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ORIGINAL ARTICLE

Anti-androgenic activity of Xylopic acid in orchidectomerized rats

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To characterize anti-androgenic properties of xylopic acid (XA) and to elucidate the possible mechanism of the antifertility activity of XA, XA was administered to orchidectomized rats following the Hershberger assay protocol. Thirty male Sprague-Dawley rats were orchidectomized or sham operated at 42 days of age. At 11 days post-castration the rats were weighed and assign to five treatment groups as follows; Group 1 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.), group 2 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 10 mg kg^{-1} of XA orally, group 3 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 30 mg kg^{-1} of XA orally, group 4 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 100 mg kg^{-1} of XA orally, group 5 received only distilled water. The animals were treated daily for 10 days and the weight of the animals taken daily. On the day after the last treatment the rats were necropsied to isolate organs and tissues for study of androgenic or anti-androgenic effects. The endpoints evaluated were the growth/ body weight, and weight of the seminal vesicles plus coagulating glands with fluid, ventral prostate, levator ani plus bulbocavernosus muscle, glans penis, Cowper's glands (bulbourethral glands), and liver all without fixation. XA exhibited anti-androgenic activity by decreasing the weight of these androgen dependent organs.

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INTRODUCTION

Xylopic acid [15-β-acetoxy-(-)-kaur-16-en-19-oic acid], a diterpene kaurane derivative obtained upon extraction of the fruits of *Xylopia aethiopica* with petroleum ether has been shown to cause a reduction in serum testosterone and LH levels as well as a significant reduction in epididymal sperm count, motility and viability in rats (Woode *et al.*, 2012) but no exact mechanism of action was proposed (Woode *et al.*, 2011). Reductions in sperm count and sperm quality as well as reproductive organ weight reduction are mostly associated with androgen antagonist or anti-androgens (Kelce *et al.*, 1995; Thompson and Wilding, 2003). Accessory sex glands and tissues are dependent upon androgen stimulation to gain and maintain weight during or after puberty (Ashby *et al.*, 2000; Yamada *et al.*, 2000).

Reproductive development and function in human and other species may be affected by chemicals that behave as anti-androgens which are linked to the increasing incidence of reproductive cancers and a worldwide decline of semen quality (Toppari, 1996). Some of these chemicals are present in the environment, drinking water and food (Toppari *et al.*, 1996). XA is a major component of the fruits of *Xylopia aethiopica* which is used locally in Ghana as cough remedy, a carminative, a post-partum tonic, and to treat uterine fibroid and amenorrhea (Burkill, 1985; Asekun and Adeniyi, 2004). It is also use as spice in the preparation of most local food in Ghana, Nigeria and Cameroon. Evaluation of the androgen and anti-androgen activity of XA is thus invaluable in our quest to establish the effect of xenobiotic on the reproductive system. The present study was carried out to characterize potential anti-

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androgenic properties of XA and to elucidate the possible mechanism of the antifertility activity of XA in animal modules.

MATERIALS AND METHODS

Plant material

The dried fruits of the *Xylopia aethiopica* [Duna] A. Rich, were obtained from the Botanical Garden, KNUST, Kumasi/Ghana and authenticated in the Department of Pharmacognosy, KNUST, Kumasi, Ghana. A voucher specimen (number FP/08/76) has been deposited in the herbarium of the faculty.

Isolation of Xylopic Acid (X.A)

Xylopic acid was isolated using the method described by Ekong and Ogan (1968). Dry fruits of *X. aethiopica* (0.36 kg) were pulverized and soaked in petroleum ether 40-60 °C for three days. The petroleum ether extract was drained and concentrated using rotary evaporator at a temperature of 50°C. Ethyl acetate (5.0 ml) was added to the concentrate to facilitate crystallization of XA. Crystals formed after three days were washed with petroleum ether 40-60 °C repeatedly. Xylopic acid was purified using recrystallization by dissolving it in ethanol. The resulting solution was filtered and left to stand for three days to recrystallize, yielding 1.41% (5.1 g) of XA with 95% purity. The purity of XA was determined using High Performance Liquid Chromatography (HPLC). The chromatograph consisted of LC-10AT Shimadzu pump with programmable absorbance detector (783A Applied Biosystems) and Shimadzu CR501 chromatopac. Phenomenexhypersil 20 micron C18 200 × 3.20 mm column was used. The mobile phase consisted of methanol and water (9:1) eluted isocratically at 0.5 ml min^{-1}. Portions of 20 µl of a suitable concentration of pure XA were loaded and injected unto the column after dissolving in the mobile phase at 60°C. The eluent was monitored at 206 nm. Portions of the Xylopic Acid Extract (XAE) and XA were loaded and injected. The peak (s) was noted as component(s) of the XAE and XA.

Drugs and chemicals

Pentobarbitone was obtained from the Sigma-Aldrich Inc., St. Louis, MO, USA. Testosterone propionate was a gift from Abeth Consult limited (Kumasi, Ghana).

Hersberger assay

Animals

All experiments were performed with immature Sprague-Dawley rats weighing 60-70 g bought from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and kept at the Animal House Facility of the Department of Pharmacology, KNUST, Kumasi. The animals were allowed to acclimatize to the laboratory condition (Temperature 24-26°C and 12 hour light-dark cycle) for two week before commencement of the experiment. The rats were allowed free access to solid pellet diet (GAFCO Trading Company, Tema) and water *ad libitum* throughout the study. Prior permission was obtained from the ethical committee of the Pharmacology Department, KNUST. All the animals were treated according to the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health and Human Services Publication no. 85-23, revised 1985).

Experimental Procedure

The experiment was carried out according to the Hershberger assay (1953) as modified by Dorfman (1962). Thirty male Sprague-Dawley rats were orchidectomized or sham operated at 42 days of age. The animals were anaesthetized with pentobarbitone and the testes were exteriorized via a midline incision. The testicular blood vessels were clamped and ligated and each testis was removed. The midline musculature was sutured and the skin was auto clipped. The condition of the animals was checked on a daily basis and the clips were removed from the healed wound 7 days after the operation. At 11 days post-castration the rats were weighed and assign to five treatment groups as follows; Group 1 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.), group 2 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 10 mg kg^{-1} of XA orally, group 3 received 0.4 mg kg^{-1} day^{-1} of testosterone propionate (s.c.) plus 30 mg kg^{-1} of XA orally, group 4 received 0.4 mg kg^{-1} day^{-1} of

testosterone propionate (s.c.) plus 100 mg kg^{-1} of XA orally, group 5 (sham operated) received only distilled water. The oral administration of X.A was done 30 minutes after the subcutaneous injection of testosterone propionate. The animals were treated daily for 10 days and the weight of the animals taken daily. On the day after the last treatment the rats were necropsied to isolate organs and tissues for study of anti -androgenic effects. The endpoints evaluated were the growth/body weight, and weight of the seminal vesicles plus coagulating glands with fluid, ventral prostate, levator Ani plus bulbocavernosus muscle (LABC), glans penis, Cowper's glands (bulbourethral glands), and liver all without fixation.

Statistical analysis

Results are expressed as mean ± SD. The significance of difference between the means was determined by one-way analysis of variance (ANOVA) with Newman-Keuls's as post-hoc test. In all statistical tests, a value of $P<0.05$ was considered significant. All analysis was performed using Sigma Plot for Windows, Version 11.0, (Systat Software, Erkrath, Germany; www.systat.com).

RESULTS

Finger print of XAE and XA in TLC and HPLC

The TLC of the extract showed several spots which indicate the presence of several compounds (figure 1a). On the contrary, XA revealed a single spot indicating the presence of a single compound (figure 1b). Several HPLC peaks were observed after loading XAE indicating the presence of several compounds in the fruits as shown in figure 2. A single peak was observed for XA indicating the presence of a single compound (Figure 3) with 95% purity.

Anti-androgenic activity

The body weight of animals that received XA did not differ significantly from vehicle control group and the reference control that received only TP as shown in figure 4. XA administration to orchidectomised testosterone-treated male rats reduced significantly both absolute and relative weight of the following tissues, seminal vesicle (14.4%), prostate (26.6%), Glans penis (22.3%), LABC (7.7%), Cowper's gland (14.6%). The number in parentheses re-

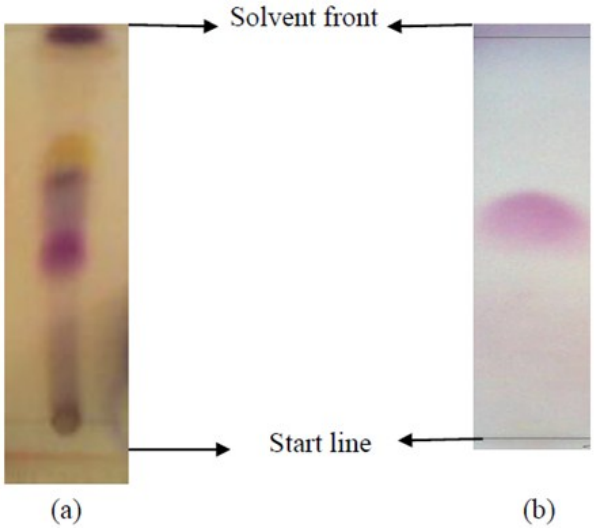

Figure 1: TLC results of (a) extract revealing several spots indicating the presence of several compounds and (b) xylopic acid showing a single spot indicating the presence of a single compound

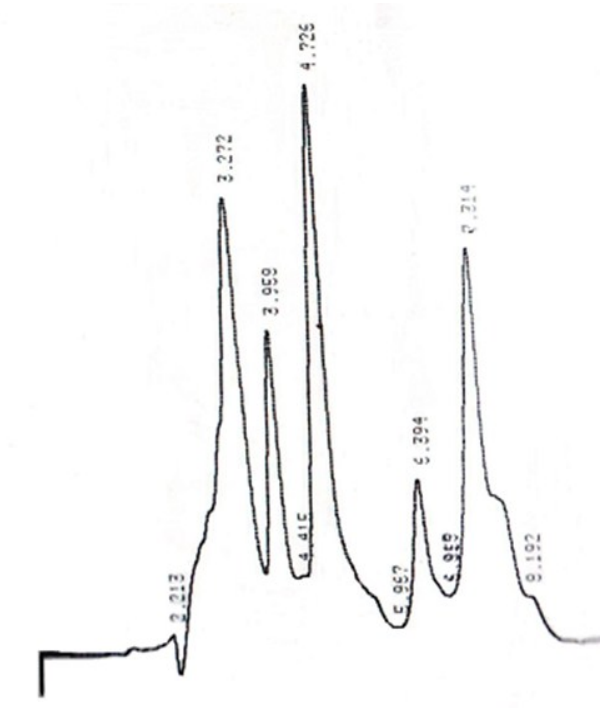

Figure 2: HPLC finger print of the extract showing peaks of the various compounds in the extract.

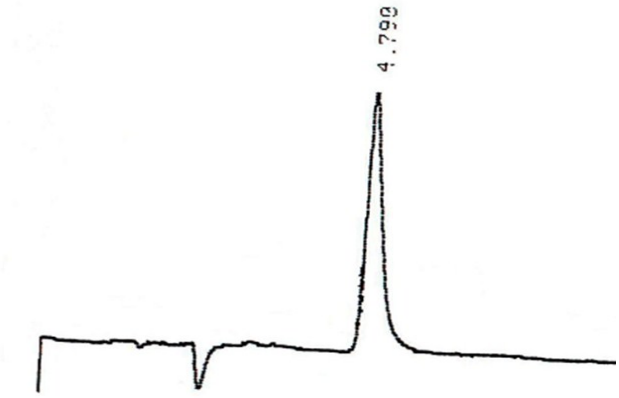

Figure 3: Chromatogram of XA showing a single peak corresponding to the isolated XA.

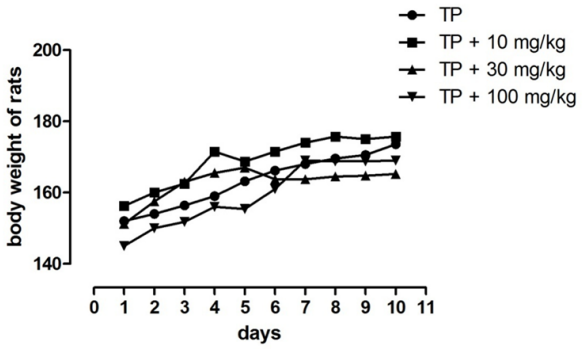

Figure 4: Effect of XA on animal body weight

fers to percentage reduction of absolute weight caused by 10 mg kg^{-1} of XA. The weight reduction were even more pronounce at the middle dose of 30 mg kg^{-1} and at the highest dose of 100 mg kg^{-1} of XA compare to the control and the testosterone propionate treated group as shown in figure 5. However, testosterone administration to orchidec-

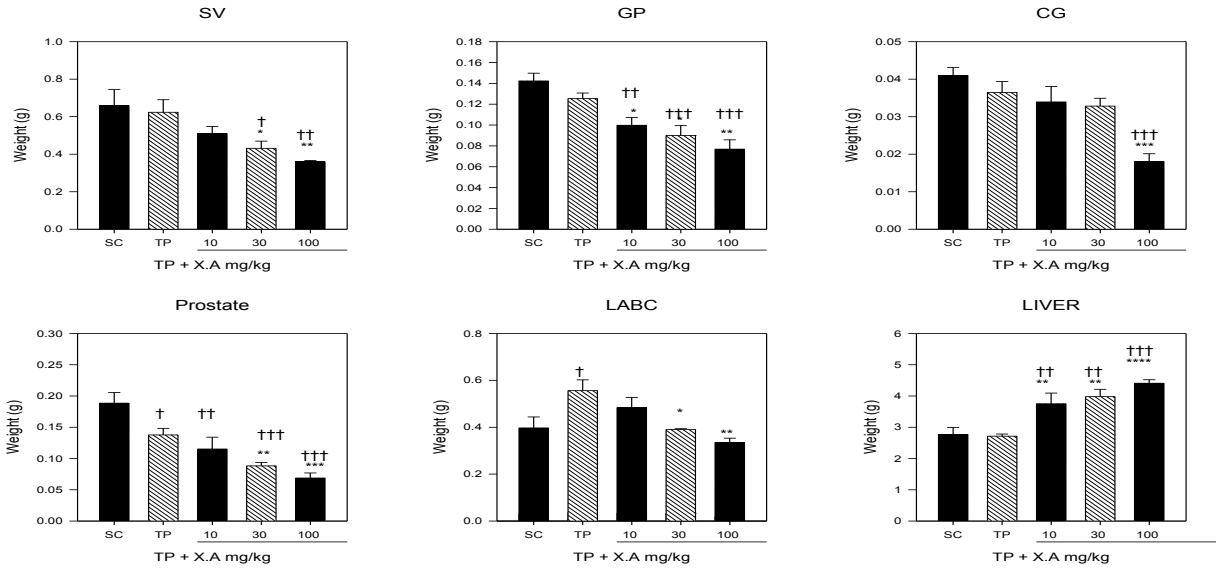

Figure 5: Relative weight of androgen dependent organs. Seminal Vesicles (SV), Glans Penis (GP), Coagulating Gland (CG), Prostate, LevatoraniBulbocavanous Muscle (LABC), Liver from sham castrated and castrated rats treated with Testosterone Propionate (0.4 mg/kg sc) with or without Xylopic Acid (X.A) at doses of 10, 30 and 100 mg/kg given orally. Results are presented as means ± SEM. *P ≤ 0.05, ** P ≤ 0.01, ***P ≤ 0.001 compared to TP treated. (one-way ANOVA followed by Newman-Keuls post hoc); †P ≤ 0.05, ††P ≤ 0.01, †††P ≤ 0.001 compared to sham castrated control rats (one-way ANOVA followed by Newman-Keuls post hoc).

tomised rats increase significantly both absolute and relative weight of the androgen dependent tissues as follows seminal vesicle (7.8%), prostate (4.6%), Glans penis (9.7%), LABC (1.4%), Cowper's gland (6.6%) compare to the vehicle treated animals as shown in figure 5. Subcutaneous administration of TP had the expected stimulatory effect on the androgen dependent tissues as stated above. Co-administration of XA with TP, as observed essentially abolished the stimulatory effects of the standard androgen on the tissues. The weight of the liver in rats receiving TP plus various doses of XA was significantly increased in a dose dependent manner compare to control and TP administered rats as shown in figure 5.

DISCUSSION

Various xenobiotics and naturally occurring compounds have been found to disrupt the endocrine system of animals (Toppari *et al*., 1996). Reduction in androgen-dominance to oestrogens and interference with androgen action are apparent mechanisms causing demasculinization and fertility decline in males (McKinnell *et al*., 2001; Williams *et al*., 2001; Rivas *et al*., 2002). In the present study, when XA. was administered to male rats orally, it exhibited anti-androgenic activity as seen by the significant decrease in the weight of the seminal vesicles, ventral prostate, LABC, glans penis, and the Cowper's gland, as these organs are dependent on androgens. This anti-androgenic action further support earlier report (Woode *et al*., 2012) which showed that administration of XA to adult male rats resulted in a significant reduction in sperm count, motility, and viability and significantly increase abnormal sperm morphology as well as decreases the number of Leydig cells and the seminiferous tubular diameter.

Anti-androgens may exhibit their activity both peripherally on androgen-dependent tissues and by feedback action at a central site (Mainwaring, 1977; Neumann *et al*., 1977; Moguilewsky and Raynaud, 1979; Raynaud *et al*., 1979; Neumann, 1985). XA may thus be competing for the peripheral androgen receptors and thus inhibit the effect of endogenous or exogenous androgens. Centrally, XA might be inhibiting gonadotropin secretion and thereby diminish testosterone production by the gonads (Neumann *et al*., 1970; Neri, 1977; Hans, 2007). Additionally, XA could also be an inhibitior of 5α-reductase, an enzyme located in tissues such as the prostate, seminal vesicle, epididymis, skin and sebaceous glands. Such inhibitors reduced the conversion from testosterone to 5α-dihydrotestosterone (DHT). Inhibition of 5α-reductase provides a selective approach to androgen deprivation in DHT-target tissues, such as the prostate (Hans, 2007).

CONCLUSION

In conclusion, XA exhibited anti-androgenic by reducing the weight of the androgen dependent organs possible by blocking androgen receptors which prevents androgens from binding to them and suppresses luteinizing hormone which in turn reduces testosterone levels thus suppressing the actions of testosterone and its metabolite dihydrotestosterone on tissues. The results thus confirm the earlier report of antifertility activity of XA in male rats.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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