



Research Article

Ameliorative Potentials of *Bombax costatum* Pellegr. & Vuillet
Chloroform Stem Bark Extract on Pentylenetetrazole-
induced Testicular Damage in Wistar Rats

^{1,2}Fali Wajuba, ¹Joseph Vandi Zirahei, ¹Sani Hyedima Garba and ^{1,*}Samaila Musa Chiroma

¹Department of Human Anatomy, University of Maiduguri, Nigeria

²Borno State College of Nursing and Midwifery, Maiduguri, Nigeria

*Corresponding author: musasamailachiroma@yahoo.com, doi.org/10.55639/607.2535

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ABSTRACT

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This study was conducted out to find out the ameliorative potentials of *Bombax costatum* Pellegr. & Vuillet chloroform extract (BCCE) on Pentylenetetrazole (PTZ)-induced testicular damage in albino Wistar rats. Eighteen adult rats were randomly allotted into three groups (n=6) after one week of acclimatization. Group one served as a control and was given normal saline. Group two (PTZ only) was given 35mgkg⁻¹ body weight PTZ. Group three received BCCE 250mgkg⁻¹ followed by PTZ 35mgkg⁻¹, the treatments lasted for one week. The rats were euthanized and testicular tissues were processed for histological study, while blood samples were collected and used for the hormonal assay. The interstitial Leydig cells have been disrupted by the PTZ toxicity which resulted in a significant decrease (p<0.05) of testosterone in the PTZ-induced rats as compared with the control group, while there was no significant difference in group three that was given BCCE when compared to the control. Also, there was no significant difference in the luteinizing hormone (LH) and follicles stimulating hormones (FSH) levels in all the rat groups. The histological features showed that PTZ has a deleterious effect on testicular tissues with seminiferous tubule lumen devoid of spermatozoa. The histology of the group that received BCCE 250mgkg⁻¹ shows the lumen of the seminiferous tubules with spermatozoa indicating that BCCE exerts a protective effect against PTZ-induced damage. This suggests that BCCE has an ameliorative effect against PTZ-induced testicular toxicity in rat models.

Corresponding author: Samaila Musa Chiroma, Email: musasamailachiroma@yahoo.com
Department of Human Anatomy, University of Maiduguri, Nigeria

INTRODUCTION

Bombax costatum is a common tree which is also called red-flowered silk cotton tree in English and kapokier in French (Mohammed *et al.*, 2018). It is known locally as Kuryaa or Gurjiyaa in Hausa language (Mohammed *et al.*, 2018). The plant is of the family Bombacaceae, found within the Savanna zone of West and Central Africa including Nigeria and Chad (Apinega *et al.*, 2018). The plant has been reported as an important medicinal herb in Nigeria and other countries of West Africa to treat many types of ailments and diseases (Mohammed *et al.*, 2018). Various parts of the plant are used for the medicinal purposes to treat diseases such fever and epilepsy besides being a stimulant (Orwa *et al.*, 2009). The root has been reported to be useful for food and for the treatment of different types of diseases (Obora and Kennedy 2017). Further, the bark of BC has been used in the condition of skin diseases, headache, fever and often times for wound healing (Belem *et al.*, 2008; Oyen 2011). The leaves are reported to have been used in the cases and management of seizures (Assogba *et al.*, 2018).

The industrial and medicinal importance of the all-medicinal plants are embedded in the bioactive phytochemical components that yield a desired physiological effect on the human body (Al-aziz *et al.*, 2019). The polyphenolic compounds which are common in many plants have been shown to enhance testosterone production by various regulatory mechanisms (Martin and Mohammed 2020). The phytochemical constituents of BC include saponins, tannis, alkaloids, flavonoids, carbohydrates, glycosides, steroids and terpenoids (Mohammed *et al.*, 2018). Saponins has been reported in a previous study to enhance serum testosterone level (Liu *et al.*, 2020). The hormone testosterone which is secreted by the interstitial Leydig cells of the testis is responsible for production, formation

and maturation of spermatozoa (Martin and Mohammed 2020). Flavonoids are low-molecular-weight common natural plants products containing polyphenolic compounds, found in fruits, vegetables and some beverages. Flavonoids have been reported to enhance androgens production in Leydig cells in addition to other health benefits (Martin and Mohammed 2020). Further, flavonoids contain a 5,7-dihydroxychromen-4-one backbone which increases steroidogenic acute regulatory gene (StAR) expression, that causes testosterone production from the Leydig cells (Martin and Mohammed 2020).

Infertility is a problem that has caused serious global family impact ranging from unhappy marriage to broken home (Agarwal *et al.*, 2015). Infertility has been defined as inability to get pregnant after 12 months or more of usual unprotected sexual intercourse (Zegers *et al.*, 2009). Data has shown 8-12% prevalence rate of infertility globally, which affected about 60-80 million couples (Leslie *et al.*, 2022). About 30 million men have been reported world-wide to be infertile with most of the cases recorded in Africa and Eastern Europe (Agarwal *et al.*, 2015). The prevalence rate of infertility in Nigeria is about 4 to 48.1% (Abiodun *et al.*, 2007; Adeyemi *et al.*, 2009; Obuna *et al.*, 2012). Men are the major cause and responsible for about 20% cases and the main contributing problem of all infertility cases (Leslie *et al.*, 2022). Male infertility occurs due to variety of pathogenic factors which could be testicular or non-testicular (Obora and Kennedy 2017). The causes could also be acquired or congenital disorders including use of drugs and alcohol (Gannon and Walsh 2015; Jugwirth *et al.*, 2014; Parcy *et al.*, 2014). The challenges of infertility have been reported to be worse in most developing countries which could be attributed to constrain medical resources and high cost of

living as well as cultural influence, taboos and various form of stigmatization (Leslie *et al.*, 2022). On the other hand, infertility could result from structural obstruction of the male reproductive system, hormonal disorders leading to abnormalities in hormone production and testicular inability to produce sperm such as in varicoceles or defective sperm-producing cells (Gore *et al.*, 2015).

Pentylenetetrazole (PTZ) exposure has been shown to be a risk factor for decrease serum level of testosterone, luteinizing hormone and increase prolactin (Mehrabinasab and Klazaei 2009). PTZ is a common convulsing drug used in animal model experiment for the induction of epilepsy which is similar to the tonic-clonic epilepsy in man (Bradford 1995). It is one of the commonest drugs with kindling effect (Sefil *et al.*, 2015). Studies have shown that seizures interfere with the activities of gonadotropin releasing hormone (GnRH) because it affects the temporal lobe regions (Sherifa and Hamed 2016). PTZ that possess convulsing effect can cause reproductive dysfunction and infertility as being attributed to epilepsy and antiepileptic drugs (Atif *et al.*, 2016). It has also been reported to cause oxidative stress affecting the functionality of many organs (Yang *et al.*, 2020). Further, PTZ has been shown to decrease serum testosterone level in a rat model (Elzharhraa and Salem 2021). Hence, this study is aimed at evaluating the protective potentials of chloroform stem bark extract of *Bombax costatum* pellegr. and vuillet on pentylenetetrazole-induced testicular damage in rats. Hormonal assay for follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (TT) was carried-out, including the histology of the testis to ascertain the protective potentials of the BC.

MATERIALS AND METHODS

Procurement of plant material and extraction

The procurement, identification and authentication, extraction and dosing of chloroform stem bark extract of *Bombax costatum* (Pellegr. & Vuillet) was earlier described (Bello *et al.*, 2022). Briefly, *Bombax costatum* stem bark was obtained from Jere Local Government Area of Borno state at Latitude: 11.8333, Longitude: 13. 1500 Coordinates: 11°49'59" North, 13°9'0". Washed with clean water, shade-dried for two weeks, grinded and subsequently extracted with chloroform through maceration method. The obtained extract was kept in a refrigerator for a later use.

Chemicals and Drugs used in the Study

Pentylenetetrazole (Sigma-Aldrich, St. Louis USA), chloroform (JHD[®] chloroform, California, USA) and ELISA kits for FSH, LH and TT (PerkinElmer Health Sciences, Inc., Hayward, USA) were bought from their respective manufacturers, while the other chemicals used were of analytical grades.

Experimental animals and ethical issues

Eighteen (18) apparently healthy male Wistar rats (100 – 160 g) were used for the experiment. They were kept in their respective cages in a well-ventilated animal house and had free access to food and water. The study protocol was approved by the Board of Postgraduate Studies University of Maiduguri and it's in line with ARRIVE guidelines and NIH publication 1996 (Council 2011).

Experimental design

For the induction of PTZ testicular damage, 18 rats were grouped randomly in to 3 (n=). Groups I and II were administered with normal saline orally and 35 mg/kg of PTZ subcutaneously every other day respectively. While group III were administered with 250 mg/kg of *Bombax costatum* extract orally followed by 35 mg/kg of PTZ subcutaneously

after 30 minutes. The rats were exposed to the substances for 28 days, euthanized on day 29, then blood samples collected for serum analysis of male reproductive hormones while the testis was harvested for histology.

Hormonal analysis

The blood for the hormonal assay was collected in plain sample container after sacrifice and allowed to clot then centrifuged to run for 5000 rpm. The serum was then separated for hormone assay. Enzyme immunoassay test kit manufactured by PerkinElmer Health Sciences, Inc. US 10 Division, 23595 Cabot Blvd., Suite 106, Hayward, CA 94545 catalog number 10001, 10004 and 10007 were used for the hormonal assay of FSH, LH and TT respectively.

Histology

The right testis of the rat was removed from respective group and was fixed in *Bouin's fluids*. The fixed tissues were then transferred to a graded degree of ethanol, cleared in xylene and then embedded in a paraffin wax. The various tissues were sectioned using microtome at 5 μ m and were stained with

Haematoxylin and Eosin (H&E) to observe the structure. The slides were then examined at magnification of X100 and X400 under optical microscope (Homayoon *et al.*, 2012).

Statistical analysis

One-way (ANOVA) analysis of variance was made using GraphPad Prism version 9.1.0 (221) software in San Diego, California. The results were examined and presented as mean \pm standard error of the mean (SEM), and those with $p < 0.05$ were considered statistically significant.

RESULTS

Testicular hormones

As shown in Fig. 1a, one way ANOVA showed a statistically significant difference in the testosterone level. Tukey's post hoc indicated a marked decrease ($p < 0.05$) in testosterone level in PTZ-induced group when compared to the control (0.2750 ± 0.1031) and BCCE 250 mg/kg (11.55 ± 0.2986) groups respectively. However, one way ANOVA does not show any statistically significant differences in the levels of FSH and LH among all the groups Fig. 1b and c.

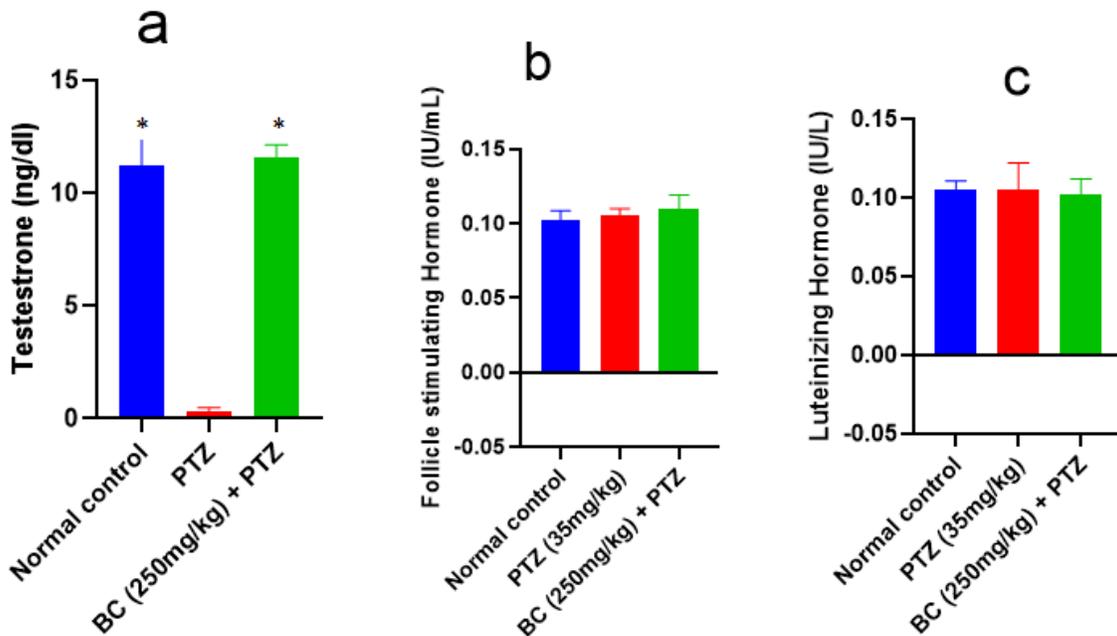


Figure 1: Graph showing the effect of PTZ on testicular hormones and protective effect of stem bark extract of BC in rat models. (a) Testosterone, (b) Follicle stimulating hormone and (c) Luteinizing hormone. Values are presented as Mean \pm SEM, $n=6$, * $p < 0.05$ vs PTZ.

Histological study

The testis of the control rats showed normal features, comprising of the coiled seminiferous tubules lined with multi-layered seminiferous epithelium of the spermatogenic cells and sustentacular Sertoli cells. The seminiferous epithelium rest on a basement membrane and are surrounded by lamellated connective tissues (Figure 2a). The interstitial Leydig cells are found in the loose vascular connective tissue separating the seminiferous tubules. The seminiferous tubules of the PTZ only group showed deleterious histological

changes including lost in the spermatids and spermatozoa with large vacuole in some areas of the section (Figure 2b). Germinal epithelium and spermatocytes have not been affected. In-between the tubules the interstitial tissue showed the presence of blood vessels with cluster of Leydig cells. The structure of seminiferous tubules showed remarkable ameliorative effect of BC extract in rats of that received BC followed by PTZ (Figure 2c). The germinal epithelium and spermatocytes are presents with organized spermatogenic cells.

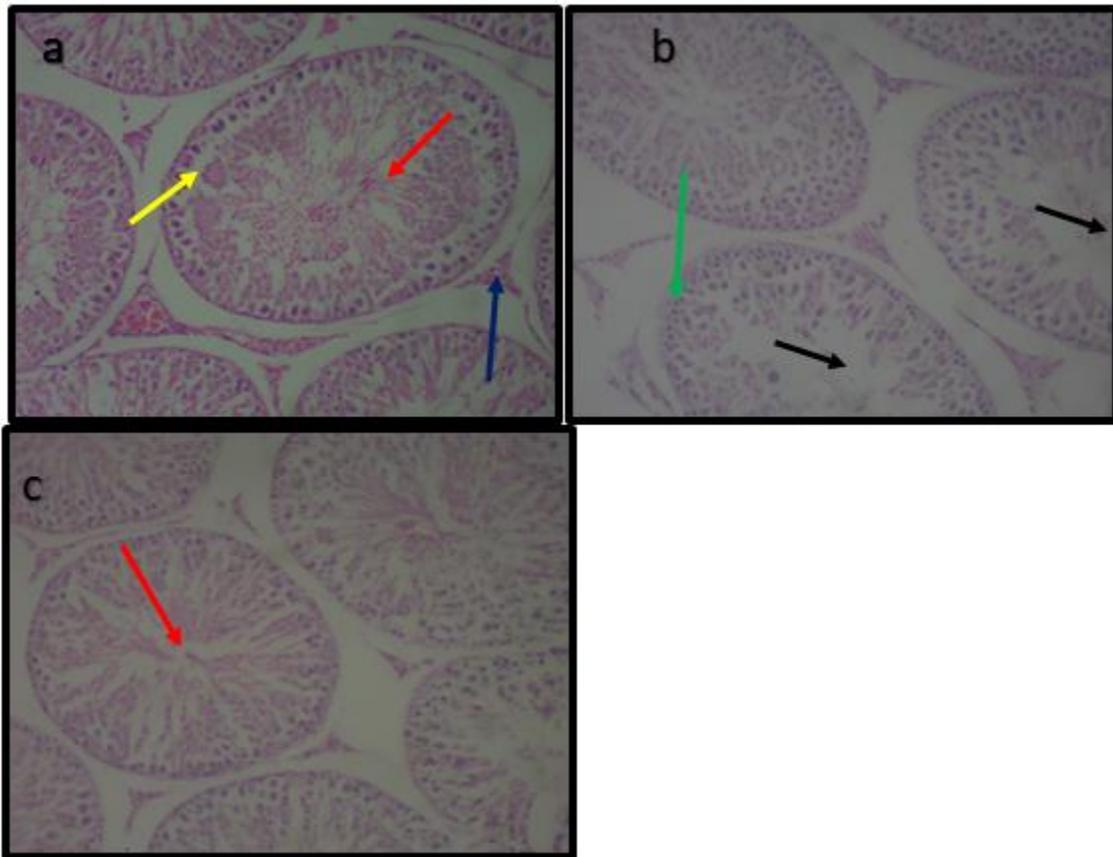


Figure 2: Photomicrograph of the testis at the end of the experiment. a) Control group, showing spermatogonia (yellow arrow), spermatozoa (red arrow) and interstitial tissue with leydig cells (blue arrow), b) PTZ alone group, showing degerated interstitial tissue (green arrow) and empty lumen of semniferous tubules (black arrow), c) BC 250 mg/kg group, showing numerous spermatozoa (red arrow). H & E (x 100)

DISCUSSION

From this study the histology of the control group (Figure 2a) showed normal architecture of the seminiferous tubules with interstitial Leydig cells, and spermatogenic cells series (from the periphery spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa to the lumen).

The PTZ only group (Figure 2b) showed interstitial peritubular necrosis, degeneration and disorganization of seminiferous epithelium cells. Also, tubular vacuolization with the lumen empty of spermatozoa noticed. This has been supported in a study carried out by (Karakaya *et al.*, 2021) where PTZ administration resulted in a severe testicular tissue damage.

The histological appearance of the group that was given BC followed by PTZ (Figure 2c) showed no changes in their tubular architecture when compared with the control group. It showed normal morphological features. Both Leydig cells and spermatogenic germ series were ameliorated by the BC extract. To our knowledge there is no enough literature on ameliorative effect of BC on PTZ-induced testicular damage. However, in the previous study, other antiepileptic agents such as valproic acid has caused degeneration of the spermatogenic germ series, necrosis of the interstitial Leydig cells and vacuolization of the seminiferous tubules (Iamsaard *et al.*, 2017).

This study was done out to evaluate the probable protective potentials of *Bombax costatum* (Pellegr. and Vuillet) Chloroform Extract on PTZ-induced testicular damage in male Wistar albino rat model. Previous studies have demonstrated that PTZ is a commonly used convulsing drugs in experimental studies (Seo *et al.*, 2020; (Chiroma *et al.*, 2022). In this study it has been revealed that PTZ causes significant decrease in serum testosterone

level ($p < 0.05$) in rat model. This is similar to previous study (Apinega *et al.*, 2018) who observed remarkable decrease in the serum testosterone level of PTZ-induced epileptic rat model. Further, Bohlonli (2020) reported that PTZ induces oxidative stress which causes reduction in the synthesis and secretion of testosterone. Therefore, the decrease in the serum testosterone level in this study could be as a result of oxidative stress.

The histology result of the present study has revealed that PTZ has interfered with the interstitial proliferation of the Leydig cells. This suggests that the decrease in the testosterone level could be due to PTZ interference in the function of the Leydig cell. Also reduced serum testosterone level could be due to increased free radicals and oxidative stress cause by PTZ (Bohlonli 2020). The low serum testosterone level has also affected spermiogenesis as the seminiferous tubules are empty of spermatozoa. Testosterone is the common hormone synthesizes by the Leydig cells and is being secreted in a pulsatile fashion (Jarow and Jonathan 2003). (Sherifa and Hamed 2016), reported that approximately 45-50% of the serum testosterone is tightly bound to sex hormone binding globulin, 50-55% is loosely bound to albumin and 1-2% is unbound or free portion. Therefore, serum testosterone is in circulation tightly bound to sex hormone binding globulin known as testosterone-binding globulin (TeBG) and albumin (Sherifa and Hamed 2016). The albumin-bound and the free portion are considered to be biologically active testosterone which act on target tissues, while the TeBG-bound fraction is tightly bound and is known to be not biologically active (Sherifa and Hamed 2016). Testosterone which is synthesizes in the Leydig cells play a key role in spermatogenesis and maturation of spermatozoa (Setyawati *et al.*, 2017). The

metabolism of the testosterone occurs in the liver with dihydrotestosterone and estradiol (E_1) as the metabolites. Increase in the serum level of E_1 is manifested with decrease in the erectile dysfunction and inhibition of gonadotropin LH due to hypogonadotropic hypogonadism (Setyawati *et al.*, 2017). Also, deficiency of testosterone leads to decrease in libido and erectile dysfunction. Testosterone acts on gene expression of neurotransmitter serotonin neurons in a way that may increase 5-hydroxytryptamine (HT) neurotransmission (Bethea *et al.*, 2014). This implies that decrease in the serum testosterone level could lead a depletion in the 5-HT content in the hypothalamus. Previous studies have revealed that PTZ cause production of reactive oxygen species (ROS) by generation of hydrogen peroxide, hydroxyl radicals and also decreasing the enzymatic antioxidant system capacity (Setyawati *et al.*, 2017; Moa *et al.*, 2019).

LH hormone is responsible for stimulation of Leydig cells to synthesize testosterone. The combined action of LH and FSH is exerted on Sertoli cells in the seminiferous tubules that support germ cells development and maturation. Meanwhile, this study showed no significant change in both LH and FSH in all the groups when compared with the control. This is not in accordance with previous study who reported marked increase of both LH and FSH in epileptic rat model (Elzharhraa and Salem 2021). In this study despite non-interference with the serum LH and FSH from the anterior pituitary gland the Leydig cells were not able to synthesize testosterone due to the impairment of the interstitial Leydig cells. This could be as a result of exposure to PTZ which has epileptic effect, as has been reported that patients with epilepsy are liable to hypergonadotropic hypogonadism due to

inadequate replacement mechanism in the hypothalamo-pituitary-gonadal-axis to normalize serum testosterone (Elzharhraa and Salem 2021). This study showed that BC significantly elevates serum testosterone level ($p < 0.05$) in a group that received 250mgkg^{-1} followed by 35mgkg^{-1} body weight of PTZ as compared to the control group. This could suggest that BC possess protective effect against the PTZ-induced testicular damage. Spermatogenesis relies on the action of the hormone testosterone. Also, at puberty the initiation and maintenance of spermatogenesis throughout life is carried out by the testosterone (Setyawati *et al.*, 2017).

CONCLUSION

This study suggested that PTZ induces testicular toxicity which led to disruption of the Leydig cells, which in turn caused decrease in the testosterone production. However, BC possess ameliorative effect and can be used to prevent PTZ-induced testicular toxicity. Further studies can be carried out to determine the mechanism of BC as a therapeutic and protective agent against the toxic effect of PTZ.

Ethical standards compliance

The study protocol was approved by School of Postgraduate Studies University of Maiduguri, which is in line with ARRIVE guidelines as well as NIH publication 1996 [29]).

Conflict of Interest

None

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