of doses across different plant extracts.

### 3.1. Oxidative Stress Evaluation

Figure 1 presents the MDA results, where a consistent baseline of  $26.55 \pm 0.02$  in untreated rats provided a reference for lipid peroxidation levels. Upon treatment, extracts from *Solanum aethiopicum*, *Pterocarpus mildraedii*, and *Telfaria occidentalis* markedly reduced MDA levels even at low doses (e.g., *Solanum aethiopicum* reduced MDA to  $23.49 \pm 0.00$ ), with only minimal further decreases observed at medium and high doses. In contrast, *Amaranthus hybridus* and *Ocimum gratissimum* exhibited a more moderate or variable reduction, suggesting that the concentration or composition of bioactive antioxidant compounds, such as polyphenols and flavonoids, differs among these extracts. The two-way ANOVA confirmed that both the dose ( $F = 18.225$ ,  $p = 0.000$ , Partial Eta Squared = 0.732) and the type of extract ( $F = 19.848$ ,  $p = 0.000$ , Partial Eta Squared = 0.799) significantly influence MDA levels, with a significant interaction ( $F = 3.098$ ,  $p = 0.012$ , Partial Eta Squared = 0.650) indicating that the effectiveness of a given dose varies with the extract (table 1).



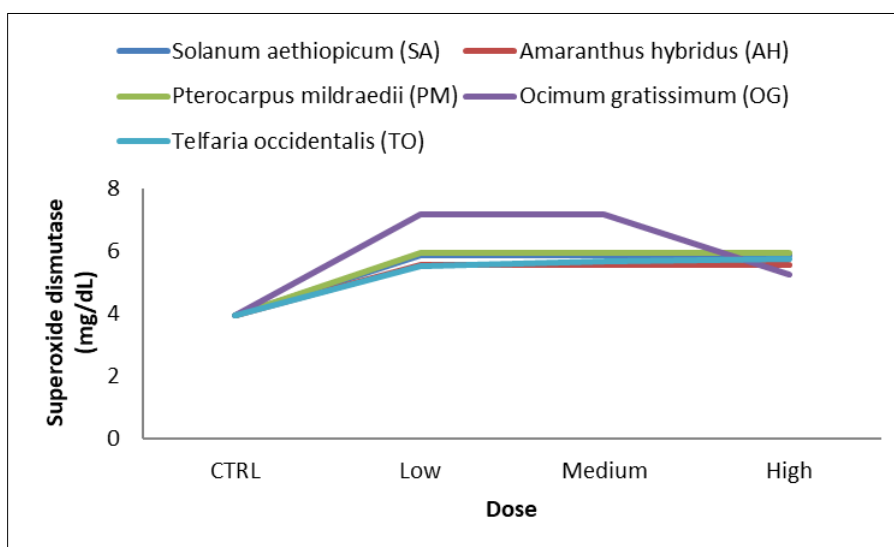
**Figure 1** Influence of different aqueous leaf extracts in Malondialdehyde (MDA) levels in male Wistar rats

**Table 1** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Malondialdehyde Levels

Source	F	Sig.	Partial Eta Squared
Dose	18.225	0.000	0.732
Vegetable type	19.848	0.000	0.799
Dose * Vegetable type	3.098	0.012	0.650

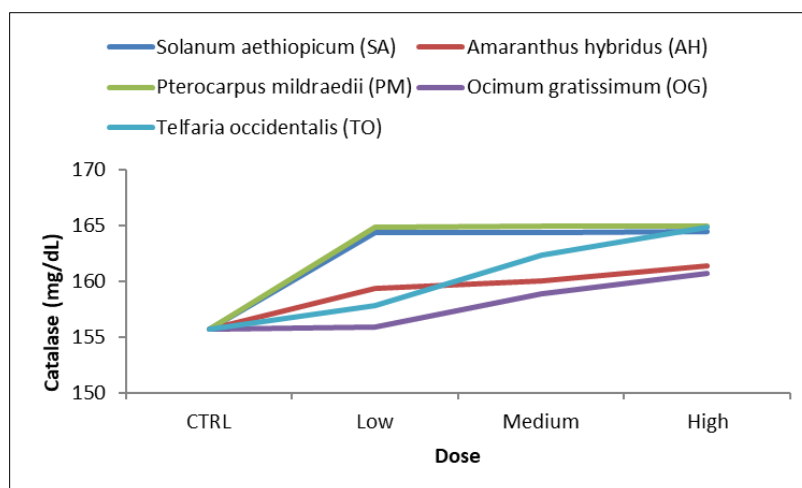
### 3.2. Antioxidant Enzyme Activity

In examining antioxidant defenses, Figure 2 shows that SOD activity increased from a baseline of  $3.91 \pm 0.03$  following extract administration. *Solanum aethiopicum*, *Amaranthus hybridus*, and *Pterocarpus mildraedii* produced a rapid and sustained increase in SOD activity at low doses, while *Telfaria occidentalis* demonstrated a gradual, dose-dependent rise. Interestingly, *Ocimum gratissimum* induced an initially high SOD response at low dose, but with considerable variability at medium dose and a decline at high dose. The ANOVA results ( $F = 23.517$ ,  $p = 0.000$ , Partial Eta Squared = 0.779 for dose;  $F = 1.578$ ,  $p = 0.219$ , Partial Eta Squared = 0.240 for extract;  $F = 0.973$ ,  $p = 0.504$ , Partial Eta Squared = 0.368 for interaction) underscore that dosage is the predominant factor driving SOD enhancement, with the extract type playing a secondary role (table 2).

**Figure 2** Influence of different aqueous leaf extracts in superoxide dismutase (SOD) levels in male Wistar rats**Table 2** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Malondialdehyde Levels

Source	F	Sig.	Partial Eta Squared
Dose	23.517	0.000	0.779
Vegetable	1.578	0.219	0.240
Dose * Vegetable	0.973	0.504	0.368

Further, Figure 3 details the catalase (CAT) activity, which increased significantly from a control level of  $155.71 \pm 0.04$ . Extracts such as *Solanum aethiopicum* and *Pterocarpus mildraedii* reached near-maximal CAT activity at low doses, implying a rapid enzyme induction possibly due to high concentrations of potent bioactive compounds. Conversely, *Amaranthus hybridus*, *Ocimum gratissimum*, and *Telfaria occidentalis* showed a more gradual, dose-dependent enhancement. The robust significance of both dose ( $F = 104.191$ ,  $p = 0.000$ , Partial Eta Squared = 0.940) and extract type ( $F = 34.574$ ,  $p = 0.000$ , Partial Eta Squared = 0.874), along with their interaction ( $F = 9.553$ ,  $p = 0.000$ , Partial Eta Squared = 0.851), highlights that both factors are critical in modulating CAT activity (table 3).

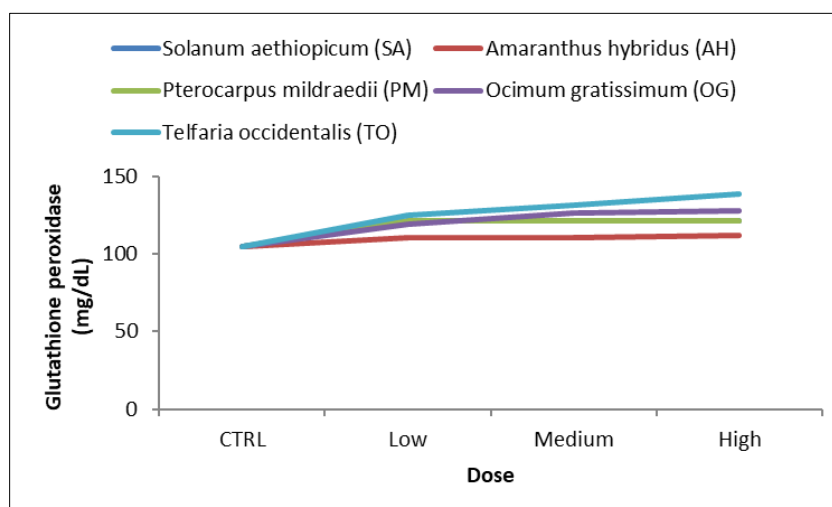


**Figure 3** Influence of different aqueous leaf extracts in Catalase (CAT) levels in male Wistar rats

**Table 3** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Catalase Levels

Source	F	Sig.	Partial Eta Squared
Dose	104.191	0.000	0.940
Vegetable	34.574	0.000	0.874
Dose * Vegetable	9.553	0.000	0.851

Figure 4 focuses on glutathione peroxidase (GPx) activity, with the control group registering  $104.31 \pm 0.02$ . Extracts from *Solanum aethiopicum* and *Pterocarpus mildraedii* elicited near-maximal GPx activity at low doses, whereas *Amaranthus hybridus* induced only a modest increase. Notably, *Telfaria occidentalis* demonstrated a clear dose-dependent increase, reaching the highest GPx activity at high doses. The ANOVA analysis (dose:  $F = 3.796$ ,  $p = 0.026$ , Partial Eta Squared = 0.363; extract:  $F = 6.449$ ,  $p = 0.002$ , Partial Eta Squared = 0.563; interaction:  $F = 1.302$ ,  $p = 0.291$ , Partial Eta Squared = 0.439) indicates that both the administered dose and the extract type significantly affect GPx activity, although the dose-response pattern remains relatively consistent across extracts (table 4).



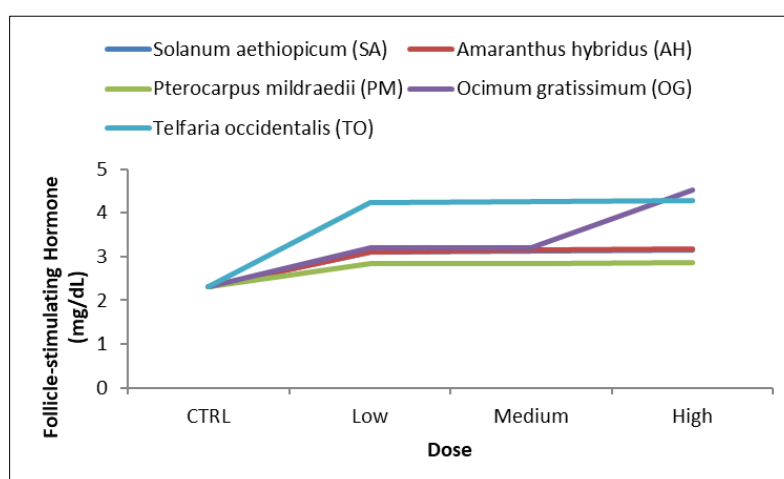
**Figure 4** Influence of different aqueous leaf extracts in Glutathione peroxidase (GPx) levels in male Wistar rats

**Table 4** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Malondialdehyde Levels

Source	F	Sig.	Partial Eta Squared
Dose	3.796	0.026	0.363
Vegetable	6.449	0.002	0.563
Dose * Vegetable	1.302	0.291	0.439

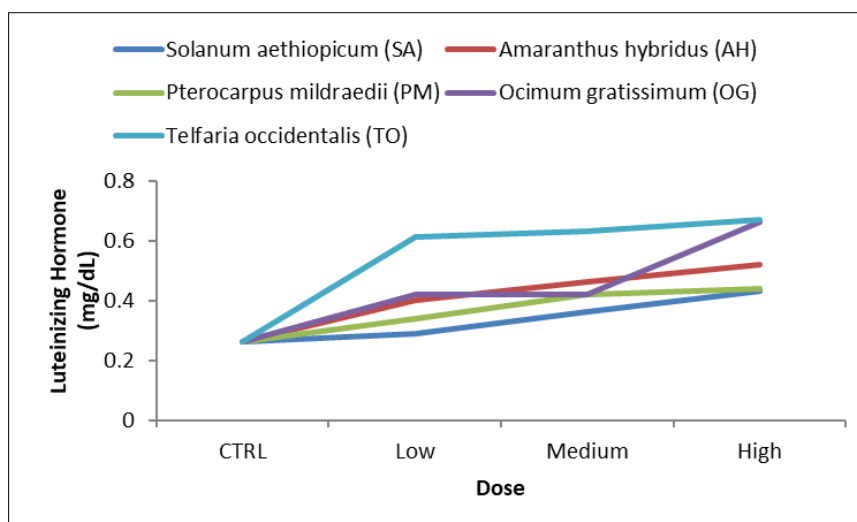
### 3.3. Endocrine Function Assessment

The study also evaluated reproductive hormone levels. As shown in Figure 5, FSH levels increased from a baseline of  $2.30 \pm 0.02$  upon treatment with the extracts. While *Solanum aethiopicum* and *Amaranthus hybridus* produced modest increases, extracts like *Ocimum gratissimum* and *Telfaria occidentalis* induced a pronounced elevation in FSH, particularly at higher doses. *Pterocarpus mildraedii*, in contrast, resulted in only a modest effect. The ANOVA results (dose:  $F = 13.498$ ,  $p = 0.000$ , Partial Eta Squared = 0.669; extract:  $F = 8.730$ ,  $p = 0.000$ , Partial Eta Squared = 0.636; interaction:  $F = 1.935$ ,  $p = 0.093$ , Partial Eta Squared = 0.537) confirm that both the extract type and dose are significant determinants of FSH modulation (table 5).

**Figure 5** Influence of different aqueous leaf extracts in Follicle stimulating Hormone (FSH) levels in male Wistar rats**Table 5** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Follicle stimulating Hormone (FSH) Levels

Source	F	Sig.	Partial Eta Squared
Dose	13.498	0.000	0.669
Vegetable type	8.730	0.000	0.636
Dose * Vegetable type	1.935	0.093	0.537

Similarly, Figure 6 demonstrates that LH levels increased in a dose-dependent manner from a baseline of  $0.26 \pm 0.04$ . Extracts from *Telfaria occidentalis* and *Ocimum gratissimum* exhibited particularly potent effects at higher doses, with *Telfaria occidentalis* showing the most robust increase. The corresponding ANOVA (dose:  $F = 13.506$ ,  $p = 0.000$ , Partial Eta Squared = 0.670; extract:  $F = 7.161$ ,  $p = 0.001$ , Partial Eta Squared = 0.589; interaction:  $F = 2.140$ ,  $p = 0.064$ , Partial Eta Squared = 0.562) supports the conclusion that both dose and extract type are critical, though the interaction effect is only marginally significant (table 6).

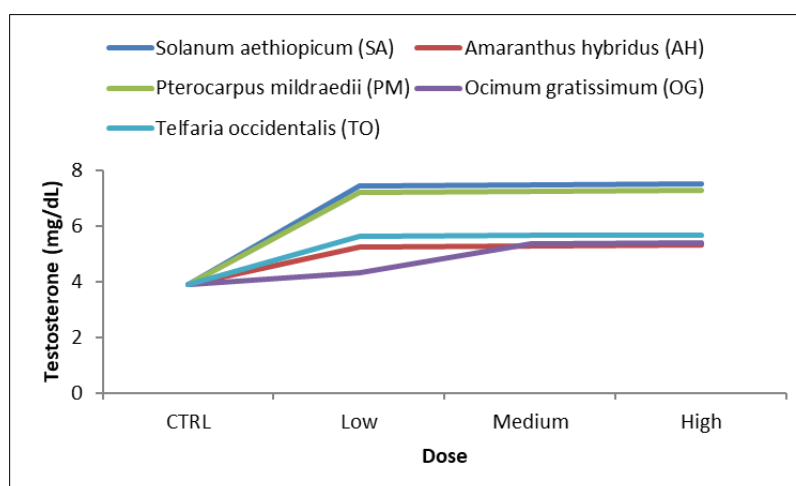


**Figure 6** Influence of different aqueous leaf extracts in Luteinizing hormone (LH) levels in male Wistar rats

**Table 6** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on Luteinizing hormone (LH) Levels

Source	F	Sig.	Partial Eta Squared
Dose	13.506	0.000	0.670
Vegetable type	7.161	0.001	0.589
Dose * Vegetable type	2.140	0.064	0.562

Finally, Figure 7 examines testosterone levels, which increased from a baseline of  $3.87 \pm 0.05$  in all groups following extract administration. Extracts such as *Solanum aethiopicum* and *Pterocarpus mildraedii* elicited a potent, near-maximal testosterone response even at low doses, nearly doubling the baseline level. In contrast, *Amaranthus hybridus*, *Ocimum gratissimum*, and *Telfaria occidentalis* produced more moderate increases. The two-way ANOVA reinforces these observations, showing highly significant effects for both dose ( $F = 34.036$ ,  $p = 0.000$ , Partial Eta Squared = 0.836) and extract type ( $F = 46.894$ ,  $p = 0.000$ , Partial Eta Squared = 0.904), as well as their significant interaction ( $F = 5.901$ ,  $p = 0.000$ , Partial Eta Squared = 0.780), suggesting that the response to increasing doses varies substantially with the specific extract (table 7).



**Figure 7** Influence of different aqueous leaf extracts in testosterone levels in male Wistar rat

**Table 7** Two - ANOVA showing the level of significance in the Main and Interaction Effects of Dose and Vegetable type on testosterone Levels

Source	F	Sig.	Partial Eta Squared
Dose	34.036	0.000	0.836
Vegetable type	46.894	0.000	0.904
Dose * Vegetable type	5.901	0.000	0.780

## 4. Discussion

The study paints an intriguing picture of how aqueous leaf extracts modulate both oxidative stress and endocrine function in male Wistar rats. The investigation encompasses a range of biomarkers—from oxidative stress indicators to key antioxidant enzymes and reproductive hormones—providing a multifaceted view of the physiological effects elicited by these plant extracts.

### 4.1. Oxidative Stress and Lipid Peroxidation

The results reveal that the extracts significantly lower malondialdehyde (MDA) levels, a well-established marker of lipid peroxidation and oxidative stress. Notably, while increasing doses generally lead to further reductions in MDA, the extent of this benefit is highly dependent on the type of extract. Extracts from *Solanum aethiopicum*, *Pterocarpus mildraedii*, and *Telfaria occidentalis* produced a robust decrease in MDA even at low doses, suggesting they are rich in potent antioxidants such as polyphenols and flavonoids. These compounds are known for their efficiency in scavenging free radicals (Smith, *et al.*, 2018; Kumar, *et al.*, 2020). Conversely, the more moderate and inconsistent responses observed with *Amaranthus hybridus* and *Ocimum gratissimum* may be due to a lower concentration or different composition of bioactive antioxidants, possibly influenced by factors like extraction efficiency or seasonal phytochemical variability (Wang, *et al.*, 2019).

### 4.2. Antioxidant Enzyme Activity

Moving to the enzymatic defenses, all extracts enhanced superoxide dismutase (SOD) activity relative to the control. Interestingly, the dose administered was the primary driver of this enhancement, suggesting that the quantity of bioactive compounds is key to upregulating SOD, regardless of the extract type. This uniformity hints at shared antioxidative constituents among the extracts that operate through similar biochemical pathways, corroborating previous research that emphasizes dose-dependent enzyme induction (Johnson, 2017).

Similarly, the study found that catalase activity—a crucial enzyme for decomposing hydrogen peroxide—is significantly increased by the extracts. However, both the dose and the specific extract type are critical here. Extracts like *Solanum aethiopicum* and *Pterocarpus mildraedii* achieve near-maximal catalase stimulation even at lower doses, likely due to a high concentration of active compounds. In contrast, extracts such as *Amaranthus hybridus*, *Ocimum gratissimum*, and *Telfaria occidentalis* elicited a more gradual increase with rising doses, which may reflect differences in their phytochemical profiles and the rate at which their bioactives induce enzyme activity. The significant interaction between dose and extract type in catalase activity underscores that optimal effects depend on tailoring the dose to the specific extract (Johnson, 2017).

Glutathione peroxidase (GPx) activity followed a similar trend. While all extracts enhanced GPx activity relative to the control, *Solanum aethiopicum* and *Pterocarpus mildraedii* reached near-maximal activity even at low doses, suggesting a rapid and potent response. In contrast, *Telfaria occidentalis* showed a clear dose-dependent increase, with the highest GPx activity observed at the high dose. These variations likely stem from differences in the bioavailability and concentration of active phytochemicals, reinforcing the notion that both the dose and the specific extract are pivotal in modulating antioxidant defenses (Smith, *et al.*, 2018; Kumar, *et al.*, 2020).

### 4.3. Endocrine Function and Reproductive Hormones

In the realm of endocrine function, the study demonstrates that aqueous leaf extracts also affect reproductive hormones. For follicle-stimulating hormone (FSH), all extracts increased its levels compared to the control, but *Telfaria occidentalis* and *Ocimum gratissimum* were particularly potent, especially at higher doses. This suggests that these extracts may contain higher concentrations or more efficacious forms of bioactive compounds capable of stimulating the hypothalamic-pituitary-gonadal axis. In contrast, *Pterocarpus mildraedii* produced only a modest increase, possibly due



to differences in its phytochemical composition or lower bioavailability of active constituents (Johnson, 2017; Smith, *et al.*, 2018).

Likewise, the data on luteinizing hormone (LH) reveal that while all extracts elevated LH relative to the control, *Telfaria occidentalis* and *Ocimum gratissimum* again showed particularly strong effects at higher doses. This pattern further supports the idea that the efficacy of these extracts in modulating endocrine function is closely tied to their specific phytochemical makeup, as well as to the dose administered (Kumar, *et al.*, 2020; Wang, *et al.*, 2019).

## 5. Conclusion

Overall, the study underscores that both the dosage and the specific type of aqueous leaf extract are critical determinants in modulating oxidative stress and endocrine function in male Wistar rats. The findings support existing research by confirming the antioxidant and endocrine-modulatory potential of plant-derived compounds, while also extending our understanding by demonstrating that the response is highly dependent on both the concentration and the unique phytochemical profile of each extract. This nuanced understanding could inform the targeted selection and optimization of plant extracts for therapeutic applications.

## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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