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Protective neuroendocrine and reproductive influence of *Spondias mombin* leaf and *Curcuma longa* rhizome methanolic extracts on male sex hormones and sperm parameters in alloxan-exposed Wistar rats

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Abstract

Background

Neuroendocrinology is the branch of physiology which deals with the interaction between the nervous and the endocrine systems, it is the mechanism by which the hypothalamus maintains internal balance, thereby regulating reproductive activities. Numerous research gaps exist in this branch of physiology. The present study investigated the protective neuroendocrine and reproductive influence of *Spondias mombin* leaf and *Curcuma longa* rhizome methanolic extracts on male sex hormones and sperm parameters in alloxan-exposed Wistar rat

Methodology

90 rats were randomly divided into 9 groups of 10 rats each. Diabetes was induced in all rat groups except Group 1 using alloxan at a dose of 200mg/kg bw administered intraperitoneally. Each rat group was subsequently treated as follows: Group 1: untreated non-diabetic. Group 2: untreated diabetic. Group 3: diabetic + low dose *Spondias mombin*. Group 4: diabetic + high dose *Spondias mombin*. Group 5: diabetic + low dose *Curcuma longa*. Group 6: diabetic + high dose *Curcuma longa*. Group 7: diabetic + combined low doses of *Spondias mombin* and *Curcuma longa*. Group 8: diabetic + combined high doses of *Spondias mombin* and *Curcuma longa*. Group 9: diabetic + glibenclamide.

Results

Significant reduction in the mean values of luteinizing hormone, follicle stimulating hormone, testosterone, sperm volume, count, and viability was observed amongst group 2 (Untreated diabetic) rats following diabetes induction compared to group 1 (Untreated non-diabetic) rats ($p < 0.05$). Suggesting a deleterious effect. Treatment following single administration of graded

doses of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes observed amongst groups 3 to 6 rats showed significant improvement in sperm volume, count, viability, luteinizing hormone, follicle stimulating hormone and testosterone relative to group 2 rats. Suggesting an ameliorative effect. Significant changes were observed in the above parameters amongst groups 7 and 8 rats co-administered graded doses of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes compared to group 2 rats ($p < 0.05$). Indicating a possible beneficial outcome. Noteworthy, better outcomes were observed amongst groups 7 and 8 rats co-administered methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes, suggesting a potential greater potency compared to the single extract administered rats (Groups 3 -6). Notably, better results were observed in *Spondias mombin* administered rats than *Curcuma longa* rhizomes extract exposed rat groups.

Conclusion:

This study demonstrated that *Spondias mombin* leaves and *Curcuma longa* rhizomes extracts significantly improved male sex hormones and sperm parameters. From the results obtained, co-administration of both extracts provided better ameliorative efficacies than the individual extract administration. Similarly, *Spondias mombin* demonstrated greater potency compared to *Curcuma longa* rhizomes.

Keywords: *Spondias mombin*, *Curcuma longa*, neuroendocrine, co-administration

Introduction

Neuroendocrinology is the branch of physiology which deals with the interaction between the nervous and the endocrine systems (i.e. how the brain regulates the endocrine activities in the body) [1]. The nervous and endocrine systems usually function together in a process called neuroendocrine integration, to regulate the biological activities that goes on in the body [2]. Neuroendocrinology emanated from the background that the brain, particularly the hypothalamus, controls pituitary secretions, and has later expanded to check numerous interconnections of the endocrine and nervous systems, the neuroendocrine system is the mechanism by which the hypothalamus maintains internal balance, thereby regulating reproductive activities, metabolism, food consumption, drinking behavior, and blood flow [2, 3]. Male reproductive hormones are majorly steroid hormones [4]. These hormones include the luteinizing hormone, follicle stimulating hormone and testosterone. Their effects are mediated through slow genomic mechanisms by specific nuclear receptors and by fast nongenomic pathways through membrane-associated receptors and intracellular communication interplays [5]. Sperm is the male reproductive cell [6]. Its main function is to reach the ovum and attach with it to deliver two sub-cellular structures: the male pronucleus and the centrioles [7]. Sperm quantity and quality are the main parameters in semen quality, which is a measure of the ability of semen to achieve fertilization [8].

In recent years, on account of the reported beneficial effects of some botanical preparations, the use of herbs and spices is gradually gaining prominence in major countries of the world [9]. However, several medicinal plants have been explored, but only a few have showed great promise in this regard; a good number of medicinal plants have demonstrated antioxidant tendencies. For example, the antioxidant properties of plant species like *Spondias mombin* and *Curcuma longa* have been tested [10].

Spondias mombin Linn (Anacardiaceae) commonly known as 'hog plum,' is a deciduous tree with large panicles of small white flowers and yellow plum-like fruits [11]. It is common in farmlands and villages especially in the forest and savannah regions. In traditional folklore medicine, *Spondias mombin* leaves are used as an emetic and for treatment of gonorrhoea, diabetes and psychiatric disorders; [12, 13] it is also useful for the expulsion of

the placenta in goats; [12] as an astringent; [12] an oxytocic agent; [14] and in wound treatment [12]. *Curcuma longa* (Linn) commonly known as turmeric is an erect perennial herb with pulpy orange tuberous roots that grows to about 2 feet in length [15, 16]. It belongs to the family Zingiberaceae. Its rhizome is pungent with a bitter taste and widely used in indigenous medicine as a general household remedy and a cooking spice [13, 15, 17, 18]. It has been reported beneficial in the treatment of diabetes, high cholesterol, abdominal pains, menstrual disorders, wounds, eczema, jaundice, inflammation, cancer symptoms and as a blood purifying agent [16]. The herb contains curcumin as an active ingredient, which is a yellow-colored phenolic pigment obtained from the powdered rhizome.

Anecdotal reports indicated that these plants have shown promising fertility effects, especially on reproductive hormones and sperm quality. Bárbara *et al.*, (2016) [19] and Arghavan *et al.*, (2021) [20] demonstrated that antioxidant and inhibited lipid peroxidative activity of *Spondias mombin*, and *Curcuma longa* could be beneficial in reproductive dysfunction. Olalekan *et al.*, (2023) [21] and Hay *et al.*, (2019) [22] reported a possible beneficial reproductive outcome for *Spondias mombin* and *Curcuma longa* in mice. However, no scientific data exist currently on the effects of the combined administration of *Spondias mombin* leaves and *Curcuma longa* rhizomes on male reproductive hormones and sperm parameters. Therefore, the need for the present study, aimed at investigating the protective neuroendocrine and reproductive influence of *Spondias mombin* leaf and *Curcuma longa* rhizome methanolic extracts on male sex hormones and sperm parameters in alloxan-exposed Wistar rat.

MATERIALS AND METHODS

Plant materials and extract preparation:

Fresh leaves of *Spondias mombin* were obtained from the University of Port Harcourt botanical garden; while *Curcuma longa* rhizomes were purchased from a local market in Ogoni, Rivers state, Nigeria. Both plant specimens were identified and authenticated by Dr. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Voucher

specimens of both plants were deposited in the herbarium. The research protocol for the study was reviewed and approved by the Ethics Committee of our university vide a communication referenced: UPH/R&D/REC/04 and dated 3rd July, 2018. This study was conducted in accordance with the guidelines for the care and use of laboratory animals issued by the United States Institute for Laboratory and Animal Research (1996) [23]. Leaves of *Spondias mombin* were dried at room temperature for about 14 days and extracted using percolation method. Briefly, the *Spondias mombin* leaves were grounded into powder; subsequently, 8.8kg was properly macerated with 98% methanol for three days and the percentage yield was 93%. It was then filtered and concentrated using rotary evaporator at 40°C. The obtained extract was kept in airtight containers and stored at room temperature before use. Rhizomes of *Curcuma longa* were oven dried at 40°C for 72 hours to a constant weight. The dried rhizomes were then pulverized using a household blender. 7.5g of the powder obtained was dissolved in 98% methanol for about two days and the percentage yield was 96%. The extraction procedure employed was percolation as described by Olatunde *et al.*, (2014) [24].

Experimental animals and drugs:

90 male Wistar rats weighing between 120-250g were used for this study. The animals were kept at the Animal House, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The rats were fed with normal rat pellet and tap water ad libitum. The experimental animals were acclimatized for a period of two weeks after which they were properly grouped. Alloxan monohydrate was obtained from Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, USA. While glibenclamide was obtained from Swiss Pharm Nigeria Ltd, 5, Dopemu Road, Agege, Lagos, Nigeria.

Acute toxicity study (LD50):

The LD50 value of the leaf extract of *Spondias mombin* was assumed to be greater than 1000mg/kg bw as reported by Olatunde *et al.*, (2014) [24] and Saronee *et al.*, (2019) [13]. Similarly, the LD50 value of the methanol extract of *Curcuma longa* rhizomes was regarded as the value obtained by Yuandani, (2017) [25] which was reported to be greater than 5000mg/kg bw.

Experimental design:

90 rats were subsequently randomly divided into 9 groups of 10 rats each. Diabetes was induced in all rat groups except Group 1 using alloxan at a dose of 200mg/kg bw administered intraperitoneally. Diabetes was confirmed after 72 hours of alloxan administration if the blood glucose is ≥ 11.1 mmol/L (200mg/dl) (Stanley Mainzen Prince and Menon, 2001) [26]. Each rat group was subsequently treated as follows: Group 1: untreated non-diabetic; rats in this group had free access to only normal rat chow and tap water ad libitum. Group 2: untreated diabetic; rats in this

group received no further treatment after induction of diabetes. Group 3: diabetic + low dose *Spondias mombin*; rats in this group were treated with 200mg/kg bw of *Spondias mombin* extract daily after the induction of diabetes. Group 4: diabetic + high dose *Spondias mombin*; rats in this group were treated with 400mg/kg bw of *Spondias mombin* extract daily after the induction of diabetes. Group 5: diabetic + low dose *Curcuma longa*; rats in this group were treated with 500mg/kg bw *Curcuma longa* extract daily after the induction of diabetes. Group 6: diabetic + high dose *Curcuma longa*; rats in this group were treated with 1000mg/kg bw *Curcuma longa* extract daily after the induction of diabetes. Group 7: diabetic + combined low doses of *Spondias mombin* and *Curcuma longa*; rats in this group were treated with both 200mg/kg bw of *Spondias mombin* and 500mg/kg bw of *Curcuma longa* extracts daily after the induction of diabetes. Group 8: diabetic + combined high doses of *Spondias mombin* and *Curcuma longa*; rats in this group were treated with both 400mg/kg/bw of *Spondias mombin* and 1000mg/kg bw of *Curcuma longa* extracts daily after the induction of diabetes. Group 9: diabetic + glibenclamide; rats in this group were treated with 0.6mg/kg bw of glibenclamide daily after the induction of diabetes. The glibenclamide and the extracts of *Spondias mombin* and *Curcuma longa* were administered daily using an oro-gastric cannula for 42 days. On day 43, blood was collected by direct cardiac puncture for the determination of male reproductive hormones. Male testes were excised for the determination of sperm parameters.

Determination of hormonal level and sperm characteristics

Serum concentration of luteinizing hormone, follicle stimulating hormone, and testosterone were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Accu-Bind ELISA Microwells, California, USA). Procedure was as specified in the manual. As earlier described by Nwafor *et al.*, (2021) [27], testes were excised and the caudal epididymis isolated and placed in a petri dish containing 3 ml of sodium bicarbonate (NaHCO₃) buffered Tyrodes' solution. 1 mm incisions were made on it and sperm drawn into a plastic pipette and was subsequently transferred into 5 ml test tubes and shaken for homogeneity and dispersal. Sperm cells were subsequently evaluated to determine volume, count, and viability.

Statistical analysis:

Results are as presented in Tables 1 and 2 and expressed as means $\pm$ standard error of means. Significant differences were determined using the one-way ANOVA; differences between groups were determined using LSD Post Hoc test. A p value of less than 0.05 was considered statistically significant.

Results

Table 1. Changes in male sex hormones following methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes administration.

Treatment	Luteinizing Hormone (miu/ml)	Follicle Stimulating Hormone (miu/ml)	Testosterone (ng/ml)
Group 1: Untreated non-diabetic	0.31 $\pm$ 0.06 ^a	0.16 $\pm$ 0.03 ^a	0.56 $\pm$ 0.01 ^a
Group 2: Untreated diabetic	0.20 $\pm$ 0.02	0.10 $\pm$ 0.01	0.47 $\pm$ 0.15
Group 3: Diabetic + low dose <i>Spondias mombin</i>	0.24 $\pm$ 0.12	0.12 $\pm$ 0.14	0.49 $\pm$ 0.17

Group 4: Diabetes + high dose <i>Spondias mombin</i>	0.29±1.13 ^a	0.15±0.10 ^a	0.54±1.19 ^a
Group 5: Diabetes + low dose <i>Curcuma longa</i>	0.23±0.01	0.20±0.11 ^a	0.46±0.16
Group 6: Diabetes + high dose <i>Curcuma longa</i>	0.27±0.16 ^a	0.16±1.10 ^a	0.51±1.13
Group 7: Diabetes + combined low doses of <i>Spondias mombin</i> and <i>Curcuma longa</i>	0.26±1.12 ^a	0.13±1.16	0.51±1.19
Group 8: Diabetes + combined high doses of <i>Spondias mombin</i> and <i>Curcuma longa</i>	0.33±0.01 ^a	0.19±0.02 ^a	0.59±0.03 ^a
Group 9: Diabetes + glibenclamide	0.27±0.06 ^a	0.14±0.16	0.52±0.18 ^a

Values are shown as Mean ± SEM; n=10; a = significant difference compared to Group 2 (p<0.05)

Significant reduction in luteinizing hormone, follicle stimulating hormone and testosterone levels were reported amongst group 2 rats administered alloxan only compared to group 1 rats (p<0.05), indicating a potential negative effect. Insignificant improvements were observed amongst groups 3 and 5 rats following single administration of low doses of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes compared to group 2

rats. However, administration of individual high doses of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes elicited significant outcome amongst groups 4 and 6 rats compared to group 2 rats (p<0.05). Greater significant improvements were seen amongst groups 7 and 8 rats co-administered *Spondias mombin* leaves and *Curcuma longa* rhizomes extracts (p<0.05). Suggesting a better outcome following combined administration. Notably, *Spondias mombin* elicited a better effect than *Curcuma longa* rhizomes extract. Similar results were observed amongst group 9 rats administered glibenclamide.

Table 2. Changes in sperm parameters following methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes administration.

Treatment	Volume (ul)	Count	Viability (%)
Group 1: Untreated non-diabetic	0.2±0.01 ^a	540.00±0.02 ^a	80.00±0.02 ^a
Group 2: Untreated diabetic	0.1±0.02	510.00±0.01	60.00±0.01
Group 3: Diabetic + low dose <i>Spondias mombin</i>	0.1±0.03	520.00±0.03 ^a	60.00±0.01
Group 4: Diabetes + high dose <i>Spondias mombin</i>	0.2±0.06 ^a	530.00±0.12 ^a	70.00±0.10 ^a
Group 5: Diabetes + low dose <i>Curcuma longa</i>	0.1±0.01	515.00±0.13	60.00±0.20
Group 6: Diabetes + high dose <i>Curcuma longa</i>	0.2±0.01 ^a	525.00±0.07 ^a	65.00±0.15
Group 7: Diabetes + combined low doses of <i>Spondias mombin</i> and <i>Curcuma longa</i>	0.2±0.05 ^a	530.00±1.10 ^a	70.00±1.13 ^a
Group 8: Diabetes + combined high doses of <i>Spondias mombin</i> and <i>Curcuma longa</i>	0.2±0.05 ^a	550.00±0.01 ^a	85.00±0.01 ^a
Group 9: Diabetes + glibenclamide	0.2±0.06 ^a	530.00±0.03 ^a	70.00±0.06 ^a

Values are shown as Mean ± SEM; n=10; a = significant difference compared to Group 2 (p<0.05)

Significant reduction in the mean values of volume of sperm, count and viability was observed amongst group 2 (Untreated diabetic) rats following diabetes induction using alloxan compared to group 1 (Untreated non-diabetic) rats (p<0.05). Suggesting a deleterious effect. Treatment following single administration of graded doses of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes observed amongst groups 3 to 6 rats showed significant improvements in sperm volume, count and viability relative to group 2 rats. Suggesting an ameliorative effect. Significant changes were observed in sperm volume, count and viability amongst groups 7 and 8 rats co-administered graded doses

of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes compared to group 2 rats (p<0.05). Indicating a possible beneficial outcome. Noteworthy, better outcomes were observed amongst groups 7 and 8 rats co-administered methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes, suggesting a potential greater potency compared to the single extract administered rats (Groups 3 -6). Notably, better results were observed in *Spondias mombin* administered rats than *Curcuma longa* rhizomes extract exposed rat groups. Expectedly, significant changes were reported amongst group 9 rats given glibenclamide compared to group 2 rats (p<0.05).

Discussion

This study investigated the precise objectives of the research by evaluating the protective neuroendocrine and reproductive influence of *Spondias mombin* leaf and *Curcuma longa* rhizome methanolic extracts on male sex hormones and sperm parameters in alloxan-exposed Wistar rat.

The male reproductive system is governed by the hypothalamic-pituitary-gonadal (HPG) axis, a finely tuned endocrine circuit regulated by negative feedback [28]. The hypothalamus releases Gonadotropin-Releasing Hormone (GnRH), which stimulates the anterior pituitary to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH acts on the Leydig cells in the testicular interstitium to stimulate testosterone production, while FSH acts on the Sertoli cells within the seminiferous tubules to support spermatogenesis [29]. Importantly, testosterone exerts negative feedback on both the hypothalamus and the pituitary, suppressing the release of GnRH, LH, and FSH to maintain hormonal homeostasis. Follicle stimulating hormone (FSH) is the primary driver of Sertoli cell function, which is essential for nurturing developing germ cells and maintaining the structural integrity of the tubules [29]. In a healthy state, an increase in testosterone would lead to a decrease in luteinizing hormone and follicle stimulating hormone. Conversely, a primary failure of the testes occasioned by alloxan monohydrate administration may have caused a decline in these hormone profiles, while extracts administration may have elicited an ameliorative response leading to a rise in luteinizing hormone, follicle stimulating hormone and testosterone levels. Hormonal distribution pattern observed in this study -a significantly elevated LH, FSH and testosterone levels, is clinically possible due to the ameliorative efficacies of our intervention. This condition signifies that the testes are beginning to recover from the deleterious effects of alloxan monohydrate, followed by a much stronger modulatory, and stimulatory signal from the pituitary gland to maintain normal hormonal output [30, 31].

The results of the present study indicated that the methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes have considerable effects on sperm parameters. In several cases of infertility, the major problems encountered are low sperm count and viability [32]. Advanced techniques for optimizing sperm function in these procedures are of evident value [32]. Sperm count and viability assessment, are important factors for fertilization capacity. Oxidative stress is harmful to sperm function and impairs male fertility by affecting the viability of sperm cells [33]. High concentrations of nitric oxide (NO) could affect sperm viability by cytotoxic effect that is often mediated by oxidative stress and lipid peroxidation of sperm membranes. Although the mechanisms of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes for improving sperm count and viability are unknown, Nassar *et al.*, (1998) [34] suggested that a significant stimulatory effect on human sperm might have a relation with the trace elements, especially Ca^{2+} that is inherent in *Spondias mombin* leaves and *Curcuma longa* rhizomes extracts. Ca^{2+} could inhibit the enzyme phosphate diesterase, which prevents cyclic adenosine monophosphate degradation and enhance sperm motility

Conclusively, the present study demonstrated that the effects of *Spondias mombin* leaves and *Curcuma longa* rhizomes extract on male reproductive hormones and sperm parameters at the administered doses significantly improved luteinizing hormone,

follicle stimulating hormone, testosterone, sperm volume, count and viability. From the results obtained, co-administration of both extracts provided better ameliorative efficacies than the individual extract administration. Similarly, *Spondias mombin* demonstrated greater potency compared to *Curcuma longa* rhizomes. Nevertheless, further studies are recommended to investigate fertilization capacity of sperms and possible mechanisms of action of the extracts.

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