

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net



doi: 10.4103/2305-0500.233574

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Antiandrogenic activity of *Calotropis procera* latex in ratsAbdelgader Binyamen Abdelgader^{1,2✉}, Adil Salim Elsheikh³¹College of Graduate Studies, University of Gezira, Sudan²Department of Physiology, Faculty of Medicine, Najran University, Saudi Arabia³Department of Applied Medical Sciences, Community College, Najran University, Saudi Arabia

ARTICLE INFO

Article history:

Received 19 November 2017

Revision 10 December 2017

Accepted 12 January 2018

Available online 31 May 2018

Keywords:

Anti-androgenic activity

Calotropis procera latex

Immature male rats

ABSTRACT

Objective: To determine the effects of *Calotropis procera* latex on pubertal traits of immature male Wistar rats. **Methods:** A total of 30 immature male Wistar rats aged 3 weeks old were grouped randomly into 5 groups: group A (control group) was offered distilled water as a placebo; group B was daily oral dosed with suspension of *Calotropis procera* latex at a dose rate of 5 mg/kg body weight (BW), group C 10 mg/kg BW; group D 15 mg/kg BW and group E 20 mg/kg BW. The rats were weighed daily to adjust the dose and record the BW changes, and the treatment continued for 4 weeks; thereafter, rats were sacrificed. Serum samples ($n=30$) were collected from all rats and kept frozen until assayed for reproductive hormones. Furthermore, the testes and epididymae were weighed; epididymal sperms were counted; sperm motility & abnormality were estimated; and histopathological sections of the testes were prepared. **Results:** The results of this study showed that oral dosing of immature male rats with *Calotropis procera* latex at doses rate 10 mg/kg BW significantly ($P<0.05$) reduced the growth rate, BW, testicular & epididymal weights, the level of most of reproductive hormones as well as the sperm traits examined; however, it significantly ($P<0.05$) augmented the abnormalities of spermatozoa and the seminiferous epithelium. **Conclusions:** Latex of *Calotropis procera* contains substances that have anti-androgenic activities and/or endocrine disrupting effects. If these substances are purified and identified, they can be used as male contraceptives.

1. Introduction

Herbal plants and treatment with herbs are worldwide popular as alternative for allopathic medicines and occupy a distinct position right from the primitive period to present day because of the expected serious side effects and the high cost of allopathic medicines. The *Calotropis procera* which belongs to the family

Asclepiadaceae is well known for its traditional medicinal uses.

This plant has many local names such as *Calotropis procera*, Sodom apple, Akund crown flower and Dead Sea fruit. Studies on the plant have revealed enormous diversity of medicinal uses in case of fevers, rheumatism, indigestion, cough, cold, eczema, asthma,

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How to cite this article: Abdelgader Binyamen Abdelgader, Adil Salim Elsheikh. Antiandrogenic activity of *Calotropis procera* latex in rats. *Asian Pac J Reprod* 2018; 7(3): 129-135.

✉Corresponding author: Abdelgader Binyamen Abdelgader, College of Graduate Studies University of Gezira, Sudan; Department of Physiology, Faculty of Medicine, Najran University, Saudi Arabia.
E-mail: bin2th@gmail.com

All authors contributed equally in this work.

elephantiasis, nausea, vomiting and diarrhea. The plant is used either as a whole, part of it or mixed with other plants' materials to enhance the efficacy[1]. *Calotropis procera* produces various chemicals that are useful for various activities[2,3]. The plant contains alkaloids, sterols, flavonoids, cardiac glycosides, triterpenoids and usharin[4]. This plant has been advocated to cure varieties of diseases: leprosy, ulcers tumors, and piles[4]. Different extracts of the leaves of *Calotropis procera* have been claimed for diverse biological activities such as antidiarrheal[5], antioxidant[6–8], antipyretic[9], analgesic[10], antimicrobial[6,11,12], spasmolytic[13], schizonticidal[13], cytotoxic[14,15], hepatoprotective[16], hypoglycemic[17], anthelmintic[18], laxative, purgative, anti-inflammatory, diuretic[19] and abortifacient[20]. The different parts of *Calotropis procera* and its latex are known to have antibacterial and wound healing properties[21,22] and anticancer, antifungal and insecticidal activity[23,24]. Although *Calotropis procera* has these favorable properties, the plant has been accused of varying toxic effects in animals[25–27]. Despite these claimed toxic effects, there are some trials to use the plant as feed stuff for fattening of sheep[28]. In Sudan, meat and milk producing animals especially the small ruminants as well as cattle are fed on dried leaves of *Calotropis procera* during drought and/or pasture scarcity. Thereafter, the human being is fed on animal products that presumably contain the plant's residues. This situation necessitates that the effects of the plant on all physiological processes in animals must be investigated. Many toxicological studies on water and ethanolic extracts of the plant were conducted to elucidate its effects on mature mammals. However, researches on the effects *Calotropis procera* on immature animals are sparse or even absent, and if present they only investigated its toxicity. Therefore, it is necessary to conduct studies on *Calotropis procera* before using it as medicine or feed stuff. Thus, the current study was carried out to elucidate the possible positive and negative effects of *Calotropis procera* latex on pubertal traits of growing male Wistar rats.

2. Materials and methods

2.1. Experimental animals

Thirty males Wistar rats were kept with their dams, weaned at the age of 3 weeks and grouped into 5 groups, with 6 rats in each group. Each group was kept in a separate cage at 20 °C–25 °C on a 12-hour light–dark cycle and offered commercial pellets and water *ad libitum*.

2.2. Preparation of *Calotropis procera* latex and calculating dose

Fresh latex of *Calotropis procera* was collected from the aerial parts

of wildy growing plants in Najran city- Saudi Arabia. Thereafter, the latex was dried under a shade at ambient temperature and ground to small granules. Five milligrams of the dried latex were triturated in 10 mL distilled water (0.5 mg/mL) to obtain an aqueous suspension. This aqueous suspension was kept as stock solution. The dose of the aqueous suspension was calculated according to the equation of Nair & Jacob 2016. According to this equation the dose is calculated as follow:

$$V \text{ (mL)} = [W \text{ (kg)} / C \text{ (mg/mL)}] \times D \text{ (mg/kg)}$$

V: the volume of the dose (mL); W: the weight of the rat (kg);

C: concentration of the suspension (mg/mL); D: the dose (mg/kg).

2.3. Experimental design

This experiment is a one factorial design experiment to examine the effects of oral administration of *Calotropis procera* latex on pubertal traits of growing male Wistar rats. The pubertal traits tested were growth rate, pubertal body weight (BW), testicular and epididymae weights, semen properties, testicular histology and reproductive hormones. Three-week-old weaned 30 male Wistar rats were weighed and grouped randomly into 5 groups. Group A is the control group ($n = 6$); group B ($n = 6$); group C ($n = 6$); group D ($n = 6$) and the last group E ($n = 6$). A daily oral dose of the prepared suspension of *Calotropis procera* latex was given to the treatment rat groups (group B 5 mg/kg/day; group C 10 mg/kg/day; group D 15 mg/kg/day and group E 20 mg/kg/day). The control group (group A) was offered distilled water orally as placebo. The rats were weighed every day to adjust the dose and the treatment continued for 4 weeks. At the end of the treatment period pubertal BW was recorded and the weekly growth rate was calculated according to the following equation:

$$\text{Growth rate \%} = (W_2 - W_1) / W_1 \times 100$$

W_1 = body weight (g) at the previous week; W_2 = body weight (g) at the following week.

The blood samples collections, hormonal biochemical analysis, testicular weighing, tissues preparation and semen analysis were determined as following.

2.4. Blood samples collections, biochemical analysis, testicular weighing and tissues preparation

Then the rats were anesthetized with chloroform and sacrificed by cervical dislocation[30]. Blood samples were collected by cardiac puncture, left to coagulate at room temperature and then centrifuged at 3 000 rpm for 30 min to separate the serum. The sera were kept frozen at -30 °C till used for estimation of serum testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The enzyme-linked immuno-sorbent assay technique was used to estimate all hormones and commercially available enzyme-linked immuno-sorbent assay kit were purchased from Shanghai Crystal

Day Biotech Co., LTD. All hormones in question were determined as per the manufacturer's instructions in the assay kits. The testes and epididymae were weighed and the relative testicular and epididymal weights were calculated as follows:

Relative organ weight (%) = [Absolute organ weight (g) / Body weight of rat on sacrifice day (g)] × 100

The testes were fixed immediately with 10% formalin and then dehydrated through ascending grade of alcohol concentration started from 70%, 90% and 100%, respectively; subsequently all the tissues were cleared with xylene, then they were impregnated and embedded with paraffin wax. Sections of 5 µm thickness were cut by using an Accu Cut SRM Microtome form SAKURA, and then prepared using standard procedures for hematoxylin and eosin staining and examined under the light microscope.

2.5. Sperm counts, sperm motility and sperm morphology

Sperms of the right epididymae were counted by a modified method of Reeves and Rossow[31]. Briefly, the right cauda epididymis from each rat was weighed and minced with a scalpel blade in a petri dish which contains physiological saline solution (0.9% NaCl). The suspension was kept at 37 °C for 5 min to allow for the sperms to disperse in the medium. Thereafter, the sperm suspensions were gently mixed and sperm count was performed by hemocytometer under a Nikon microscope (Nikon Eclipse E600) at a final magnification of × 400 as described elsewhere[32]. The total number of sperms was calculated as per milliliter and converted as per 0.1 g weight. The percent of motile sperm was evaluated using the standard method mentioned elsewhere[33]. The cauda epididymae were squeezed with aid of 31-gauge needle into 2 mL of Tris buffer solution and were kept at 37 °C. A slide was placed on phase contrast microscope and an aliquot of this solution was placed on the slide and percent of motile sperms was evaluated visually at a magnification of 400 ×. Motile sperms out of 200 sperms in each examined sperm suspension were counted and the means of 3 trails of the samples examined was used as the final motility score.

To determine the percentage of morphologically abnormal spermatozoa, the method described by Sonmez *et al*[34] was used. Briefly, a drop of Eosin stain was added to the sperm suspension, which was kept for 5 min, at 37 °C. Then, a drop of sperm suspension was placed on a clean slide and gently spread to make a thin film. The film was air dried and the slides were then examined under a phase contrast microscope at 400 ×. A total of 200 sperm cells were examined on each slide and sperms with absent head, absent tail, bending tail, coiled tail, curved mid-piece, bending mid-piece were scored. The abnormalities of spermatozoa were expressed as percentage.

2.6. Statistical analysis

Data were analyzed with analysis of variance (ANOVA) using SPSS-16.020 statistical package (Chicago, USA). The data were expressed as mean ± SD. The level of significance was set at $P < 0.05$.

3. Results

3.1. Effects of *Calotropis procera* on gross pubertal traits

As from Figure 1, all the gross pubertal traits were significantly ($P < 0.05$) reduced by increasing the latex concentration in the oral dose. Also, the growth was significantly ($P < 0.05$) retarded by increasing the latex dose beyond 5 mg/kg BW (Figure 1 A). Figure 1B showed the means of body weights of the rats at the end of the treatment period in group B, C, D, E. The rats' body weights were significantly ($P < 0.05$) decreased, compared to that of the control. The means of testicular weights and epididymal weights (Figure 1 C) and the mean percentages of the relative testicular weights as well as the mean percentages of the relative epididymal weights (Figure 1 D) of group B, C, D, E were significantly ($P < 0.05$) decreased compared to those of the control.

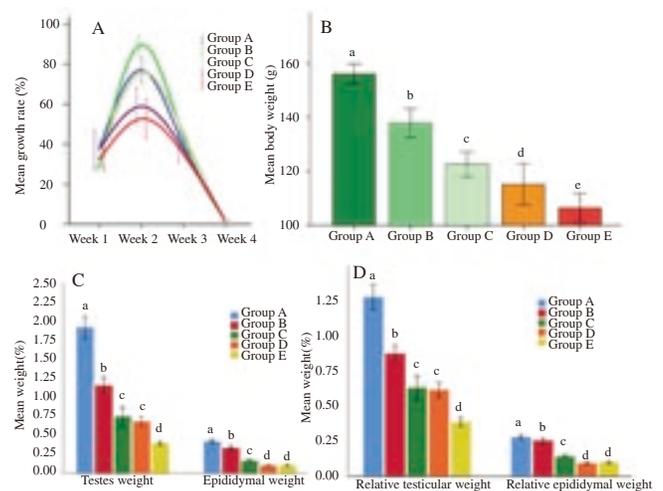


Figure 1. Effect of *Calotropis latex* on growth rate and gross pubertal traits of male Wistar rats.

A: growth rates; B: body weights; C: testis and epididymal weights; and D: relative testicular and epididymal weights. Data are means ± SD of 6 replicates. Group A = control; Group B = 5 mg/kg; Group C = 10 mg/kg; Group D = 15 mg/kg; Group E = 20 mg/kg. a-d: different letters mean significant difference, $P < 0.05$.

3.2. Effects of *Calotropis latex* on pubertal rats' semen parameters

The pubertal rats' semen motility and sperm counts were significantly ($P < 0.001$) reduced (Figure 2) and the sperm abnormalities were significantly ($P < 0.001$) increased (Figure 3) by increasing the concentration of *Calotropis latex*. These seminal changes happened in a dose dependent manner.

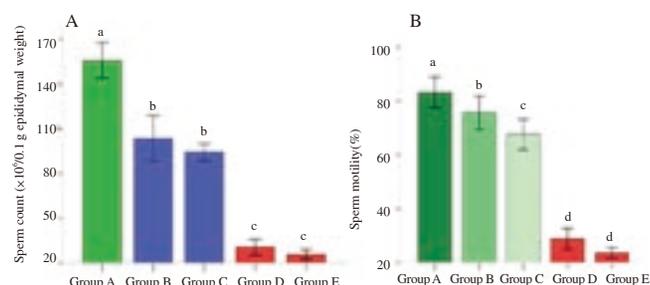


Figure 2. Effect of *Calotropis latex* on epididymal sperm count.

A: sperm count; B: sperm motility. Data are mean \pm SD for 6 replicates. Group A = control; Group B = 5 mg/kg; Group C = 10 mg/kg; Group D = 15 mg/kg; Group E = 20 mg/kg. a- d: different letters mean significant difference, $P < 0.05$.

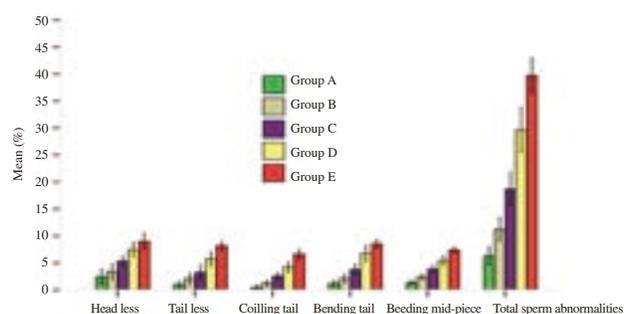


Figure 3. Effect of *Calotropis latex* on epididymal sperm normality.

Group A = control; Group B = 5 mg/kg; Group C = 10 mg/kg; Group D = 15 mg/kg; Group E = 20 mg/kg.

3.3. Effects of *Calotropis latex* on male rats' reproductive hormones

As shown in Figure 4, high doses of *Calotropis latex* significantly ($P < 0.05$) increased the serum levels of FSH in the treated male rats compared to that of the controls. In contrast, the serum concentrations of LH were significantly ($P < 0.05$) decreased. Furthermore, the serum concentrations of testosterone of treated rats decreased significantly ($P < 0.05$).

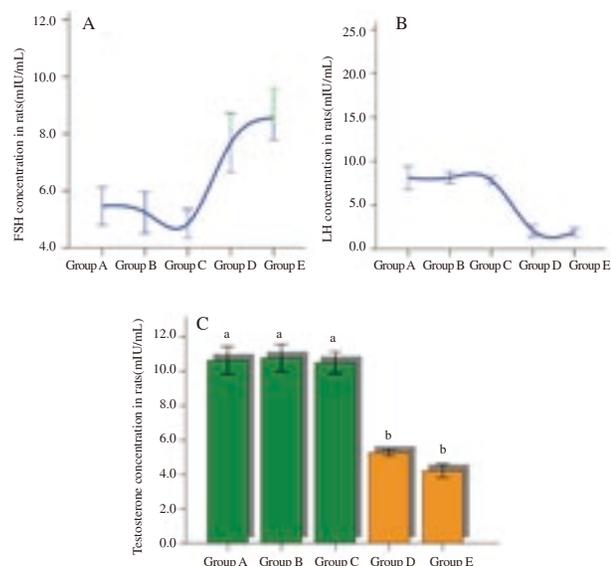


Figure 4. Effect of different doses of *Calotropis latex* on reproductive hormones of pubertal male rats.

A: FSH levels; B: LH levels; C: testosterone levels. Data are mean \pm SD of 6 replicates. a,b: different letters mean significant difference, $P < 0.05$.

3.4. Effects of different doses of *Calotropis latex* on pubertal male rats' testicular histology

Figure 5 showed H&E stained histological sections of testes of rats dosed with different concentration of *Calotropis latex*. The seminiferous tubules (STs) of the control rats were completely differentiated with obvious layers of spermatogenic epithelium, wide lumen and were of normal shape. The STs had clear basement membranes and their lumens contain mature sperms. The spermatogenic cells including spermatogonia, primary spermatocytes, early spermatids, late spermatids and sertoli cells were obvious, abundant and healthy. In the treatment groups, when the concentration of *Calotropis latex* was increased, the STs histology was distorted. When the concentration of the latex reached 10 mg/kg BW (Figure 5 C), the spermatogenic epithelial layers were remarkably reduced and the spermatogenesis was significantly affected. The lumens of some of the STs contain masses of degenerated cells. More severe reduction in STs epithelium and spermatogenesis was witnessed when *Calotropis latex* was increased to 15 or 20 mg/kg BW. (Figure 5 D & C).

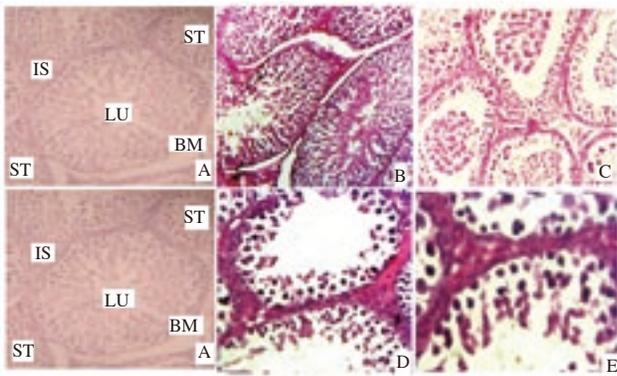


Figure 5. Light photomicrographs of rat's testes parenchyma.

Control rat testis (A) and experimental rat testis ingroup B, C, D & E ($\times 100$). ST: seminiferous tubules, IS: interstitial spaces, BM: intact basement membrane and LU: lumen.

4. Discussion

The results of this study showed that oral dosing of immature male Wistar rats with doses ≥ 10 mg/kg BW of *Calotropis procera* latex reduced their growth rate, pubertal and consequently the testicular and epididymal weights were reduced. Additionally, the pubertal testicular histology and semen traits of treated rats were abnormal. Contrarily to these deleterious effects of high doses of *Calotropis procera* latex, a dose of 5 mg/kg BW weight improved rats' growth rate.

The abnormal pubertal traits that result from dosing rats with high concentrations of *Calotropis procera* latex are probably due to the effects of toxic components of *Calotropis procera* particularly the cardiac glycosides (calotropin), anthraquinones, flavonoids, alkaloids, tannins and phenol glycosides[9]. Similar findings were reported by Srivastava *et al*[35] who employed *Calotropis rostrata* extract; and El-Shahat *et al*[36] who evaluated the effects of ethanolic crude extract of *Calotropis procera* leaves. These authors have reported reductions in the total BW and the weights of vital organs (liver, kidney, and testis). The positive effect of the low dose of *Calotropis procera* latex on growth rate of rats agrees with the finding of Madruga *et al*[28], who reported that feeding low concentrations of *Calotropis procera* latex improved the growth and quality of meat in growing lambs. Contrarily to these findings, Neto *et al*[37] have reported that the extract of dried leaves of *Calotropis procera* has no effect on rat BW; however, they reported that the extract of dried leaves of *Calotropis procera* has beneficial effect on certain parts of the rats' body especially the epididymal adipose tissue and soleus and extensor longus digitorum muscles. Neto *et al* have concluded that *Calotropis procera* leaves extract has reduced the blood glucose and improved the metabolic status of the rats thus they explained why *Calotropis procera* leaves extract has a beneficial effect on certain tissue masses, while it has no effect on the total BW of the rats. Additionally, Neto *et al* have reported that the *Calotropis procera* extract caused a

decrease in protein catabolism through increasing glucose uptake. The difference between the findings of the current study and that of Neto *et al* is probably due to the differences in the part of *Calotropis procera* employed, dose given, age of rats employed and/or the environment. In the current study, *Calotropis procera* latex was given to immature rats, while Neto *et al* offered extract of the dried leaves of *Calotropis procera* to mature rats.

Degenerative changes were observed in testicular structure of the testes of rats treated with the latex of *Calotropis procera* and consequently reduced testicular and epididymal weights were observed. Similar findings were reported by Akinloye *et al*[38] who investigated the effect of aqueous extract of *Calotropis procera* latex on the BW and reproductive organs weights of male Wistar rats. In this study, most of the testes of treated rats examined have narrow interstitial spaces with a reduced number of Leydig cells. The degenerative changes and the reduction in the testicular weights could be attributed to the decrement in the number of Leydig cells that are responsible for synthesis and secretion of testosterone, which is known to maintain normal testicular architecture. In accordance with the findings of this study, Akinloye *et al*[39] have observed varying degrees of testicular lesions as well as severe reduction in ST diameters in rats treated with aqueous extract of *Calotropis procera* latex. These lesions, coupled with the reduced number of Leydig cells and the consequent expected reduction in testosterone production, probably may result in a reduced sperm production or even aspermia when the dose of *Calotropis procera* latex is increased. These observations explain why *Calotropis procera* have been reported as possible antifertility candidate[40,41]. The pattern of cellular damage and hypoplasia of the STs observed in this study is consistent with the effects of different endocrine disrupting chemicals that were reported to have similar effects on testis histology and functions[42–46]. Therefore, *Calotropis procera* latex could be a possible member of the endocrine disrupting chemicals that could adversely affect the endocrine glands and consequently the male reproduction. Whether the latex of *Calotropis procera* works on endocrine glands or directly on the testis remain to be elucidated.

In the current study, increment in sperm abnormalities, decrement in sperm count and motility were observed in male rats treated with latex of *Calotropis procera*. These abnormalities in sperm quality could be due to multi-factors; however, the possible candidates that could induce these changes are the cardiac glycosides that are found in the latex extract of *Calotropis procera*[47]. Additionally, these abnormalities in quality and count of sperms could be attributed to the low level of testosterone that followed the decrement in Leydig cells. The reduction in sperm counts and motilities recorded here are in accordance with the findings of Shafey *et al*[48] who reported a similar phenomenon in mature rats treated with extract of latex of *Calotropis procera*.

The disturbances of reproductive hormones of rats treated with

the latex of *Calotropis procera* can be explained by 3 theories: 1) The latex of the *Calotropis procera* has affected the hypothalamo-pituitary-gonadal axis especially the LH releasing hormone of the hypothalamus leading to LH reduction and consequently reduced the serum level of testosterone; meanwhile the serum level of FSH is elevated. 2) The latex of *Calotropis procera* affected the pituitary gland and/or the hypothalamus resulting in a reduced serum level of LH and consequently a reduced testosterone level. 3) Latex of *Calotropis procera* intoxicated the Leydig and/or Sertoli cells of the testes and impaired the synthesis and secretion of testosterone. Contrarily to the finding of this study, Shafey *et al*[48] found that the ethanol extract of *Calotropis procera* has no effect on serum testosterone level of mature male rats although they recorded a mild reduction. This discrepancy is presumably due to the differences of the plants' parts used and/ or the rats' age, since in the current study immature rats were employed. The findings of Toson *et al*[36] agree with the results of the current study where they reported that ethanolic extract of the leaves of the plant reduced the concentrations of testosterone, LH, epididymal sperms count, percent of motile sperms, percent of live sperms besides an increment in the percent of abnormal sperms. However, they reported a reduced FSH that in contradiction with the findings of this study, where the FSH level is found high. Sharma and Jacob[42] reported functional sterility without loss of libido and sexual behavior in male mice treated with an intramuscular aqueous of ethanolic extracts of the plant's flowers. This finding presumably contradicts the findings of this study since libido is controlled by testosterone hormone, which is reduced by *Calotropis procera* latex in this study; however, this study did not investigate rats' libido. The discrepancy between the study of Sharma & Jacob and this study seems to be due to species difference and the different parts of the plant used. It is concluded that latex of *Calotropis procera* has anti-androgenic activity and/or an endocrine disrupting effect. Furthermore, the latex of *Calotropis procera* presumably contains substances that if extracted, identified and proven safe can be employed to regulate and control male reproductive traits and behaviors.

Conflict of interest statement

The author declares that there is no conflict of interest.

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