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In vivo anti-androgenic, anti-estrogenic and antioxidant activities of the aqueous extract of *Eremomastax speciosa*

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ABSTRACT

Objective: To evaluate the *in vivo* anti-androgenic, antioxidant activity and anti-estrogenic activities of *Eremomastax speciosa* (*E. speciosa*) in order to find new method for fighting against chronic diseases such as cancer.

Methods: Evaluations of antiandrogenic and antioxidant activities were carried out on male rats receiving simultaneous daily administration of testosterone and different doses of aqueous extract of *E. speciosa*, during a period of 10 d. The evaluation of antiestrogenic activity was carried out on mature ovariectomized female rats receiving simultaneous daily administration of estradiol and different doses of extract, for a week. Then reproductive organs were weighted, levels of prostatic acid phosphatase, superoxide dismutase and catalase as well as some hematologic parameters were measured.

Results: The treatment significantly reduced ($P < 0.01$) the weight of ventral prostate, penis, Cowper's gland and the level of serum prostatic acid phosphatase; while a significant decrease ($P < 0.01$) of the catalase activity was observed. A significant increase ($P < 0.05$) in the lymphocyte number and significant decrease of monocyte ($P < 0.05$) were noticed. The uterine relative weight were significantly reduced ($P < 0.01$).

Conclusions: Generally, these results denote the antiandrogenic, antiestrogenic and immunomodulatory potential of *E. speciosa*.

1. Introduction

Free radicals can cause oxidative damage to DNA, eventually leading to many chronic diseases, such as cancer. Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. Cancer is characterized by deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues[1].

Prostate, like any organ, can be a seat of a tumoral development. These tumors are generally not malignant

and the most frequent ones are benign tumors known as adenomas, which correspond to an increase in size of the prostate center (prostate benign hypertrophy). After 50 years of age, the prostate adenoma is very frequent, it is the fifth cause of male tumors of any age in the world and the second cause in the industrialized countries; and it is responsible for 10000 deaths per year[2].

Herbal medicine has become a focus to meet the present and future health needs against cancer. This is due to the toxic and adverse side effects of synthetic drugs, and the fact that tumor cell proliferations have not been appreciably prevented by conventional drugs[3]. Many plant secondary metabolites such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, alkaloids and other exhibit significant antioxidant activities. Studies have shown that many antioxidant compounds possess anti-

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inflammatory, antitumor, anti-mutagenic, anti-carcinogenic, anti-androgenic and antiestrogenic activities[4]. In order to find new method for fighting against initiation and promotion of prostate cancer, several researchers have focused their work on the study of anti-androgenic, antiestrogenic and antioxidant activities of medicinal plants. Thus, Liu *et al.* have demonstrated the inhibiting activity of *Ganoderma lucidum* on the 5 α -reductase in castrated adult male rats[5]. The work of Nelson *et al.* on the induction of catalase and superoxide dismutase in man showed that this induction is more effective to fight against oxidant agents than an antioxidant supplementation[6].

Eremomastax speciosa (*E. speciosa*) is a medicinal plant used in traditional system of medicine in Cameroon for the treatment of dysentery, anemia, irregular menstruation, spurious labor pains, fracture, hemorrhoids and urinary tract infection. Phytochemicals studies showed the presence of tannins alkaloids, flavonoids, saponin and tannins[7]. Those metabolites could present significant antioxidant activities as well as anti-estrogenic and anti-androgenic activities.

The present study, therefore investigated the *in vivo* anti-estrogenic, anti-androgenic and antioxidant potential of the aqueous extract of *E. speciosa*.

2. Materiel and methods

2.1. Plant and extract preparation

The fresh leaves of *E. speciosa* collected in May 2011 from the botanical garden of the University of Dschang (Cameroon) have been identified in the National Herbarium of Cameroon under voucher specimen code 23604/SFR/Cam. They were washed and dried at room temperature. The dried leaves were ground in a mortar and 10 g of the powder obtained was boiled in 100 mL of distilled water for 30 min. After cooling, the extract was filtered before being dried in a ventilated oven at 45 °C. The powder extract was stored in the refrigerator at – 20 °C for further used.

2.2. Animals

The animals used in this study were albino Wistar rats, 42–45 d old, weighing 80–150 g. They were bred in the animal house of the Biochemistry Department (University of Dschang, Cameroon), housed under natural conditions of light (12 h cycles) and temperature [22 \pm 2 °C] and fed a standard laboratory diet and tap water *ad libitum*. The animal care and handling as well as the experiments performed were in accordance with the internationally accepted standard guidelines for laboratory animal use and care as described in the European Community guidelines[8].

2.3. Evaluation of the anti-androgenic and antioxidant activities

The test was carried out on adult castrated male rats. Ten days after castration, rats were randomized and separated

into seven groups, each of which consisted of six animals. Groups I to IV received simultaneously testosterone (0.4 mg/kg) and increasing doses (100, 400, 800, and 1600 mg/kg) of the plant extract, and Group V received flutamide (positive control) at dose of 3 mg/kg and testosterone (0.4 mg/kg). The sixth group received only testosterone (0.4 mg/kg) and the last one only distilled water. An additive group of six uncastrated animals was also used as control. The treatment of the animals was carried out over a period of ten days. Twenty-four hours after the last dose, the animals were sacrificed under chloroform anesthesia and their blood extracted from the heart. The serum was separated and used for serum biochemistry. Reproductive tissues like ventral prostate gland, seminal vesicle, bulbourethral gland and levator ani (LABC), Cowper's glands, the glands penis (GP) and vital organs (liver, kidney, heart) were excised, blotted free of blood, weighed and used for tissue biochemistry. Prostatic acid phosphatase (PAP), was evaluated by using a colorimetric kit (Cat.No:AP460S01), the hematologic parameters were measured, the superoxide dismutase and catalase activities in tissues was determined in this study by the method used by Pavlović *et al*[9].

2.4. Evaluation of anti-estrogenic activity

Experiments were conducted on mature ovariectomized female albino Wistar rats aged 42 d. One week after ovariectomy, the ovariectomized rats were distributed in six groups with six animals in each group. Groups I, II, III and IV received daily subcutaneously estradiol (0.05 μ g/kg) for a week and orally the aqueous extract of the plant at doses 200, 400, 800, and 1600 mg/kg respectively. Group V received raloxifene (positive control) at dose of 0.1 mg/kg and estradiol (0.05 μ g/kg). The sixth group received only distilled water (0 mg/kg). Twenty-four hours after the last dose, the animals were sacrificed under chloroform anesthesia. Uteri were excised and its various weights were then recorded.

2.5. Statistical analysis

The data obtained from biological assays were analysed by the One way analysis of variance (ANOVA) test and expressed as mean \pm SEM. The Fisher's exact test was used for the comparison of means. The analysis of percentages was done by *Chi*-square test.

3. Results

3.1. Anti-androgenic activity of aqueous plant extract

Figure 1 shows the variations of the weight of androgen-dependent glands and tissues according to various treatments. A significant reduction was observed in weights of ventral prostate gland ($P<0.01$); GP ($P<0.05$); Cowper's glands ($P<0.01$) for animals treated with aqueous extract of *E. speciosa*. However for the animal treated with flutamide (an anti-androgen of reference), a significant reduction was observed ($P<0.001$) in the weights of seminal vesicle, GP

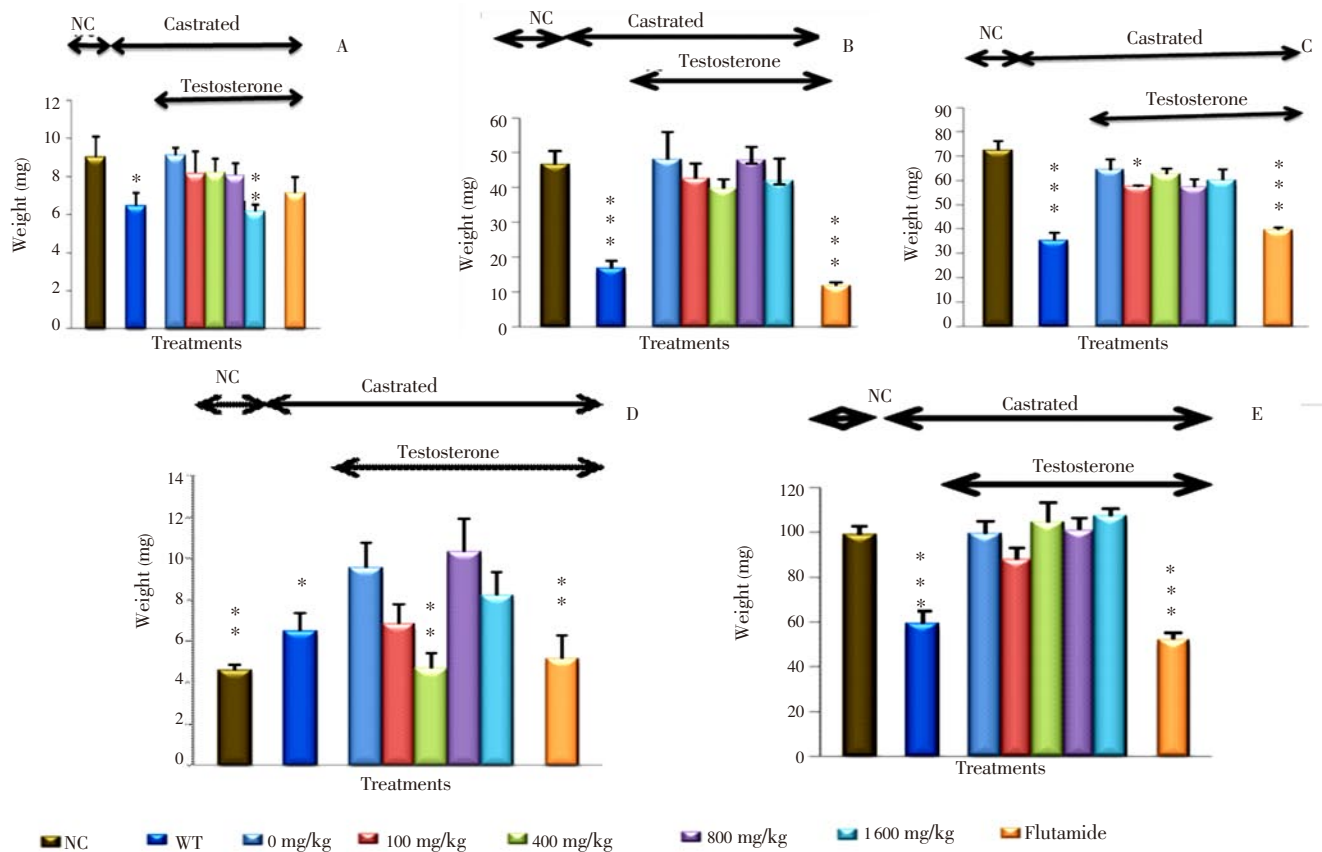


Figure 1. Variation of the weights of androgen depending organs and tissues according to the various groups of treatment. A: Ventral prostate; B: Seminal vesicle; C: GP; D: Cowper’s glands; E: LABC. NC: Not castrated; WT: Without testosterone. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

and LABC, and Cowper’s glands ($P<0.01$) (Figure 1). All these groups were compared with dose 0 mg/kg (control).

3.2. Effect of the plant extract on the serum PAP

Figure 2 shows the variations in the serum PAP activity of androgen–dependent tissues and glands. The data revealed that the plant extract have significantly reduced ($P<0.01$) the level of the serum PAP at different doses: 100 mg/kg; 400 mg/kg and 1600 mg/kg. Flutamide has also reduced the level of the serum PAP but the variation was not significant.

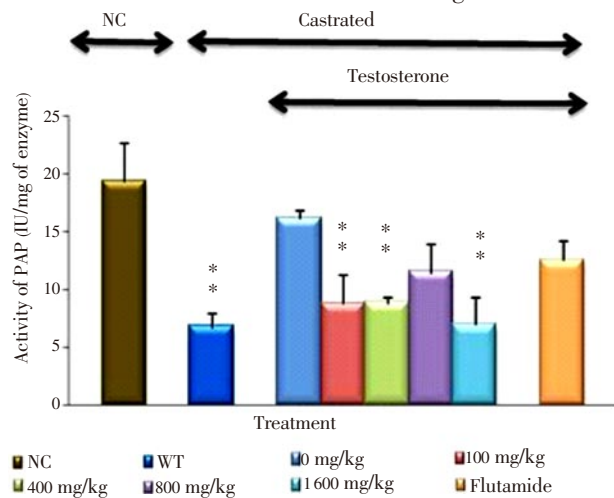


Figure 2. Variation of the PAP activity according to the various groups of treatment. **: $P<0.01$. NC: Not castrated; WT: Without testosterone.

3.3. Effect of plant extract on hematologic parameters

Table 1 shows the variations of hematocrite, red blood cells and white blood cells in animals treated with the aqueous extract of *E. speciosa*. These parameters were not much altered after the treatment of all groups compared to the control.

Table 1

Variations of the hematocrite, red blood cells and the white blood cells according to the various groups of animals.

Treatments	Hematocrite (%)	RBC ($\times 20000$)	WBC ($\times 200$)
NC	31.91 \pm 1.07	200.33 \pm 1.68	16.30 \pm 0.61
WT	26.88 \pm 0.79	201.83 \pm 2.02	19.50 \pm 2.75
0 mg/kg	26.97 \pm 1.73	202.00 \pm 1.29	21.66 \pm 2.96
100 mg/kg	30.59 \pm 3.03	202.16 \pm 2.99	29.00 \pm 5.01
400 mg/kg	28.15 \pm 0.79	206.33 \pm 5.89	30.16 \pm 4.31
800 mg/kg	32.07 \pm 2.46	204.83 \pm 5.61	24.66 \pm 3.85
1600 mg/kg	29.11 \pm 2.24	192.16 \pm 4.73	22.33 \pm 1.97
Flutamide	26.74 \pm 0.38	199.00 \pm 2.58	20.30 \pm 1.65

NC: Not castrated; WT: Without testosterone; RBC: red blood cells; WBC: white blood cells. Data are presented as mean \pm SEM.

The aqueous extract of *E. speciosa* was found to significantly increase the percentage of lymphocytes (60.83% \pm 3.09%) (mean \pm SEM), at the dose of 100 mg/kg; It also increased the percentage of monocytes (6.83% \pm 1.07%, 5.5% \pm 1.17%) at the doses of 100 mg/kg and 400 mg/kg compared with dose 0 mg/kg (Table 2).

However, although flutamide has significantly reduced the percentage of lymphocytes ($P<0.05$); monocytes ($P<0.01$) and increase neutrophils ($P<0.05$), no significant difference was observed between the eosinophils and basophils, compared with dose 0 mg/kg (Table 2).

Table 2

Percentage of the various types of white blood cells of the immune system according to the various groups of treated animals.

Treatments	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	Basophil (%)	Eosinophil (%)
NC	54.33±1.89	7.33±0.88	36.66±1.02	0.83±0.16	0.66±0.21
WT	58.66±2.47	7.33±0.95	31.50±2.84	1.33±0.21*	0.50±0.22
0 mg/kg	55.80±1.72	9.33±0.61	34.66±1.56	0.66±0.21	0.83±0.16
100 mg/kg	60.83±3.09*	6.83±1.07*	30.83±3.58	0.66±0.21	0.83±0.30
400 mg/kg	62.83±1.47	5.50±1.17**	30.33±0.42	0.83±0.16	0.83±0.16
800 mg/kg	57.16±3.78	7.83±0.90	31.33±2.61	1.16±0.16	0.83±0.30
1 600 mg/kg	66.00±1.18	9.50±0.67	25.16±0.87	0.50±0.22	0.66±0.21
Flutamide	46.50±3.98*	5.00±0.36**	48.33±5.07*	0.83±0.16	1.00±0.00

NC: Not castrated; WT: Without testosterone. *: $P<0.05$; **: $P<0.01$. Data are presented as mean±SEM.

3.4. Effect of the aqueous extract of *E. speciosa* on activity of catalase and superoxide dismutase

Table 3 shows the effect of the extract on superoxide dismutase and catalase activities in tissues. Animals receiving the plant extract at 100 mg/kg showed a significant decrease in the activity of catalase in the liver [$21.16±2.80$ IU/mg of enzyme] compared to the control [$36.82±2.51$ IU/mg of enzyme], while administration of different doses of extract had no significant effect on the activity of superoxide dismutase. Flutamide has significantly decreased the activity of catalase ($P<0.01$) in the kidneys, but increase the activity ($P<0.05$) in the heart compared with dose 0 mg/kg.

Table 3

Variation of the activity of the superoxide dismutase (IU/mg enzyme) and the catalase (IU/mg enzyme) in the liver, the kidneys and the heart according to the various treatments.

Treatments	CAT			SOD		
	Liver	Kidneys	Heart	Liver	Kidneys	Heart
NC	28.34±3.62	33.03±5.96	30.88±1.56	13.28±1.75	13.28±0.96	10.64±1.96
WT	35.67±4.47	37.28±5.05	35.86±2.82	12.50±1.22	13.94±0.90	12.61±1.13
0 mg/kg	36.82±2.51	36.65±5.19	28.74±4.95	14.24±0.73	13.35±0.75	12.68±0.57
100 mg/kg	21.16±2.80**	26.84±4.32	25.52±6.82	11.58±2.10	14.06±1.25	13.05±0.31
400 mg/kg	27.19±3.59	31.16±4.06	24.54±5.17	12.45±1.28	14.52±0.47	14.29±1.43
800 mg/kg	38.51±3.83	39.30±5.17	18.68±2.77	13.38±1.01	14.55±1.20	12.29±1.10
1 600 mg/kg	26.96±2.55	34.68±4.44	17.53±1.63	12.85±1.49	14.28±1.01	11.25±1.38
Flutamide	34.14±5.05	18.60±0.82**	41.57±4.49*	11.79±1.42	14.61±0.75	12.01±1.31

CAT: Catalase; SOD: Superoxide dismutase; NC: Not castrated; WT: Without testosterone. *: $P<0.05$; **: $P<0.01$. Data are presented as mean±SEM.

3.5. Antiestrogenic activities

Figure 3 shows the variations in the relative weight of the uteri of animals treated with the extract of plant. The data revealed that the plant extracts had significantly reduced ($P<0.01$) the relative weight of the uteri in all treated group

while raloxifene has also reduced it ($P<0.001$).

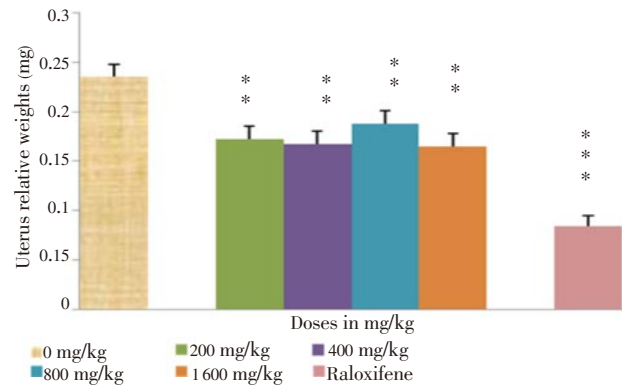


Figure 3. Variation of the relative weights of the uteri according to the various groups of treatment. **: $P<0.01$; ***: $P<0.001$.

4. Discussion

Oral administration of aqueous extract of *E. speciosa* has resulted in the reduction of ventral prostate, gland penis and Cowper's gland weights. Since androgens are involved in cell proliferation, the decrease in the weight of organs and glands may be due to the decrease in the circulating levels of this hormone. The anti-androgenic effect of the aqueous extract of *E. speciosa* could be expressed by an inhibition of the effect of testosterone propionate. This shows that the extract would contain non steroid anti-androgenic substances which inhibit the effect of the testosterone propionate. Anti-androgens bind to their androgenic receptors, thus preventing their recognition of androgens^[10]. These substances contained in aqueous extracts of *E. speciosa* could also have anti-estrogens activity, which by binding to the estrogen receptors would interact at the early stages of the synthesis of estrogen. They could also be inhibitors of 5 α -reductase, an enzyme which converts testosterone to dihydrotestosterone in prostatic cells, the principal agonist of androgen receptor^[11].

In this study, the decrease of PAP in animals treated with the plant extract at different doses (100 mg/kg; 400 mg/kg; and 1 600 mg/kg) means that this extract could present anti-androgenic properties, an observation related to the decrease observed in the weight of androgen-dependent tissues and glands mentioned above.

The significant increase of the percentage of lymphocytes (60.83%±3.09%) at the dose 100 mg/kg could be due to a stimulation of the immune system by co-stimulant compounds present in the extract. Indeed, the lymphocytes answer to antigens by proliferation, developing antigen-specific clones or producing lymphokines, thus amplifying the immune response^[12]. This effect can be imitated by the use of phytoimitogenes such as the phytohemagglutinine which binds to some glycoproteins on the surface of T lymphocyte including the T cells receptor and protein CD3 and stimulates the proliferative response of the cells T^[12].

In the defense against oxidative stress, cells are equipped with enzymatic (superoxide dismutase and

catalase) antioxidant mechanism that plays an important role in the elimination of free radicals^[13]. However a high testosterone level can induce the oxidative stress by the production of reactive species of oxygen and nitrogen which can damage tissues^[14]. The significant decrease of the cellular level of catalase in the liver [(21.16±2.80) IU/mg of enzyme] at the dose 100 mg/kg, could be attributed to an increased production of superoxide anion radical which has been reported to inhibit catalase activity in case of excessive production^[15].

The significant decrease in the relative weight of the uteri observed in animals receiving various doses of extract could translate the fact that this extract would contain steroid or non steroid anti-estrogenic substances. Indeed these compounds can act either by blocking the estrogen receptors (ER α and ER β) on the level of the uterus, or by inhibiting the enzyme responsible for the conversion of the androgens into estrogens. Furthermore steroid saponin has been found to inhibit estrous cycle in animals upon continuous administration. Flavonoids are known to have anti-inflammatory effects which are believed to result from inhibition of cyclo-oxygenase enzyme^[16]. The aqueous extract of *E. speciosa* as shown by Oben *et al.* contains both saponins and flavonoids which may also explain its anti-estrogenic effects^[7].

Thus the present investigation showed that the *E. speciosa* extracts exerts antiandrogenic, immunomodulatory, anti-estrogenic and weak antioxidant activity. The reduced androgen-dependent organs weight in male and uterine weight in female rats could be attributed to a direct effect of the chemical constituents of the extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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