

Protective role of *Jatropha tanjorensis* on Testicular Architecture and Reproductive Hormone Profile in Dichlorvos exposed Male Mice

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Abstract

This study aimed at evaluating the protective role of *Jatropha tanjorensis* on testicular architecture and reproductive hormone profile in Dichlorvos exposed Male Mice. Thirty sexually matured male mice with mean weight of 22.45 ± 3.05 g were used in the study. The animals were selected randomly into six groups of five mice in each group. Group A was the control group, while group B was administered DDVP at 5mg/kg/bw/day. C, and D were administered 250mg/kg/bw/day of fresh and dried *J. tanjorensis* leaves respectively. E and F were coadministered DDVP+250mg/kg/bw/day of fresh and dried *J. tanjorensis* leaves respectively. At the end of 35 days treatment period, blood samples were collected in plain bottles for analysis of testosterone, Follicle Stimulating hormone, Luteinizing hormone and Estradiol. Histological sections of the testes were mounted on slides, stained with hematoxylin and eosin (H&E) and Photomicrographs were generated.

Results: In the DDVP-only group (B), all the reproductive hormones significantly ($p < 0.05$) decreased compared with the control. Coadministration of *J. tanjorensis* significantly reversed these hormonal disturbances across the treated groups. Both fresh and dry extracts elevated FSH, LH, testosterone, and estradiol relative to the DDVP-only group. Histological result revealed DDVP-only group displayed severe degeneration of the seminiferous epithelium, including loss of spermatogenic layers, while co administration of *J. tanjorensis* significantly reversed these hormonal disturbances and restored testicular architecture across the treated groups. Conclusively, exposure to dichlorvos (DDVP) resulted in significant alterations in reproductive hormones alongside severe histopathological lesions. While *Jatropha tanjorensis* not only enhanced hormonal regulation but also promoted structural recovery of the testes especially in groups co administered dried extract of *J. tanjorensis*.

Keywords: Dichlorvos, hormones, *Jatropha tanjorensis*, testicular architecture, Testosterone

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I. INTRODUCTION

Male fertility refers to the reproductive capability of males, and any dysfunction in this process can lead to reduced reproductive outcomes in mammals. Over the past few decades, the incidence of infertility has risen dramatically, with environmental toxicants and associated hormonal disruptions emerging as significant contributing factors [1,2,3,4,5]. Humans are increasingly exposed to endocrine-disrupting chemicals—including pesticides, bisphenols, and dioxins—through air, water, and food. Once bioaccumulated, these compounds can profoundly affect general health, particularly reproductive function [4,5,6].

The testis is central to male reproduction, responsible for producing spermatozoa and synthesizing testosterone. Spermatogenesis occurs within the seminiferous tubules and depends on the structural integrity of testicular tissue as well as the proper functioning of Sertoli and Leydig cells. Exposure to toxicants such as pesticides can result in degeneration of the seminiferous epithelium, reductions in germ cell populations, and impaired sperm maturation, ultimately compromising male fertility [7].

Plants, beyond their role as food, have long been recognized for their medicinal properties [3,8]. It is estimated that over 400,000 species of tropical flowering plants possess therapeutic potential, contributing to the lower cost and widespread use of traditional medicine compared to modern medical treatments [9].

Although herbal and plant-derived therapies are generally considered safe, comprehensive toxicological evaluation is necessary to validate their efficacy and safety. *Jatropha tanjorensis* (family Euphorbiaceae) is a perennial herb that exhibits intermediate morphological characteristics between *Jatropha curcas* and *Jatropha gossypifolia* [10]. Locally referred to as “Iyana ipaja” in Yoruba and popularly known as

the “catholic vegetable” or “hospital too far,” the leaves are widely consumed as a vegetable in southern Nigeria, especially in Edo State, and are traditionally used in managing diabetes [10,11]. In addition to their culinary use, the leaves are incorporated into soups or used as tonics believed to increase blood volume and are traditionally employed in the treatment of anemia, diabetes, and cardiovascular disorders [10,11,12]

Like many members of the Euphorbiaceae family, *J. tanjorensis* contains potentially toxic compounds, including lectins, saponins, carcinogenic phorbol esters, and trypsin inhibitors. Fresh leaves are rich in water but low in protein and contain trace elements such as zinc, iron, and selenium. Phytochemical analyses have revealed the presence of flavonoids, tannins, terpenoids, saponins, and cardiac glycosides [13,14] which have been associated with hypolipidemic, antioxidant, and anti-inflammatory activities. Anecdotal evidence also suggests that the whitish latex exuded from leaf stems and stalks, though mildly irritant, may enhance hematopoiesis.

Given the increasing exposure to reproductive toxicants and the rich phytochemical profile of *J. tanjorensis*, this study was designed to investigate the effect of *Jatropha tanjorensis* on some reproductive hormones and the histological architecture of the testes in male mice exposed to dichlorvos.

II. Materials And Methods

2.1 Experimental Location

The experiment was carried out in the animal house of the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt. (Coordinates 4° 48'14''N 6° 59' 12''E).

2.2 Experimental animals and Management

Thirty sexually matured male mice (mean weight 22.45±3.05g) were used in the study. The mice were housed individually in cages under standard conditions. They were provided with clean water and rodent pellet *ad libitum*. All experiment were conducted according to the Institutional Animal Care Protocols at the Rivers State University Port Harcourt, Nigeria and follow guidelines for the ethical treatment of experimental animals.

2.3 Experimental Design and Procedure

A total of thirty (30) Swiss matured male mice were assigned to six (6) groups (A - F) of five (5) mice each. Group A served as control. Group B received 5mg/kg/bw/day of DDVP only. Group C received 250mg/kg/bw of fresh *Jatropha tanjorensis* leaves extract. Group D received 5mg/kg/bw/day of DDVP and 250mg/kg/bw/day of dried *Jatropha tanjorensis* leaves. Group E received 5mg/kg/bw/day of DDVP and 250mg/kg/bw/day of fresh *Jatropha tanjorensis* extract. Group F received 5mg/kg/bw/day and 250mg/kg/bw/day of dried *Jatropha tanjorensis*. All the groups were exposed to their treatment by oral gavage for thirty - five (35) days.

2.4 Blood collection

After the final treatment, the animals were fasted for 24 hours with free access to water. Blood samples were collected under isoflurane inhalation anesthesia by retro-orbital puncture using a heparinized capillary tube inserted into the medial canthus of the eye. Blood was collected into labeled plain tubes, allowed to clot, and centrifuged at 3,000 rpm for 10 minutes to separate serum for hormonal assays. The hormones include: Testosterone, Progesterone, Lutenizing hormones, Estradiol, Follicle stimulating hormone according to [4,5]. Analysis for the quantitative determination of all androgens was carried out using enzyme-linked immunosorbent assay (ELISA).

2.5. Histological analysis of the testis

For each mouse, 0.5g of the testis was fixed in 10% v/v buffered formaldehyde and dehydrated through ascending grades of ethanol, cleared in xylene and embedded in paraffin wax and sectioned with a digital microtome at 5µm thick. Histological sections mounted on slides were stained with Hematoxylin and Eosin (H&E). Photomicrographs were generated with a digital Microscope Biosphere Miller B with an image processor DN2-Microscopy Image Processing Software at X40 magnification.

2.6. Statistical analysis

Data analysis was conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Graphical representations and data visualizations were carried out using the JMP statistical discovery™ software version 14.3.

III. Results

3.1. Effects of oral administration of *Jatropha tanjorensis* on hormonal profile of male mice exposed to dichlorvos.

The effect of oral administration of *Jatropha tanjorensis* extract on the concentrations of reproductive hormones in male mice exposed to dichlorvos are presented in Fig. 1-4 The control group (A) recorded an FSH level of

1.36 \pm 0.03 IU/ml, which significantly ($p < 0.001$) decreased to 0.44 \pm 0.03 IU/ml in the DDVP-only group (B). All treatment groups receiving *J. tanjorensis* showed increased FSH levels compared to group B (Fig. 1). For LH, the control group had 1.26 \pm 0.11 IU/ml, while group B showed a marked reduction to 0.63 \pm 0.13 IU/ml. LH concentrations significantly increased in the treated groups, reaching 2.30 \pm 0.06 IU/ml in group C and 1.80 \pm 0.02 IU/ml in group D, with further increase observed in groups E and F (Fig. 2). Testosterone levels declined from 2.50 \pm 0.22 IU/ml in the control group to 1.43 \pm 0.21 IU/ml in group B. However, treatment with *J. tanjorensis* significantly ($p < 0.05$) elevated testosterone levels across the other groups (Fig. 3). Estradiol concentration in the control group was 52.60 \pm 3.97 IU/ml, decreasing to 42.20 \pm 1.48 IU/ml in group B. A significant increase occurred in group C and group D while groups E and F showed moderate increase compared to group B (Fig. 4).

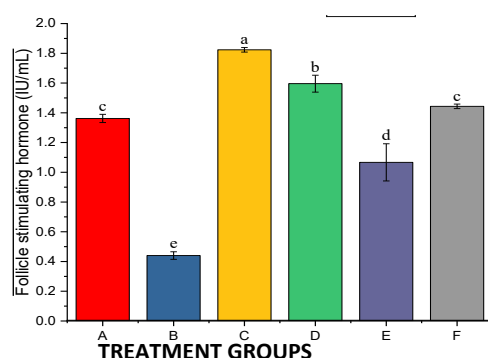


Fig 1: Effect of *Jatropha tanjorensis* on FSH in male mice exposed to Dichlorvos

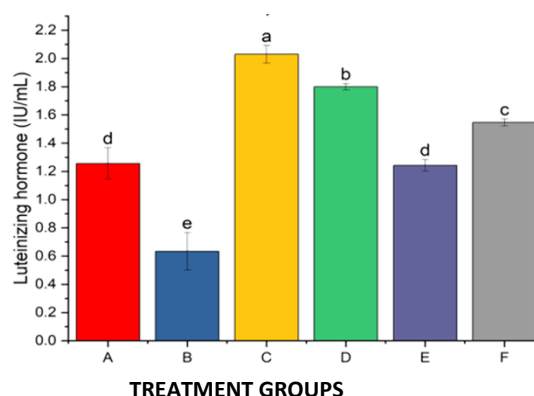


Fig 2: Effect of *Jatropha tanjorensis* on LH in male mice exposed to Dichlorvos

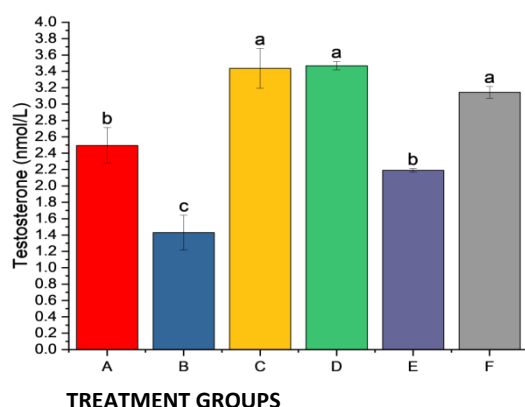


Fig 3: Effect of *Jatropha tanjorensis* on Testosterone in male mice exposed to Dichlorvos

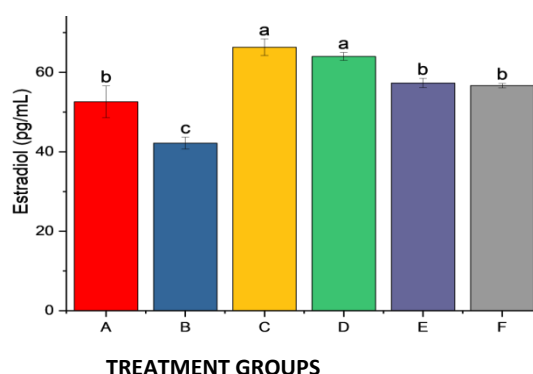


Fig 4: Effect of *Jatropha tanjorensis* on Estradiol in male mice exposed to Dichlorvos

3.2: Histopathological assessment of Swiss Mice exposed to DDVP and co-administered *Jatropha tanjorensis* extract

The histological examination of testicular tissues revealed marked differences in the seminiferous epithelium among the experimental groups, indicating the effects of dichlorvos (DDVP) toxicity and the protective role of *Jatropha tanjorensis* (Fig. 3.2a-Fig. 3.2f)

In the control group (Fig. 3.2a), the seminiferous epithelium appeared normal, showing active spermatogenesis. The basal compartment was lined with spermatogonia, while the adluminal compartment contained primary spermatocytes and maturing spermatozoa within the lumen,

In contrast, the DDVP-only group (Fig. 3.2b) showed massive degeneration of the seminiferous epithelium, characterized by the loss of spermatogenic cells, scanty spermatogonia, few primary spermatocytes, absence of secondary spermatocytes, and an empty lumen.

The group treated with fresh *Jatropha tanjorensis* alone (Fig. 3.2c) showed partial restoration of spermatogenic activity. Although the interstitial spaces remained sparse, spermatogonia were visible along the basement membrane, and some mitotic activity was observed in the adluminal compartment, suggesting mild stimulation of spermatogenesis.

The group treated with dry *Jatropha tanjorensis* alone (Fig. 3.2d) exhibited a well-recovered seminiferous epithelium. The presence of numerous Leydig cells in the interstitial space indicates enhanced androgenic activity and improved testicular function.,

In the group exposed to both DDVP and fresh *Jatropha tanjorensis* (Fig. 3.2e), partial recovery of the seminiferous epithelium was observed. Sertoli cell nuclei were visible, and the epithelial lining appeared regenerated, although the lumen remained empty, indicating incomplete spermiation and partial restoration of spermatogenesis.

Finally, the group exposed to DDVP and dry *Jatropha tanjorensis* (Fig. 3.2f) showed a fully restored seminiferous epithelium, with normal cellular organization and repopulation of germ cells.

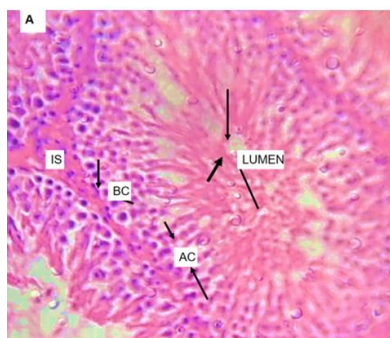


Fig 3.2a: Photomicrograph of testis from group A animals X40

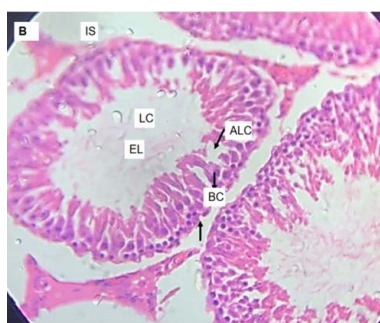


Fig 3.2b: Photomicrograph of testis from group B animals administered DDVP only X40

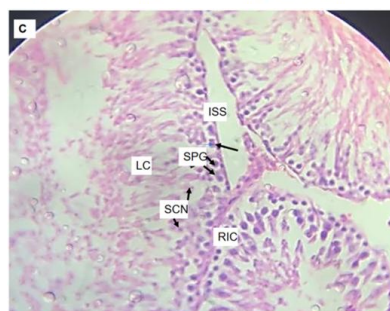


Fig 3.2c: Photomicrograph of testis from group C animals administered fresh *J. tanjorensis* leaves only X40

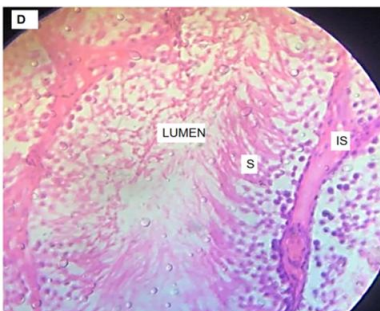


Fig 3.2d: Photomicrograph of testis from group D animals administered dried *J. tanjorensis* leaves only X40

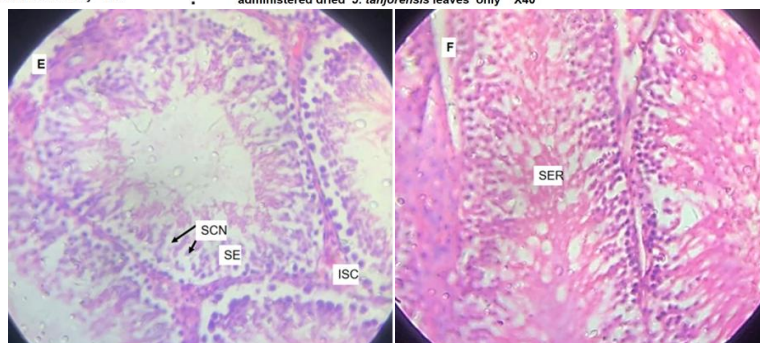


Fig 3.2e: Photomicrograph of testis from group E animals administered fresh *J. tanjorensis* leaves + DDVP X40

Fig 3.2f: Photomicrograph of testis from group F animals administered dried *J. tanjorensis* leaves + DDVP X40

IV. Discussion

DDVP exposure caused a marked decline in FSH, LH, testosterone, and estradiol levels, confirming its gonadotoxic and endocrine-disrupting properties. In the DDVP-only group (B), FSH and LH significantly decreased compared with the control (fig 1 & 2). These reductions suggest that DDVP impairs the hypothalamic–pituitary–gonadal (HPG) axis, likely by interfering with gonadotropin-releasing hormone (GnRH) secretion and suppressing pituitary output of FSH and LH. This is consistent with previous reports that organophosphate pesticides inhibit reproductive hormones by inducing oxidative stress and disrupting central neuroendocrine regulation. [14, 15,16].

The decline in testosterone (from 2.50 ± 0.22 to 1.43 ± 0.21 IU/ml) further supports DDVP-induced testicular dysfunction. Since LH stimulates Leydig cells, the reduced LH level is directly linked to impaired testosterone synthesis. Similarly, estradiol levels decreased substantially in the DDVP group, suggesting reduced aromatase activity and compromised Sertoli cell function.

However, co administration of *J. tanjorensis* significantly reversed these hormonal disturbances across the treated groups. Both fresh and dry extracts elevated FSH, LH, testosterone, and estradiol relative to the DDVP-only group. Notably, testosterone levels increased to more than double the control values in some treatment groups (3.47 ± 0.05 IU/ml in group D), indicating strong androgenic support. This hormonal recovery can be attributed to the plant's rich phytochemical composition—flavonoids, vitamins, antioxidants, and alkaloids—which are known to ameliorate oxidative stress, enhance steroidogenesis, and restore endocrine balance.[13,17].

The increase in FSH and LH following *J. tanjorensis* treatment suggests stabilization of the HPG axis. Elevated FSH enhances spermatogenic activity by stimulating Sertoli cell function, while increased LH supports Leydig cell activity and testosterone production. The concurrent rise in estradiol, especially in groups C and D, may reflect improved Sertoli cell aromatase function and renewed spermatogenic activity.

The histological results strongly support the hormonal findings. Control animals showed normal seminiferous tubules with active spermatogenesis, indicating intact testicular function. Conversely, the DDVP-only group displayed severe degeneration of the seminiferous epithelium, including loss of spermatogenic layers, absence of secondary spermatocytes, scanty spermatogonia, and empty lumina. These structural changes parallel the endocrine disruptions observed, as reduced FSH and testosterone are directly associated with impaired Sertoli cell support and diminished germ cell maturation. This finding supports [18,19,20]report scanty spermatogonia, loss of primary and secondary spermatocytes, and empty lumina as a typical hallmarks of organophosphate-induced testicular injury.

Treatment with *J. tanjorensis* led to progressive histological recovery. Fresh leaf extract alone (group C) restored the lining of the seminiferous tubules with visible spermatogonia and early spermatogenic activity, although the interstitial space remained sparse. Dry leaf extract alone (group D) resulted in near-complete restoration of seminiferous structure, with abundant Leydig cells—consistent with the elevated testosterone levels recorded. Increased Leydig cell population reflects enhanced steroidogenic activity, aligning with the high LH and testosterone concentrations.

In groups treated with both DDVP and *J. tanjorensis*, partial to full restoration was observed. The DDVP + fresh extract group (E) showed regenerated seminiferous epithelium and visible Sertoli cell nuclei, despite the lumen remaining empty, indicating recovering but incomplete spermiation. The DDVP + dry extract group (F) showed fully restored seminiferous architecture, with normal germ cell layering and reestablished spermatogenesis. This aligns with the highest hormone levels recorded in these groups, confirming that structural repair of the testis occurred concurrently with normalization of endocrine function.

V. Conclusion

The findings of this study demonstrate that exposure to dichlorvos (DDVP) resulted in significant alterations in reproductive function, as reflected by disrupted hormonal balance, impaired spermatogenesis, degeneration of seminiferous tubules, and reduced antioxidant capacity. DDVP-exposed mice showed decreased testosterone, LH, and FSH levels, alongside severe histopathological lesions, confirming its toxic effects on the male reproductive system. Conversely, the restoration of testicular architecture in the groups treated with *Jatropha tanjorensis*—particularly the dry extract—aligns with the observed elevation of reproductive hormone levels. This relationship suggests that *Jatropha tanjorensis* not only enhanced hormonal regulation but also promoted structural recovery of the testes, thereby facilitating normal spermatogenic activity after DDVP-induced damage.

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