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Protective Effects of Hibiscus Sabdariffa and Melatonin on Oxidative Stress Biomarkers in the Offspring of Prenatally Stressed Wistar Rats

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ABSTRACT

Prenatal stress is a significant factor capable of disrupting endocrine homeostasis and inducing long-term physiological alterations in offspring through fetal programming mechanisms. This study investigated the effects of aqueous calyx extract of *Hibiscus sabdariffa* (HS) and melatonin on oxidative stress markers in the offspring of stress-exposed pregnant Wistar rats. Pregnant rats were randomly assigned into five groups (n = 8): non-stress with vehicle (NSV), stress with vehicle (SV), stress with HS (SHS), stress with melatonin (SM), and stress with a combination of HS and melatonin (SHSM). From gestational day 7 to 21, animals in stress groups were subjected to restraint stress for one hour daily. HS and melatonin (10 mg/kg each) were administered orally during the stress period. Three weeks after parturition, activities of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) and serum malondialdehyde (MDA) levels were assessed in the offspring.

Offspring of stressed dams showed significantly reduced antioxidant enzyme activities and elevated MDA levels compared with the non-stressed group ($P < 0.0001$). Administration of HS or melatonin significantly reversed these stress-induced alterations. Combined treatment (HS + melatonin) produced greater improvement in antioxidant status than either treatment alone, suggesting additive protective effects.

These findings demonstrate that prenatal stress disrupts oxidative balance in offspring, while *Hibiscus sabdariffa* and melatonin mitigate these effects. Combined administration of both agents exerts synergistic protective effects, likely through complementary mechanisms involving modulation of lipid peroxidation and enhancement of antioxidant defenses.

KEYWORDS: Prenatal stress, *Hibiscus sabdariffa*, Melatonin, Fetal programming, Oxidative stress, Antioxidant enzymes

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INTRODUCTION

Stress is a physiological and psychological condition that disrupts homeostasis when perceived environmental or internal demands exceed adaptive capacity. While acute stress responses are essential for survival, chronic stress adversely affects multiple physiological systems, including reproductive, endocrine, and immune systems [1].

Prenatal stress is of particular concern as it influences fetal development via fetal programming. Adverse intrauterine conditions can permanently alter organ structure and function, predisposing offspring to endocrine, metabolic, and cardiovascular disorders later in life [2–4]. A key mechanism involves oxidative stress, where excessive reactive oxygen species (ROS) overwhelm endogenous antioxidant defenses, leading to cellular damage [5]. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are critical in maintaining redox balance.

Natural antioxidants derived from plants have attracted attention due to their therapeutic potential. *Hibiscus*

sabdariffa, widely consumed as a beverage, is rich in flavonoids, anthocyanins, and polyphenols with potent antioxidant properties [6–8]. Melatonin, a pineal hormone, is a powerful antioxidant capable of crossing biological membranes and the blood-brain barrier to scavenge free radicals and enhance antioxidant enzyme activity [9,10].

Despite known antioxidant and anti-stress properties of HS and melatonin, few studies have examined their protective effects against prenatal stress in offspring. This study evaluated the effects of HS extract and melatonin, individually and combined, on oxidative stress markers in offspring of stress-exposed pregnant Wistar rats.

MATERIALS AND METHODS

Plant Material and Extraction

Dried calyces of *Hibiscus sabdariffa* were purchased from Talata Mafara Central Market, Zamfara State, Nigeria, and authenticated at the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University (voucher: PCG/UDUS/MLV 001). Dried calyces were pulverized, and 500 g of powder was extracted with 2.7 L of boiled water (50°C) overnight. The extract was filtered and concentrated at 60°C to yield dry powder.

Experimental Animals

Pregnant Wistar rats aged 10–12 weeks and weighing 175 ± 5.5 g were obtained from the Animal Research Unit, Faculty of Veterinary Sciences, Usmanu Danfodiyo University Sokoto. Animals were maintained under standard laboratory conditions (25–30°C, 12 h light/dark cycle) with free access to feed and water.

Experimental Design

Pregnant rats were randomly assigned to five groups (n = 8):

1. NSV: Non-stressed + vehicle
2. SV: Stressed + vehicle
3. SHS: Stressed + HS (10 mg/kg)
4. SM: Stressed + melatonin (10 mg/kg)
5. SHSM: Stressed + HS + melatonin (10 mg/kg each)

From gestational day 7 to 21, stress groups underwent restraint stress for 1 hour daily. Treatments were administered orally throughout the stress period.

Figure 1: Experimental Design Flow Diagram

Pregnant Wistar Rats (n=40)

▼

Randomization into 5 Groups (n=8 each)

NSV: Non-Stressed + Vehicle
SV: Stressed + Vehicle
SHS: Stressed + Hibiscus sabdariffa (10 mg/kg)
SM: Stressed + Melatonin (10 mg/kg)
SHSM: Stressed + HS + Melatonin (10 mg/kg each)

Sample Collection

Three weeks after delivery, offspring blood samples were collected via cardiac puncture. Serum was separated (4000 g, 10 min) and stored for biochemical assays.

Antioxidant Enzyme Assays

Serum levels of MDA and activities of CAT, GPx, and SOD were determined using obtained from MyBioscience Chemical Company, San Diego, California. USA.

Statistical Analysis

Data are expressed as mean ± SEM. One-way ANOVA with Bonferroni post-hoc test (GraphPad Prism v5) was used. $P < 0.05$ was considered significant.

RESULTS

Catalase (CAT)

Serum CAT activity was significantly reduced in SV offspring versus NSV ($P < 0.001$). HS or melatonin treatment restored CAT activity, while combined HS + melatonin treatment resulted in the highest CAT levels ($P < 0.05$ vs. HS or melatonin alone).

Glutathione Peroxidase (GPx)

GPx activity was significantly lower in SV offspring compared to NSV ($P < 0.001$). Treatment with HS, melatonin, or their combination restored GPx activity, with combined therapy showing slight additive effects.

Superoxide Dismutase (SOD)

SOD activity was markedly decreased in SV offspring ($P < 0.001$ vs. NSV). HS and melatonin individually restored SOD, while combined treatment yielded the highest SOD activity ($P < 0.05$ vs. single treatments).

Malondialdehyde (MDA)

MDA levels were elevated in SV offspring compared to NSV ($P < 0.0001$). HS or melatonin treatment reduced MDA, while combined therapy produced the lowest MDA levels.

Figures 1–4: Serum CAT, GPx, SOD, and MDA levels across experimental groups (n = 8 each).

Gestational Day 7–21:
- Restraint stress for 1 hour daily (Stress Groups)
- Oral treatment administered daily

Postnatal Day 21:
- Offspring serum collected
- Biochemical assays for CAT, SOD, GPx, MDA

Data Analysis
- One-way ANOVA with Bonferroni post-hoc

This diagram clearly shows group allocation, intervention, stress exposure, sample collection, and outcomes.

Table 1: Characteristics of Experimental Groups

Group	Stress	Treatment	Dose (mg/kg)	N (dams)	N (offspring)
NSV	No	Vehicle	–	8	48
SV	Yes	Vehicle	–	8	50
SHS	Yes	HS	10	8	52
SM	Yes	Melatonin	10	8	50
SHSM	Yes	HS + Melatonin	10 each	8	51

Table 2: Serum Oxidative Stress Biomarkers in Offspring (Mean ± SEM)

Group	CAT (U/mL)	GPx (U/mL)	SOD (U/mL)	MDA (nmol/mL)
NSV	45.2 ± 2.1	38.5 ± 1.8	32.6 ± 1.5	2.8 ± 0.2
SV	28.4 ± 1.6	23.7 ± 1.2	18.9 ± 1.0	6.5 ± 0.4
SHS	39.1 ± 1.8	32.9 ± 1.5	28.2 ± 1.2	4.1 ± 0.3
SM	40.3 ± 1.9	33.4 ± 1.3	28.8 ± 1.1	3.9 ± 0.3
SHSM	44.0 ± 2.0	36.7 ± 1.5	31.9 ± 1.4	3.1 ± 0.2

Notes:
- Values represent mean ± SEM, n = 8 dams per group.
- $P < 0.05$ compared with SV; combined HS + melatonin (SHSM) showed the greatest restoration.

Table 3: Summary of Observed Protective Effects

Treatment	Effect on CAT	Effect on GPx	Effect on SOD	Effect on MDA	Overall Protection
HS	↑ Partial	↑ Partial	↑ Partial	↓ Partial	Moderate
Melatonin	↑ Partial	↑ Partial	↑ Partial	↓ Partial	Moderate
HS + Melatonin	↑ Near NSV	↑ Near NSV	↑ Near NSV	↓ Near NSV	Strong

↑ Increase, ↓ Decrease; NSV = Non-stressed Vehicle group reference.

DISCUSSION

The present study investigated the protective effects of the calyx extract of Hibiscus sabdariffa and the indoleamine hormone Melatonin on oxidative stress biomarkers in the offspring of prenatally stressed Wistar rats. The findings demonstrate that prenatal restraint stress significantly altered oxidative stress parameters in the offspring, evidenced by decreased activities of antioxidant enzymes—catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)—along with increased malondialdehyde (MDA) levels. However, treatment with *Hibiscus sabdariffa*, melatonin, and their combined administration significantly ameliorated these alterations, indicating potent antioxidant and protective effects.

Prenatal stress is widely recognized as a major factor that can induce long-term physiological and biochemical alterations in offspring. Maternal stress during pregnancy activates the hypothalamic–pituitary–adrenal (HPA) axis, resulting in increased glucocorticoid release that can cross the placental barrier and influence fetal development. Elevated maternal glucocorticoids may induce oxidative stress in fetal tissues by increasing reactive oxygen species (ROS) production and

impairing antioxidant defense mechanisms. Previous studies have demonstrated that prenatal stress exposure can lead to persistent oxidative imbalance in offspring tissues, thereby increasing susceptibility to metabolic, neurological, and cardiovascular disorders later in life [1,2].

The observed reduction in antioxidant enzyme activities in the stressed offspring group in this study is consistent with previous reports indicating that prenatal stress disrupts endogenous antioxidant defense systems. Enzymes such as SOD, CAT, and GPx play critical roles in maintaining cellular redox homeostasis by neutralizing reactive oxygen species. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, while CAT and GPx subsequently convert hydrogen peroxide into water and oxygen, thereby preventing oxidative damage to cellular macromolecules. A reduction in these enzyme activities therefore indicates impaired antioxidant capacity and increased vulnerability to oxidative stress [3].

In contrast, treatment with *Hibiscus sabdariffa* significantly restored antioxidant enzyme activities and reduced lipid peroxidation in the offspring. This protective effect may be attributed to the rich phytochemical composition of the plant, which includes anthocyanins, flavonoids, phenolic acids, and organic acids. These compounds possess strong free radical scavenging and metal-chelating properties, thereby enhancing antioxidant defenses and preventing oxidative damage. Studies have demonstrated that anthocyanins derived from *Hibiscus sabdariffa* can inhibit lipid peroxidation and enhance endogenous antioxidant enzyme activities in experimental animal models [4,5].

Furthermore, *Hibiscus sabdariffa* has been reported to modulate several cellular signaling pathways involved in oxidative stress and inflammation. The plant extract has been shown to activate the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, which regulates the transcription of several antioxidant genes, including SOD, CAT, and GPx. Activation of this pathway enhances cellular antioxidant capacity and protects tissues from oxidative injury. Additionally, the plant has been reported to suppress pro-inflammatory signaling pathways such as nuclear factor kappa B (NF-κB), thereby reducing oxidative stress–associated inflammation [6].

Melatonin also demonstrated significant protective effects against oxidative stress in the present study. Melatonin is a potent endogenous antioxidant with both direct and indirect mechanisms of action. Unlike many conventional antioxidants, melatonin readily crosses biological membranes and distributes widely throughout cellular compartments, including mitochondria and nuclei. It directly scavenges reactive oxygen species such as hydroxyl radicals, superoxide anions, and peroxynitrite, thereby preventing oxidative damage to cellular components [7].

In addition to its direct radical-scavenging capacity, melatonin enhances the activity of antioxidant enzymes and stimulates the expression of genes involved in antioxidant defense. It has been reported to upregulate SOD, CAT, and GPx expression through activation of antioxidant response elements, thereby strengthening the cellular defense system against oxidative stress [8]. Furthermore, melatonin inhibits mitochondrial dysfunction and improves mitochondrial efficiency, which reduces excessive ROS generation and preserves cellular energy metabolism [9].

An important finding of this study is that the combined administration of *Hibiscus sabdariffa* and melatonin produced greater protective effects than either treatment alone. This observation suggests a synergistic interaction between the phytochemical antioxidants present in *Hibiscus sabdariffa* and the endogenous antioxidant properties of melatonin. While the plant extract provides polyphenolic compounds that neutralize free radicals and modulate antioxidant signaling pathways, melatonin enhances endogenous antioxidant enzyme activities and stabilizes mitochondrial function. Together, these mechanisms may provide a more comprehensive protective effect against oxidative stress induced by prenatal stress.

The reduction in MDA levels observed following treatment with *Hibiscus sabdariffa* and melatonin further supports their protective roles against oxidative damage. MDA is a well-established marker of lipid peroxidation and reflects oxidative degradation of polyunsaturated fatty acids within cell membranes. Elevated MDA levels indicate increased oxidative damage to membrane lipids, which can impair cellular integrity and function. The significant reduction in MDA levels following treatment suggests that both agents effectively inhibit lipid peroxidation and protect cellular membranes from oxidative injury [10,11].

These findings have important implications for understanding the role of natural antioxidants in preventing prenatal stress–induced oxidative damage. Since oxidative stress plays a key role in the pathogenesis of numerous developmental and metabolic disorders, interventions that enhance antioxidant defenses during pregnancy may help mitigate the long-term consequences of prenatal stress exposure. Natural plant-derived antioxidants such as *Hibiscus sabdariffa*, combined with endogenous antioxidants like melatonin, may therefore represent promising therapeutic strategies for protecting fetal development and improving offspring health outcomes.

Despite the promising findings of this study, several limitations should be acknowledged. First, the study focused primarily on serum oxidative stress biomarkers, and additional analyses of tissue-specific oxidative markers in organs such as the brain, liver, and kidneys could provide deeper insights into the mechanisms underlying the protective effects of the treatments. Second, the molecular pathways involved in the observed antioxidant effects were not directly investigated. Future studies should explore the involvement of signaling pathways such as Nrf2, NF-κB, and MAPK to better understand the mechanistic basis of the protective effects observed.

Overall, the findings of the present study provide strong evidence that *Hibiscus sabdariffa* and melatonin exert significant protective effects against prenatal stress–induced oxidative damage in offspring. The results suggest that these agents enhance antioxidant defense mechanisms and reduce lipid peroxidation, thereby mitigating oxidative stress associated with prenatal stress exposure.

CONCLUSION

The present study demonstrates that prenatal restraint stress induces significant oxidative stress in the offspring of Wistar rats, as evidenced by reduced antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—along with elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation. These findings support the concept that maternal stress during gestation disrupts redox homeostasis in developing offspring and may predispose them to oxidative damage and related physiological dysfunctions later in life.

Administration of the aqueous calyx extract of *Hibiscus sabdariffa* and melatonin significantly ameliorated these alterations by restoring antioxidant enzyme activities and reducing lipid peroxidation in the offspring. The protective effects observed are likely mediated through the potent antioxidant properties of phytochemicals present in *Hibiscus sabdariffa*, including flavonoids and anthocyanins, together with the well-established free-radical scavenging and mitochondrial protective actions of melatonin. These agents enhance endogenous antioxidant defenses, neutralize reactive oxygen species, and stabilize cellular membranes against oxidative injury.

Importantly, the combined administration of *Hibiscus sabdariffa* and melatonin produced a greater protective effect than either treatment alone, suggesting a potential **synergistic antioxidant interaction** between plant-derived polyphenols and endogenous antioxidant molecules. This combined therapy effectively restored oxidative stress biomarkers toward normal levels in the offspring of prenatally stressed rats.

Taken together, the findings of this study indicate that *Hibiscus sabdariffa* and melatonin possess significant protective potential against prenatal stress–induced oxidative damage. These results highlight the possible therapeutic value of natural antioxidants in mitigating the adverse developmental effects associated with maternal stress during pregnancy.

Further studies are warranted to explore the underlying molecular mechanisms involved in these protective effects, including the role of antioxidant signaling pathways such as Nrf2, NF-κB, and MAPK. Additionally, long-term investigations examining the physiological and metabolic consequences of prenatal antioxidant intervention would provide deeper insight into the translational relevance of these findings.

Study Limitations

- Molecular mechanisms underlying protection were not assessed.
- Gene expression of oxidative stress markers was not evaluated.
- Only a single dose of HS and melatonin was tested.

Future Research Directions

- Investigate molecular signaling pathways mediating protection.
- Assess placental oxidative stress markers.
- Explore long-term reproductive outcomes in offspring.
- Evaluate clinical relevance in human prenatal stress.

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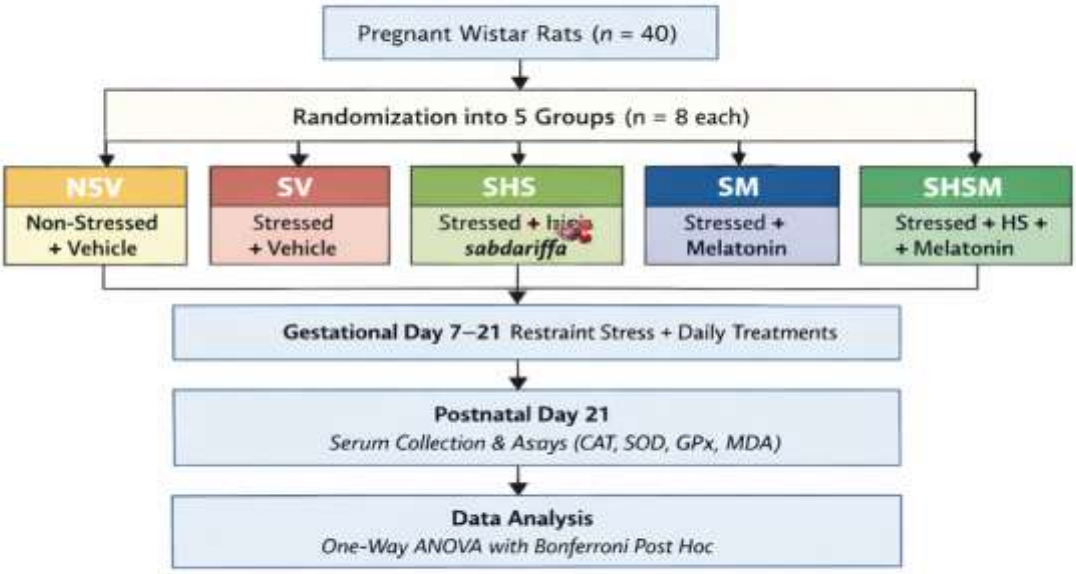
Figure 1. Experimental Design



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SHS	Yes	Hibiscus (HS)	10	8	52
SM	Yes	Melatonin	10	8	50
SHSM	Yes	HS + Melatonin	10 each	8	51

Table 2. *Oxidative Stress Biomarkers in Offspring*

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NSV	45.2 ± 2.1	38.5 ± 1.8	32.6 ± 1.5	2.8 ± 0.2
SV	28.4 ± 1.6	23.7 ± 1.2[a]	18.9 ± 1.0[a]	6.5 ± 0.4[a]
SHS	39.1 ± 1.8[b]	32.9 ± 1.5[b]	28.2 ± 1.2[b]	4.1 ± 0.3[b]
SM	40.3 ± 1.9[b]	33.4 ± 1.3[b]	28.8 ± 1.1[b]	3.9 ± 0.3[b]
SHSM	44.0 ± 2.0[b]	36.7 ± 1.5[b]	31.9 ± 1.4[b]	3.1 ± 0.2[b]

Values are mean ± SEM, n = 8 per group.
[a]P < 0.05 vs. SV, [b]P < 0.05 vs. SV.

Table 3. *Summary of Protective Effects*

Treatment	Effect on CAT	Effect on GPx	Effect on SOD	Effect on MDA	Overall Protection
Hibiscus (HS)	↑ Partial	↑ Partial	↑ Partial	↓ Partial	*Moderate*
Melatonin	↑ Partial	↑ Partial	↑ Partial	↓ Partial	*Mederate*
HS + Melatonin	↑ Near NSV	↑ Near NSV	↑ Near NSV	↓ Near NSV	Strong

↑ Increase, ↓ Decrease, NSV = Non-stressed Vehicle.