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Effects of *Cissus populnea* and *Panax ginseng* on Flutamide-Induced Testicular Defect in Pre-Pubertal Male Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author VOA designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors ENA and RBB reviewed and managed the analyses of the study. Author AAO supervised the whole process. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: This study was carried out to determine the effects of *Cissus populnea* and *Panax ginseng* on flutamide-induced testicular toxicities in pre-pubertal rats.
Place and Duration of Study: Department of Anatomy, College of Medicine of the University of Lagos, Lagos – Nigeria, between May and December 2010.
Methodology: 20 male immature (25 days old) Wistar rats were used. They were randomly divided into 4 groups; 1 control and 3 treatment groups. Group A served as control, group B was administered flutamide and *Cissus populnea*, group C was administered flutamide and *Panax ginseng* and group D was administered flutamide alone. Body weight and testicular weights were measured. Hormonal assay for testosterone, FSH and LH were done using Enzyme-Linked Immunosorbent Assay (ELISA). Histopathology of the testis was also investigated.
Result: There were no statistically significant differences in serum testosterone levels in all three treatment groups when compared with the control group. There was a significant increase in the serum LH level in group D when compared with the control group ($p<0.05$). Serum FSH level in group B showed a significant increase when

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compared with the control group ($p<0.05$). The histological evidences of testis in group D showed a reduction in lining cells of the seminiferous tubules; however, in the other three treatment groups they were similar to the control group.
Conclusion: The results suggest that *Cissus populnea* and *Panax ginseng* ameliorates the adverse effects of flutamide on the testis.

Keywords: Cissus populnea; Panax ginseng; flutamide; testis.

1. INTRODUCTION

Dorfman [1] defined an anti-androgen "as a substance which prevents androgens from expressing their activity at target sites". The precise mechanism whereby anti-androgens inhibit androgen activity both peripherally and centrally, and the overall effects produced *in vivo* by the drugs have evoked numerous studies [2,3,4,5,6,7,8]. Flutamide is a non-steroidal anti-androgen which competitively inhibits the binding of androgens to the androgen receptor. Perobelli et al. [9] reports that the exposure to flutamide during the prepubertal period compromises the function of the epididymis along with epididymal sperm quality at adulthood.

However, it has been reported that *Cissus populnea (C. populnea),* a herbaceous climber which belongs to *Vitaceae* family has some fertility potentials [10]. The aqueous extract of its stem bark is associated with aphrodisiac or fertility potentials among the Yoruba-speaking people of South West Nigeria, where it is observed that men consume the aqueous and ethanolic extracts copiously and consistently for long periods of time either in mono or poly herbal formulations [10].

The use of various herbal remedies, including *C. populnea,* as an aphrodisiac and fertility enhancer amongst the males has been attributed to the declining fertility trend that has been established over the years [11]. Flavonoids, saponins, tannins and steroidal nucleus have been reported to be constituents of *C. populnea* [10].

Panax ginseng a member of the *Araliaceae* family of plants, which includes the closely related American ginseng and less similar *Siberian ginseng* is also reported to increase libido and sexual satisfaction [12]. *Panax ginseng* commonly grows on mountain slopes and is usually harvested in the fall. The root of *Panax ginseng* is used preferably from plants older than six years of age [13]. Taking *Panax ginseng* orally may enhance male fertility by acting directly on the pituitary gland as it reduces prolactin production or on the central nervous system increasing dopaminergic actions [14].

This study was therefore carried out to investigate effects of *Cissus populnea* and *Panax ginseng* on Flutamide-induced alterations on the pituitary-testis axis of pre-pubertal Wistar rats.

2. METHODOLOGY

2.1 Animals

20 Wistar rats, 25 days old, were obtained from the animal unit of the College of Medicine, University of Lagos. They were kept in plastic cages and allowed to acclimatize for two

weeks under standard laboratory conditions of temperature 27– 30ºC, with a photoperiodicity of twelve hours light alternating with twelve hours of darkness; at which time they would have attained pre-pubertal (40 days old) stage before experimentation. They were fed with commercially available rat chow and had access to water *ad libitum.*

2.2 Preparation of Extract

Cissus populnea stem barks were purchased from a local market in Lagos. The samples were authenticated at the Pharmacognosy Department of the College of Medicine, University of Lagos, Nigeria. The bark samples were chopped into tiny bits, rinsed thoroughly and then blotted. The fresh, blotted, weighed samples were steeped in sterile distilled water at a concentration of 43 g/ 100 ml for 72 hours with constant stirring and then filtered. The resulting crude extract was refrigerated (4ºC) until needed. Flutamide manufactured by Sovereign Medical and *Panax ginseng* manufactured by Hatay Pharmaceuticals J.S.C., Vietnam.

2.3 Experimental Protocol

The rats were randomly divided into 4 groups; 1 control and 3 treatment groups, then labeled and treated as follows: group A (control) was administered only tap water and feed. Group B was administered flutamide and *Cissus populnea*, group C was administered flutamide and *Panax ginseng* and group D was administered flutamide alone. Flutamide was given at a dose of 10 mg/kg/day [15]; *Panax ginseng* at a dose of 4 mg/kg/day; and *Cissus populnea* at a dose of 200 mg/kg/day [16]. The animals were dosed orally once daily for 15 days using a canula. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [17] and were approved by the Departmental Committee on the Use and Care of Animals in conformity with internationally acceptable standards.

2.4 Necropsy Schedule

At the end of the experimental period, the animals were serially sacrificed using intra-peritoneal Ketamine hypochloride with ketamine base of 50 mg at a dose titrated against consciousness starting with 0.01 ml. Incisions were made by a ventral laparotomy which was extended into the scrotum. The testes were removed and prepared for histological processing.

2.5 Testicular Weight

The weights of the testes were taken before fixing in Bouin's solution, using an electronically controlled scale.

2.6 Hormonal Assay

The serum samples collected and refrigerated were taken to the diagnostic laboratory in the Department of Obstetrics and Gynaecology, Lagos University Teaching Hospital (LUTH) where hormonal assay for testosterones, FSH and LH was done using Enzyme-Linked Immunosorbent Assay (ELISA) with kits supplied by Biotech laboratories Ltd, UK.

2.7 Tissue Processing

The tissues were processed in accordance with modifications of the method described by Akang et al. [18]. The testicular tissues were fixed in Bouin's solution. The fixed tissues were transferred to a graded series of ethanol and then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58ºC. Serial sections of 5 µm in thickness were obtained from a solid block of tissue, cleared, fixed in clean slides, stained with Haematoxylin and Eosin stains and examined with the light microscope at a magnification of x400.

2.8 Statistical Analysis

The data were analyzed with one way ANOVA at 5% level of significance.

3. RESULTS

3.1 Testicular Weight

There were no statistically significant differences in testicular weights of group B and C, and, in group D, it was lower, but not significantly different ($p>0.05$), when compared with the control group as shown in Table 1.

3.2 Hormone Assay

There was an increase the serum levels of testosterone (ng/ml) in group B which was administered flutamide and *Cissus populnea* (2.92 ± 0.31) however, it was not significantly different ($p>0.05$) when compared to control (2.50 ± 0.28), group C which was administered flutamide and *Panax ginseng* (2.22 ± 0.13) and group D which was administered flutamide alone (2.00 ± 0.19) showed a decrease which was not significantly different ($p>0.05$) when compared with control. There was also no statistically significant difference in LH (ng/ml) levels of group B (0.34±0.04) and C (0.47±0.07); however, there was a statistically significant increase in the serum levels of LH in group D (0.61±0.08) when compared with control (0.33±0.03). There was no statistically significant difference in the FSH (ng/ml) levels in groups C (0.23 ± 0.03) and D (0.25 ± 0.02); whereas FSH level in group B (0.28±0.05) showed a statistically significant increase when compared with the control group (0.23±0.04), respectively ($P < 0.05$) as shown in Table 1.

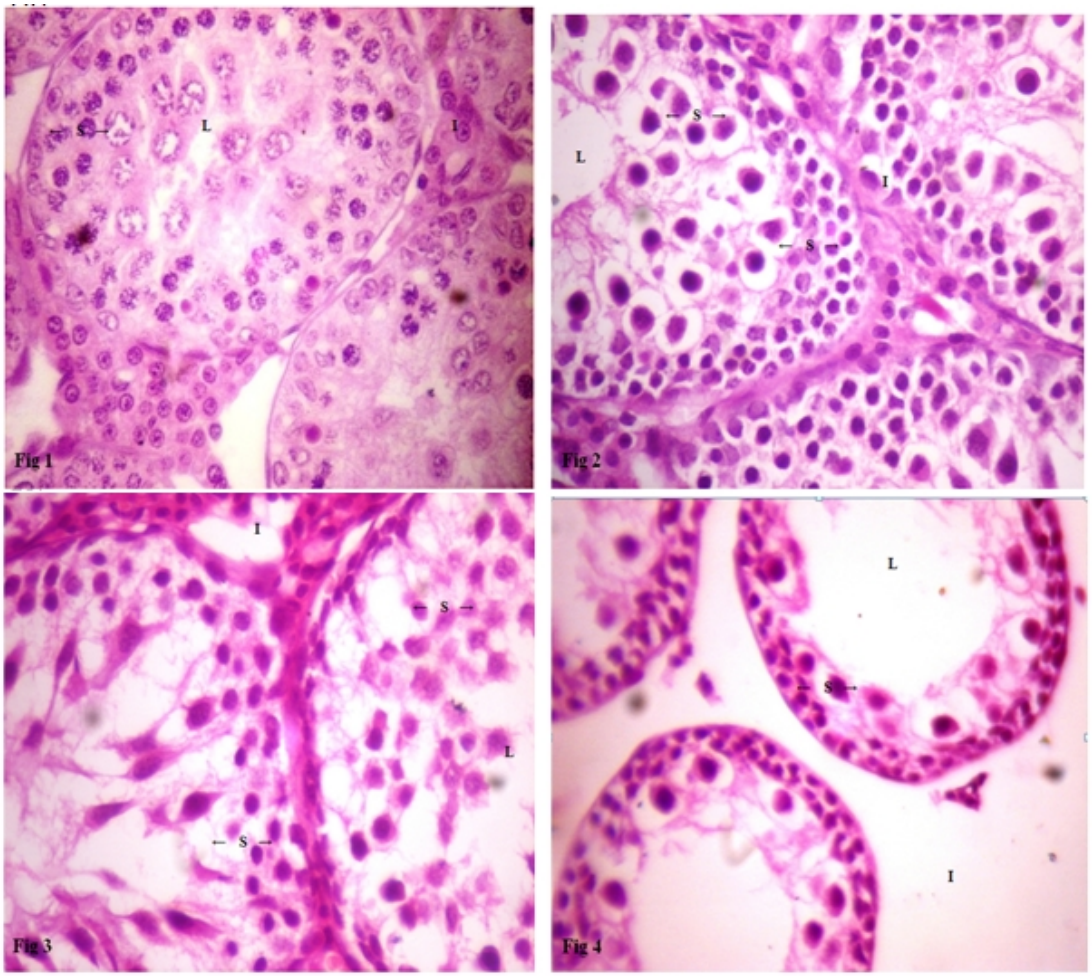
Table 1. The mean weights of prepubertal rats testes in grams

Groups	Testicular weight
A	1.20±0.03
B	1.15±0.01
C	1.21±0.05
D	0.70±0.02

Values are expressed in Mean ± SEM

3.3 Histology

The prepared slides were examined under the light microscope and sections photographed. The findings of all the groups were presented as photomicrographs (Fig. 1).



Photomicrographs showing sections of testes administered: Food and tap water (Fig. 1), flutamide and *Cissus populnea* (Fig. 2), flutamide and *Panax ginseng* (Fig. 3) and flutamide alone (Fig. 4); stained with H and E at magnification x400
I = Interstitium; L = Lumen; S = Spermatogonic cell series

The slides in groups B (Fig. 2) and C (Fig. 3) showed that the seminiferous tubules were lined by 4 – 5 cell layer thick germinal cells, devoid of luminal spermatids. This is the same finding in the control group (Fig. 1). However the group D (flutamide alone) showed that there was reduction in lining cells of the seminiferous tubules, to about 3 cell layers thick. They had sparse spermatogenic cell series, wide seminiferous tubular lumen and extensive interstitial spaces with scanty cellular components. No luminal spermatids were seen (Fig. 4).

4. DISCUSSION

Flutamide (4'-nitro-3'-trifluoromethyl-isobutyranilide) is a potent non-steroidal androgen receptor antagonist that has been used therapeutically to treat androgen-dependent prostate cancer [19,20] and as a tool to study male reproductive development.

Studies in rats have demonstrated that pre- or postnatal flutamide (6.5 to 50 mg/kg) exposure alters androgen dependent reproductive development [15,21] and has been shown to produce decreased reproductive organ weights, which was shown in this study although not statistically significant.

Vo et al. [22] reported the anti-androgenic effects of flutamide to alter reproductive function and exert distinct effects on developing male reproductive organs. This is in concert with our result as flutamide disrupted germ cells of the seminiferous tubules. It is believed that flutamide disrupts the function of the hypothalamo-pituitary-gonadal (HPG) axis [15]. This could be the reason for the increase in LH of flutamide treatment alone in this study. Decreased testosterone level is usually accompanied with increased LH production by the anterior pituitary gland [24]. Hence, the significant increase in luteinizing hormone in this study may have resulted from the decreased testosterone feedback.

It has been reported by Cheng [24] that testosterone plays an important role in maintaining quantitatively normal spermatogenesis. This is in concert with our study as decreased testosterone was accompanied with disrupted spermatogenesis. Cheng [24] also relates sperm production with gram of testis. This suggests that the decreased testicular weight of the animals that received flutamide alone is as a result of disrupted spermatogenesis.

O'Shaughnessy et al. [25] reports that FSH acts to stimulate spermatogenesis through an increase in spermatogonial number. Cheng [24] also reports that FSH is required for the initiation, maintenance and restoration of spermatogenesis. This study corroborates these reports as aqueous extract of *Cissus populnea* increased FSH level and increased cells of spermatogonic series. Hence, it ameliorated the alterations induced by flutamide. This is in consonance with the result from Smith and Ogunfeibo [16] who reported *Cissus polpunea* to increase sperm concentration in mainstream smoked weaned rabbits. Akpantah et al. [26] reported flavonoids which is constituent in *Cissus polpulnea* to act as antioxidants. It also invigorates the reproductive system [27]. This appears to be the reason for *C. polpulnea* ameliorative effect in this study.

There is evidence to support the use of *Panax ginseng* in the treatment of male sexual dysfunction [12]. Taking *Panax ginseng* orally may enhance male fertility by increasing sperm count, quality, and movement, as it activates the body system that increases production of certain hormones [12].

The use of *Panax ginseng* extract showed an increase in spermatozoa number/ml and progressive oscillating motility, an increase in serum testosterone, FSH and LH levels. It is suggested that ginsenosides may have an effect at different levels of the hypothalamus-pituitary-testis axis [28].

Jang et al. [29] reported that administration of ethanol plus red ginseng extract appeared to minimize the negative effects of ethanol toxicity on male fertility. This is in concert with the findings from this study as *Panax ginseng* ameliorated the toxic effects of flutamide on the

testis. *Panax ginseng* normalized FSH, LH and testosterone levels this may be due to a direct or an indirect effect on the HPG axis [30].

5. CONCLUSION

Flutamide is detrimental to spermatogenesis and alters the HPG axis but a concomitant use of adaptogens like *Cissus populnea* or *Panax ginseng* can preserve cellular morphology of testes and reproductive functions.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

The authors report no conflicts of interest.

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