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Prenatal Stress Alters Hormonal Profiles in Rat Offspring: Protective Roles of Hibiscus Sabdariffa and Melatonin

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Abstract

Background: Prenatal stress can disrupt endocrine homeostasis and induce long-term physiological alterations in offspring through fetal programming mechanisms. Such disturbances may impair reproductive hormone balance and elevate stress-related hormones, predisposing offspring to metabolic and neuroendocrine dysfunctions. Natural antioxidants such as Hibiscus sabdariffa and melatonin have been reported to possess protective properties against oxidative and stress-related damage.

Objectives: This study investigated the effects of aqueous calyx extract of Hibiscus sabdariffa and melatonin on the hormonal profile of offspring from stress-exposed pregnant Wistar rats.

Methods: Pregnant rats were randomly assigned into five groups (n = 8): non-stress with vehicle (NSV), stress with vehicle (SV), stress with Hibiscus sabdariffa (SHS), stress with melatonin (SM), and stress with combined Hibiscus sabdariffa and melatonin (SHSM). From gestational day 7 to 21, animals in stress groups were subjected to restraint stress for one hour daily. Hibiscus sabdariffa extract and melatonin (10 mg/kg each) were administered orally during the stress period. Three weeks after parturition, serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), cortisol, prolactin, adrenaline, and noradrenaline were measured in the offspring.

Results: Offspring of stressed dams exhibited significantly reduced FSH and LH levels alongside elevated prolactin, cortisol, adrenaline, and noradrenaline compared with the non-stressed group ($P < 0.0001$). Treatment with Hibiscus sabdariffa or melatonin significantly attenuated these stress-induced hormonal alterations. Combined treatment produced a greater improvement in hormonal balance than either treatment alone.

Conclusion: Prenatal stress disrupts endocrine regulation in offspring, whereas Hibiscus sabdariffa and melatonin mitigate these effects. Their combined administration appears to provide enhanced protective benefits, possibly through complementary mechanisms involving modulation of gonadotropins and stress hormones.

Introduction

Stress represents a physiological and psychological condition that disrupts the homeostatic balance of biological systems. It occurs when an organism perceives environmental or internal demands as exceeding its adaptive capacity. Although acute stress responses are essential for survival, prolonged or chronic stress can adversely affect multiple physiological systems including the reproductive, endocrine, and immune systems [1].

Exposure to stress during pregnancy is of particular concern because it may influence fetal development through mechanisms collectively referred to as fetal programming. Fetal programming describes the phenomenon whereby adverse intrauterine conditions permanently alter the structure and function of developing organs, thereby predisposing offspring to diseases later in life [2]. Maternal stress can activate the hypothalamic–pituitary–adrenal (HPA) axis and increase circulating glucocorticoids, which may cross the placenta and alter fetal endocrine development [3].

Prenatal stress has been associated with a wide range of adverse outcomes in offspring, including alterations in reproductive hormones, impaired neurodevelopment, metabolic dysfunction, and increased susceptibility to cardiovascular and metabolic diseases [4,5]. These effects are partly mediated through increased production of reactive oxygen species (ROS) and the resulting oxidative stress, which can damage cellular structures and disrupt normal physiological processes [6]. Oxidative stress occurs when the generation of reactive oxygen and nitrogen species exceeds the capacity of endogenous antioxidant defense systems [7]. Excessive ROS production has been implicated in reproductive disorders, pregnancy complications, and impaired fetal development [8]. Natural antioxidants derived from plants have attracted increasing scientific interest due to their potential therapeutic benefits. Hibiscus sabdariffa, a medicinal plant widely consumed as a beverage in many parts of the world, is rich in flavonoids, polyphenols,

and other bioactive compounds with potent antioxidant properties [9]. Previous studies have demonstrated its antihypertensive, cardioprotective, and hypolipidemic effects [10,11].

Melatonin, a hormone primarily secreted by the pineal gland, is also recognized as a powerful antioxidant. It readily crosses biological membranes and the blood–brain barrier, enabling it to scavenge free radicals and enhance antioxidant enzyme activities [12]. Unlike many antioxidants, melatonin does not undergo redox cycling, making it an effective terminal antioxidant [13].

Although both *Hibiscus sabdariffa* and melatonin possess anti-stress properties, limited studies have investigated their effects on prenatal stress and the hormonal status of offspring. Therefore, the present study aimed to evaluate the effects of *Hibiscus sabdariffa* extract and melatonin, individually and in combination, on hormonal profile in the 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. The plant material was authenticated at the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, where a voucher specimen (PCG/UDUS/MLV 001) was deposited. The dried calyces were pulverized into powder, and 500 g of the powder was extracted with 2.7 L of boiled water (50°C) using continuous stirring overnight. The extract was filtered and concentrated using a water bath at 60°C to obtain a dry powder.

Experimental Animals

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

Experimental Design

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

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

Restraint stress was applied daily from gestational day 7 to 21 for one hour. Treatments were administered orally via gavage throughout the stress exposure period.

Sample Collection

Three weeks after delivery, blood samples were collected from offspring via cardiac puncture. Serum was separated by centrifugation at 4000 g for 10 minutes and stored for biochemical analysis.

Hormonal Assays

Serum levels of FSH, LH, prolactin, cortisol, adrenaline, and noradrenaline were determined using ELISA kits obtained from Cusabio Biotech Company (Wuhan, China).

Statistical Analysis

Data were expressed as mean $\pm$ SEM. Statistical analysis was

performed using one-way ANOVA followed by Bonferroni post-hoc test using GraphPad Prism version 5. Statistical significance was set at $P < 0.05$.

Results

Prenatal restraint stress significantly altered hormonal profiles in the offspring of pregnant Wistar rats. Offspring of stressed dams exhibited significantly reduced levels of FSH and LH compared with the non-stressed group ($P < 0.0001$). However, treatment with *Hibiscus sabdariffa*, melatonin, or their combination significantly increased these gonadotropin levels.

Similarly, prolactin, cortisol, adrenaline, and noradrenaline concentrations were significantly elevated in the offspring of stressed dams. Administration of HS and melatonin significantly reduced these stress hormone levels. The combined treatment produced greater reductions than either treatment alone.

Follicle Stimulating Hormone (FSH)

The serum follicle-stimulating hormone (FSH) levels of the offspring of stress-exposed pregnant Wistar rats are presented in [Figure 1]. Prenatal exposure to restraint stress significantly reduced the FSH concentration in the offspring of the stress-with-vehicle (SV) group compared with the offspring of the non-stress with vehicle (NSV) group ($P < 0.0001$). This result suggests that maternal stress during pregnancy adversely affects the reproductive hormonal axis of the offspring.

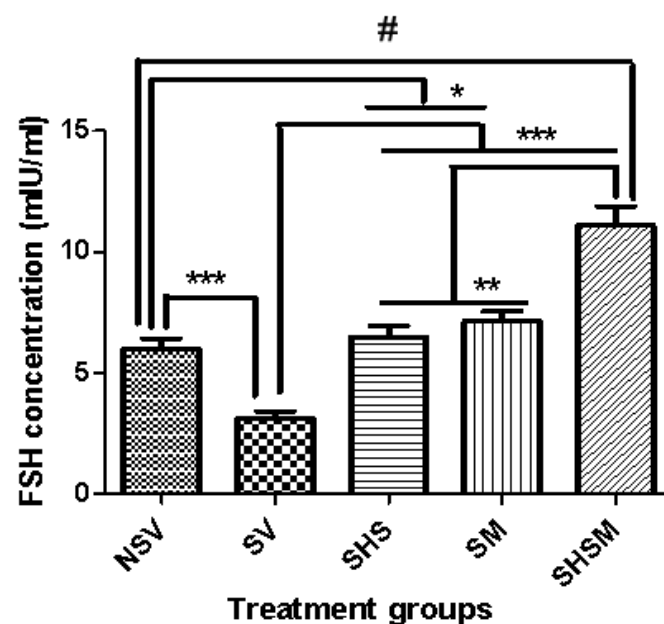


Figure 1: The serum FSH levels of the offspring of stress exposed pregnant Wistar rats treated with vehicle, *Hibiscus sabdariffa*, melatonin and their combination compared to the offspring of non-exposed group given vehicle (n = 8 each). *** = $P < 0.0001$ SV vs NSV, SHS, SM and SHSM; # = $P < 0.0001$ NSV vs SHSM; * = $P < 0.05$ NSV vs SHS and SM; ** = $P < 0.0001$ SHSM vs SHS and SM;. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

FSH= Follicle Stimulating Hormone, NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS =Stress with 10 mg/kg *Hibiscus sabdariffa* SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg *Hibiscus sabdariffa* and 10 mg/kg melatonin.

Administration of Hibiscus sabdariffa (SHS) and melatonin (SM) significantly increased FSH levels in the offspring compared with the SV group ($P < 0.0001$). Notably, the FSH levels observed in the offspring of SHS and SM groups were significantly higher than those of the NSV group ($P < 0.05$). This indicates that both treatments not only reversed the stress-induced reduction but also enhanced FSH levels beyond basal values.

Furthermore, the offspring of rats treated with the combined HS and melatonin therapy (SHSM) exhibited significantly higher FSH levels compared with those treated with either HS or melatonin alone ($P < 0.001$). These findings suggest a possible additive or synergistic interaction between HS and melatonin in modulating gonadotropin secretion.

Luteinizing Hormone (LH)

Serum luteinizing hormone (LH) levels showed a similar pattern to that observed for FSH [Figure 2]. The offspring of the SV group displayed significantly reduced LH levels compared with those of the NSV group ($P < 0.0001$), indicating that prenatal stress impairs the hypothalamic–pituitary–gonadal axis.

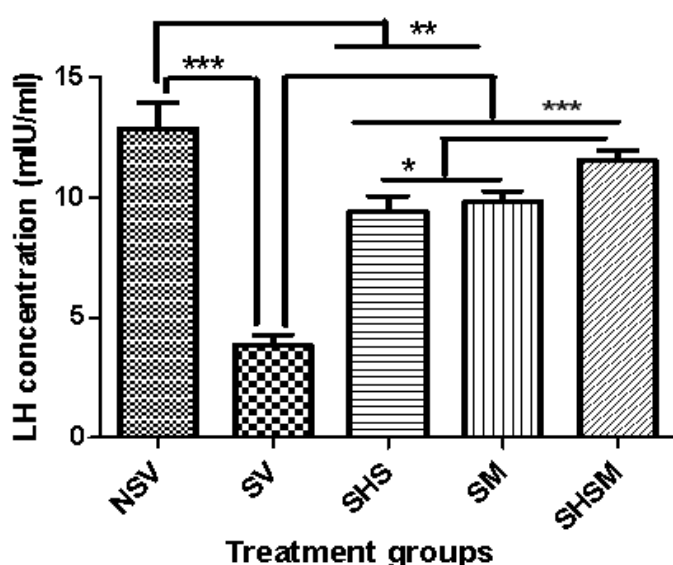


Figure 2: Serum LH levels of the offspring of stress exposed pregnant Wistar rats given vehicle, Hibiscus sabdariffa, melatonin and their combination compared to the offspring of non-exposed group given vehicle ($n = 8$). *** = $P < 0.0001$ SV vs NSV, SHS, SM and SHSM; ** = $P < 0.001$ NSV vs SHS and SM; * = $P < 0.05$ SHSM vs SHS and SM. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

LH = Luteinising Hormone, NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS = Stress with 10 mg/kg Hibiscus sabdariffa, SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg Hibiscus sabdariffa and 10 mg/kg melatonin.

Treatment with HS or melatonin significantly increased LH levels relative to the SV group ($P < 0.0001$). However, LH levels in the SHS and SM groups remained significantly lower than those observed in the NSV group ($P < 0.001$). Interestingly, the combined treatment group (SHSM) restored LH levels to values comparable with those observed in the NSV group.

Comparison among treatment groups revealed that LH levels in the SHSM group were significantly higher than those observed in the SHS and SM groups ($P < 0.05$), further supporting the additive effect of the combined therapy.

Prolactin

The serum prolactin levels of the offspring are illustrated in [Figure 3]. Offspring from stressed dams receiving vehicle (SV) exhibited significantly elevated prolactin levels compared with the NSV group ($P < 0.0001$). Elevated prolactin levels are commonly associated with stress-induced endocrine disturbances.

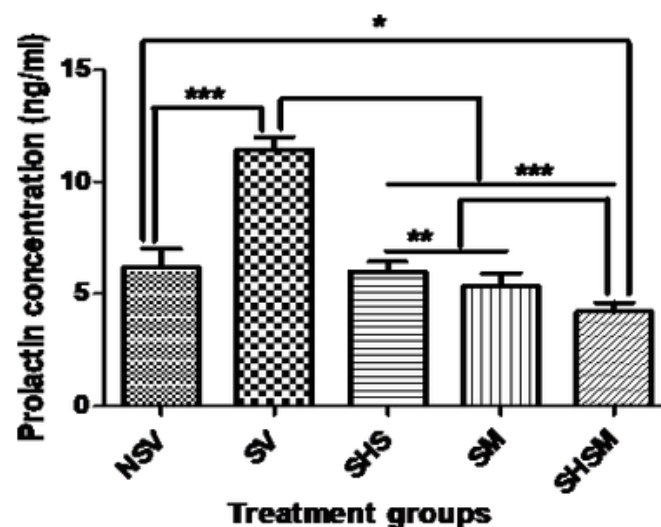


Figure 3: Serum Prolactin levels of the offspring of stress exposed pregnant Wistar rats in the presence of vehicle, Hibiscus sabdariffa, melatonin and their combination compared to the offspring of non-exposed group given vehicle ($n = 8$ each). *** = $P < 0.0001$ SV vs NSV, SHS, SM and SHSM; * = $P < 0.05$ SHSM vs NSV; ** = $P < 0.05$ SHSM vs SHS and SM. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS = Stress with 10 mg/kg Hibiscus sabdariffa, SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg Hibiscus sabdariffa and 10 mg/kg melatonin.

Treatment with HS or melatonin significantly reduced prolactin concentrations compared with the SV group ($P < 0.0001$). The prolactin levels in SHS and SM groups were not significantly different from those observed in the NSV group, indicating that both treatments effectively normalized prolactin secretion.

Interestingly, the SHSM group exhibited significantly lower prolactin levels compared with the NSV group ($P < 0.05$) and also lower than those observed in the SHS and SM groups ($P < 0.05$). These findings further support the enhanced efficacy of combined HS and melatonin therapy.

Cortisol

Prenatal stress markedly increased serum cortisol levels in the offspring of the SV group compared with those of the NSV group ($P < 0.0001$), confirming activation of the hypothalamic–pituitary–adrenal (HPA) axis [Figure 4].

Treatment with HS, melatonin, and their combination significantly reduced cortisol levels compared with the SV group ($P < 0.0001$). However, cortisol levels in SHS and SM groups remained significantly higher than those observed in the NSV group.

In contrast, the combined treatment group (SHSM) exhibited

cortisol levels comparable with those observed in the NSV group, indicating near-complete reversal of the stress-induced hormonal disturbance.

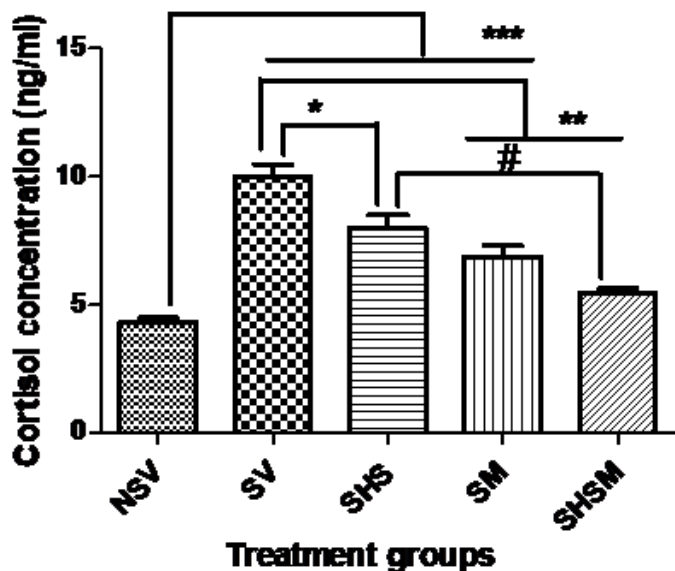


Figure 4: Serum Cortisol levels of the offspring of stress exposed pregnant Wistar rats given vehicle, Hibiscus sabdariffa, melatonin and their combination compared to the offspring of non-exposed group given vehicle (n = 8 each). * = $P < 0.01$ SV vs SHS; ** = $P < 0.0001$ SV vs SM and SHSM; *** = $P < 0.0001$ NSV vs SV, SHS and SM; # = $P < 0.05$ SHSM vs SHS. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS = Stress with 10 mg/kg Hibiscus sabdariffa, SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg Hibiscus sabdariffa and 10 mg/kg melatonin.

Adrenaline

[Figure 5], showed Serum adrenaline levels in the offspring followed a similar pattern to cortisol. Prenatal stress significantly increased adrenaline levels in the SV group compared with the NSV group ($P < 0.0001$).

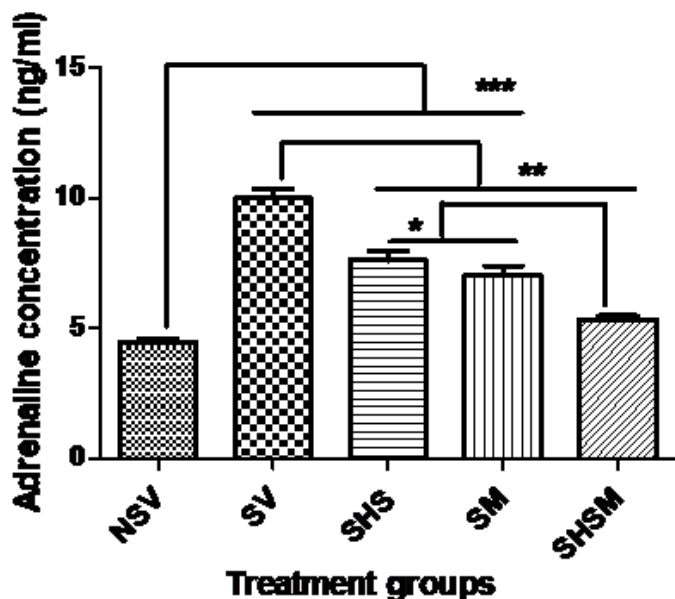


Figure 5: Serum adrenaline levels of the offspring of stress exposed pregnant Wistar rats in the presence of vehicle, Hibiscus sabdariffa, melatonin and their combination compared to the offspring of non-exposed group administered with vehicle (n=8 each). ** = $P < 0.0001$ SV vs SHS, SM and SHSM; *** = $P < 0.0001$ NSV vs SV, SHS and SM; * = $P < 0.05$ SHSM vs SHS and SM. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS = Stress with 10 mg/kg Hibiscus sabdariffa, SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg Hibiscus sabdariffa and 10 mg/kg melatonin.

Treatment with HS and melatonin significantly reduced adrenaline levels relative to the SV group ($P < 0.0001$). However, adrenaline levels in SHS and SM groups remained slightly elevated compared with the NSV group.

The combined treatment group (SHSM) demonstrated adrenaline levels comparable to those of the NSV group, suggesting a more potent anti-stress effect of the combined therapy.

Noradrenaline

Noradrenaline concentrations were significantly elevated in the offspring of the SV group compared with the NSV group ($P < 0.0001$), reflecting increased sympathetic nervous system activity [Figure 6].

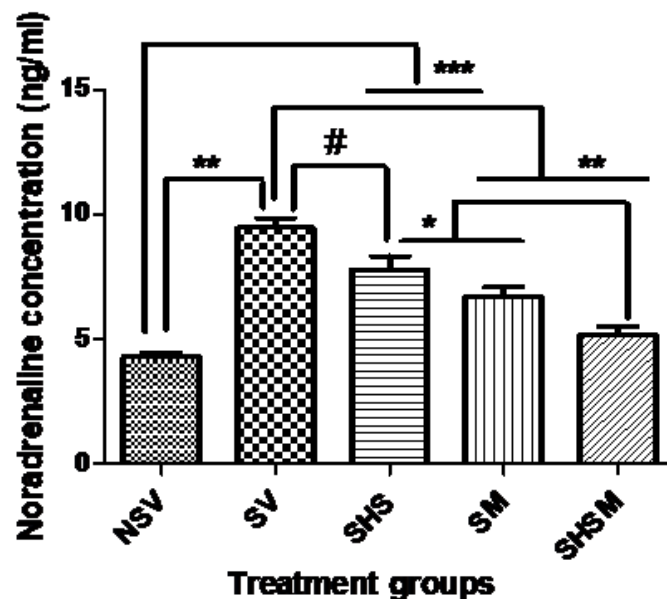


Figure 6: Serum noradrenaline levels of the offspring of stress exposed pregnant Wistar rats administered with vehicle, Hibiscus sabdariffa, melatonin and their combination compared to the offspring of non-exposed group given vehicle (n = 8 each). # = $P < 0.001$ SV vs SHS; *** = $P < 0.0001$ NSV vs SHS and SM; ** = $P < 0.0001$ SV vs NSV, SM and SHSM; * = $P < 0.05$ SHSM vs SHS and SM. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

NSV = Non Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SHS = Stress with 10 mg/kg Hibiscus sabdariffa, SM = Stress with 10 mg/kg melatonin, SHSM = Stress with combination of 10 mg/kg Hibiscus sabdariffa and 10 mg/kg melatonin.

Treatment with HS and melatonin significantly reduced noradrenaline levels compared with the SV group ($P < 0.0001$). The SHSM group exhibited significantly lower noradrenaline levels than the SHS and SM groups ($P < 0.05$), further supporting the additive effect of combined treatment.

Discussion

Prenatal stress significantly reduced circulating levels of the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the offspring. These findings suggest that maternal stress disrupts the hypothalamic–pituitary–gonadal (HPG) axis of the developing fetus. Previous studies have reported that elevated glucocorticoids resulting from activation of the maternal hypothalamic–pituitary–adrenal (HPA) axis may cross the placenta and influence fetal endocrine development [14,15]. Chronic exposure of the fetus to high levels of maternal glucocorticoids has been associated with impaired reproductive development and altered endocrine function later in life [16,17].

The reduction in gonadotropin levels observed in this study may also be linked to stress-induced activation of gonadotropin-inhibitory hormone (GnIH), which suppresses gonadotropin secretion by inhibiting the activity of gonadotropin-releasing hormone (GnRH) neurons [18].

Administration of *Hibiscus sabdariffa* significantly improved gonadotropin levels, suggesting that the plant extract exerts protective effects on reproductive endocrine function. The beneficial effects of *Hibiscus sabdariffa* may be attributed to its rich phytochemical composition, particularly anthocyanins, flavonoids, and polyphenols, which possess potent antioxidant properties [9,19,20].

Melatonin also restored gonadotropin levels in the offspring. Melatonin is widely recognized as a powerful endogenous antioxidant and free radical scavenger capable of crossing biological membranes and the blood–brain barrier [12, 21]. In addition to its antioxidant properties, melatonin regulates circadian rhythms and modulates reproductive hormone secretion through its actions on the hypothalamus and pituitary gland [22].

Conclusion

Prenatal restraint stress disrupts hormonal balance and induces oxidative stress in the offspring of pregnant Wistar rats. Administration of *Hibiscus sabdariffa* extract and melatonin effectively mitigates these alterations by restoring gonadotropin levels and reducing stress hormones. The combined administration of HS and melatonin produced additive protective effects, suggesting that the two agents may act through distinct but complementary mechanisms. These findings highlight the potential therapeutic value of *Hibiscus sabdariffa* and melatonin as natural interventions for mitigating prenatal stress-induced physiological disturbances.

Further studies are required to elucidate the precise molecular mechanisms underlying these protective effects and to explore their potential clinical applications in human reproductive health.

Study Limitations

- The molecular mechanisms underlying the protective effects were not investigated.

- Gene expression of oxidative stress markers was not assessed.
- Only one dose of HS and melatonin was tested.

Future Research Directions

Future studies should investigate:

- Molecular signaling pathways involved
- Placental oxidative stress markers
- Long-term reproductive outcomes in offspring
- Clinical relevance in human pregnancy stress

Authors' Contribution

Aliyu Buhari conceptualized and designed the study, conducted the literature review, developed the methodology, performed data analysis and interpretation, and drafted the original manuscript. Aliyu Buhari also coordinated the overall research process and prepared the final version of the manuscript.

KabiruLadan contributed to the study design, provided critical intellectual input during manuscript development, assisted in data interpretation, and critically reviewed and revised the manuscript for important intellectual content.

Both authors read, approved, and agreed to the final version of the manuscript and are accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict Of Interest

The authors declare that there is no known conflict of interest associated with this publication and no significant financial support that could have influenced its outcome.

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