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*Full Length Research Paper*

# Combined effects of testosterone propionate and *Leptadenia hastata* Pers. (Decne) aqueous extracts on immature castrated male rats

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**To evaluate the competition between testosterone propionate (TP) and *Leptadenia hastata* aqueous extracts, immature castrated male Wistar rats were divided into two groups. *L. hastata* aqueous extracts reduced significantly the weight of androgen-dependant sex glands, the level of phosphatase acid prostatic (PAP) and fructose in seminal vesicles and prostate, and the serum testosterone level. When TP was administered simultaneously with 100, 200 and 400 mg/kg of *L. hastata* aqueous extracts, a potentiate action was observed with 100 mg/kg of *L. hastata* by the increase of androgen-dependant sex glands weights, fructose and PAP levels in seminal vesicles and prostate, and the serum testosterone level. The anti-androgenic effect of *L. hastata* appeared with the doses of 200 and 400 mg/kg which reduced significantly the weight of androgen-dependant sex glands, fructose and PAP levels in seminal vesicles and prostate and serum testosterone level. From these findings, it was concluded that the effect of TP was potentiated with low doses of *L. hastata* and antagonized with high doses. This study confirmed the anti-androgenic effects of *L. hastata* aqueous extracts in immature castrated male rats.**

**Key words:** *Leptadenia hastata*, testosterone propionate, anti-androgenic activity, rats.

## INTRODUCTION

The increasing interest in the effects of the components of plant extracts on humans and wildlife has been reflected in the number of recent papers and international conferences devoted to this topic (Weisburger, 1999; Arbonnier, 2000; Vasudeva and Sharma, 2006). Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible beneficial as well as adverse effects (Gamache and Acworth, 1998). *Leptadenia hastata* (Pers.) Decne (Asclepediaceae) is an indigenous medicinal plant of West Africa, commonly used by traditional healers for the treatment of many human and animal diseases (Kerharo and Adam, 1974; Berhaut, 1979; Nacoulma/Ouédraogo, 1996; Arbonnier, 2000). In the north of Burkina Faso, where livestock is very important, the breeders claimed the loss of fertility of their animals after the consumption of *L. hastata* leaf stems. In a previous study, we showed the anti-androgenic effect of *L. hastata* leaf stems aqueous extracts in immature castrated male rats by Hershberger assay (Ashby and Lefevre, 2000; OECD, 2001, 2006). This study proved that *L. hastata* aqueous extracts contained substances which can be taken as anti-androgens. The purpose of the present study was: first, to determine the competition between *L. hastata* extracts and testosterone propionate (TP) as androgen reference;

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and second, to authenticate the anti-androgenic activity of *L. hastata* extracts. For the anti-androgenic activity validation tests, immature castrated male rats were co-treated with TP and different doses of *L. hastata* aqueous extracts for 10 consecutive days. After the treatment, androgen-dependant sex glands weight, biochemical parameters and hormonal parameters were examined.

## MATERIALS AND METHODS

### Plant collection and preparation of extract

The leaf stems of *L. hastata* were collected from Kamboinsè (25 km in the North of Ouagadougou), during April and May 2010. The plant was authenticated by the Department of Botany, of the University of Ouagadougou. Herbaria are made and their voucher specimen retained in the Department.

The leaf stems of *L. hastata* were first washed with large amount of water then dried in a ventilated room, away from dust and direct sunlight. 150 g were coarsely powdered and macerated in 1 L of distilled water at 40°C. The obtained macerated product was filtrated, run through Rotavapor (Buchi/R-114), lyophilised, and kept in a drier until ready for use. Yield of the extraction was 24.6%.

### Chemicals

Testosterone propionate (Purity: 97%) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the Aldrich Chemical Co., and was shipped and stored in glass containers at room temperature.

### Animal model

Wistar male rats from Charles River Laboratories (France) were checked for overt signs of illness and anomalies. Animals were acclimatized to the Laboratory environment for 7 days before use. During the experiment, rats were housed six animals per cage in polycarbonate cages under controlled environmental conditions, including a temperature 22°C, a relative humidity of 55%, and a 12 h light/12 h dark cycle. Pellet rodent diet and drinking water were available *ad libitum*. The castration was performed on 3 week-old animals via a midline incision, and test compounds treatment were started 7 days later to allow the animals time for complete recovery. All the experiments have been carried out under approval of institutional ethics committee.

### Study design

Seven days after castration, the rats were randomised into 2 treatment groups according to body weight. Each group was divided into 4 subgroups of 6 animals. The four subgroups of the first group received respectively distilled water, 100, 200 and 400 mg/kg of *L. hastata* aqueous extracts. For the second group, each subgroup received simultaneously 0.4 mg/kg of TP and the same amount of *L. hastata* aqueous extracts.

Testosterone propionate was administered subcutaneously and *L. hastata* extracts by oral gavage during 10 consecutive days.

### Measurement of organ weights

24 h after the last administration, the rats were killed by decapitation. After necropsy, androgen-dependent sex glands were removed and weighed without blotting. The excised tissues were trimmed of any fat. The excision procedures used were reproducible over time, and particular attention was paid to the prevention of tissue fluid loss variations during processing. A standard operating procedure was followed for the excision of sex accessory tissues. The weight of the following androgen-dependant sex glands was measured: seminal vesicles, prostate, cowper's glands, levator anis and bulbocavernous muscles (LABC).

### Tissue biochemistry

Seminal vesicles and prostate were kept at -20°C until assayed for fructose and prostatic acid phosphatase (PAP) (Mann, 1964).
PAP was assayed by the method of Jacobson using p-nitrophenyl phosphate as substrate and by specific tartrate inhibition method as described (Anonymous, 1995).

### Hormonal assay

Serum testosterone levels were assayed from samples using radio-immuno-assay (RIA) method (Belanger et al., 1980). The sensitivity of the assay was 10 pg/ml.

### Statistical analysis

Data were analyzed using the statistical package SYSTAT (Version 10). Data are presented as mean ± standard error of the mean (S.E.M.). If variances were homogeneous, differences between groups were assessed by one-way analysis of variance. Differences between pair of means were assessed by the LSD test. A value of $p < 0.05$ was considered as statistically significant.

## RESULTS

### Effect of *L. hastata* on androgen-dependant sex glands weight

*L. hastata* extracts induced a significant *($p < 0.05$)* decrease (dose effect) of glands weight (seminal vesicles, prostate, cowper's glands and LABC) in comparison with distilled water control group (Figures 1 to 5).

The testosterone propionate combined with 200 and 400 mg/kg of *L. hastata* induced a significant ($p < 0.05$) decrease of organs weight compared to controls. The dose of 100 mg/kg of *L. hastata* increased non-significantly the weight of the glands.

The weight of seminal vesicles, prostate, cowper's glands and LABC, of rats treated with testosterone propionate combined to different doses of *L. hastata* was increased significantly ($p < 0.01$) than rats treated only with *L. hastata* extracts (Figures 1 to 5).

### Effect of *L. hastata* on fructose and PAP level

The fructose level was significantly ($p < 0.05$) decreased in prostate and seminal vesicles compared to control

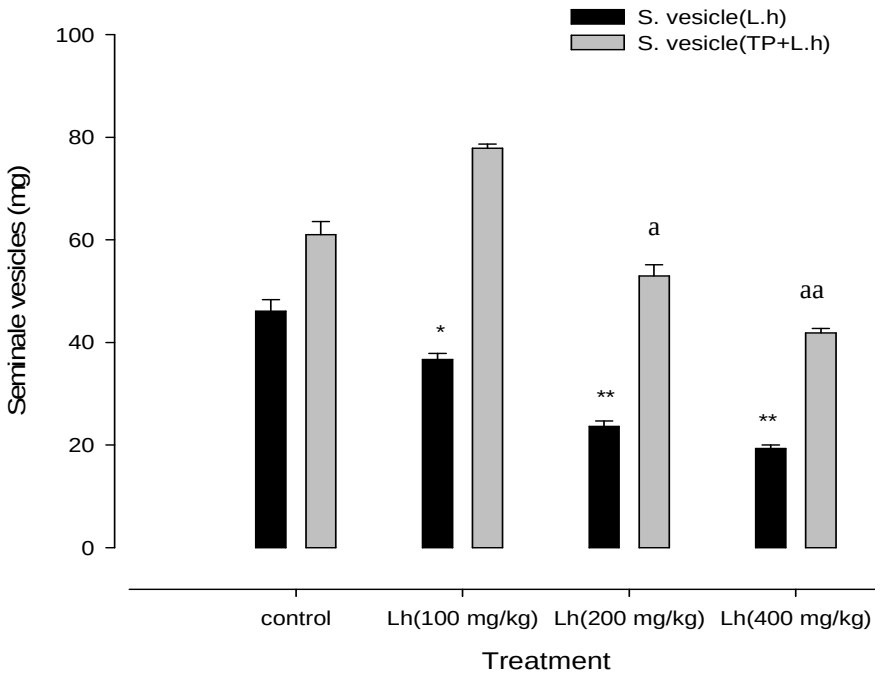


**Figure 1.** Seminal vesicles weights from immature castrated Wistar male rats given 10 consecutive daily treatments of *L. hastata* (100, 200, 400 mg/kg per day) and the same doses of *L. hastata* combined to testosterone propionate (0.4 mg/kg per day), commencing 8 days after castration. *P < 0.05, **P < 0.01. a: P < 0.05; aa: P < 0.01.

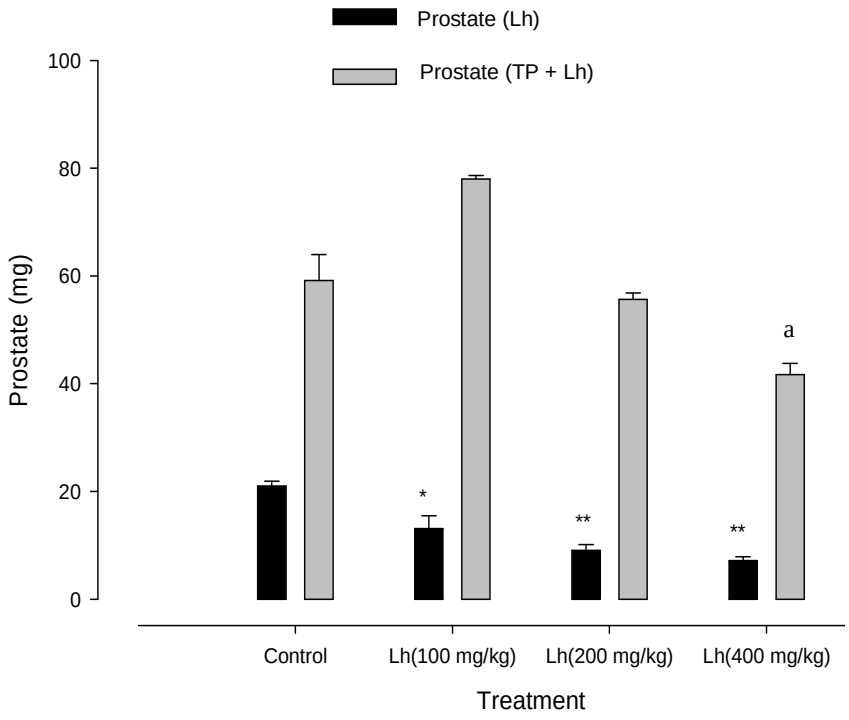


**Figure 2.** Prostate weights from immature castrated Wistar male rats given 10 consecutive daily treatments of *L. hastata* (100, 200, 400 mg/kg per day) and the same doses of *L. hastata* combined to testosterone propionate (0.4 mg/kg per day), commencing 8 days after castration. **P* < 0.05, ***P* < 0.01. a: P < 0.05; aa: P < 0.01.

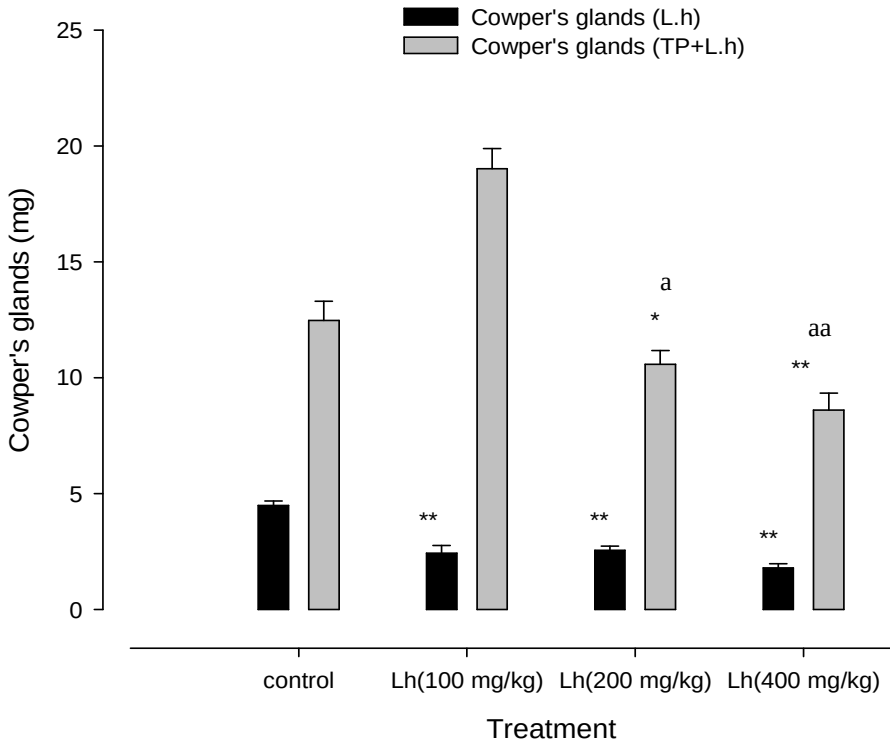


**Figure 3.** Cowper's glands weights from immature castrated Wistar male rats given 10 consecutive daily treatments of *L. hastata* (100, 200, 400 mg/kg per day) and the same doses of *L. hastata* combined to testosterone propionate (0.4 mg/kg per day), commencing 8 days after castration. **P* < 0.05, ***P* < 0.01. a: P < 0.05; aa: P < 0.01.

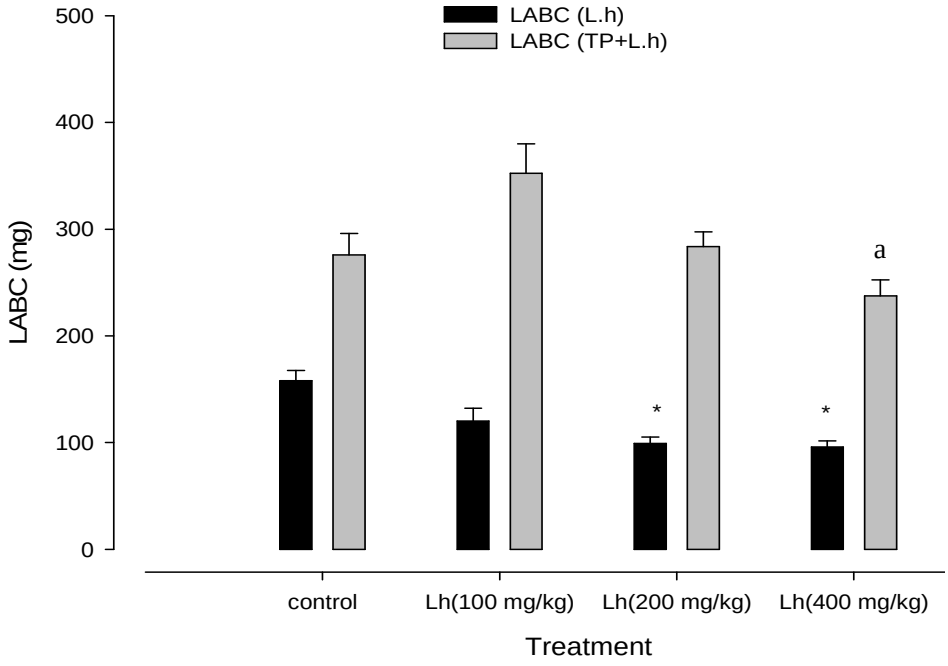


**Figure 4.** LABC weights from immature castrated Wistar male rats given 10 consecutive daily treatments of *L. hastata* (100, 200, 400 mg/kg per day) and the same doses of *L. hastata* combined to testosterone propionate (0.4 mg/kg per day), commencing 8 days after castration. **P* < 0.05; a: P < 0.05. LABC: Levator Ani and Bulcavernous muscle.

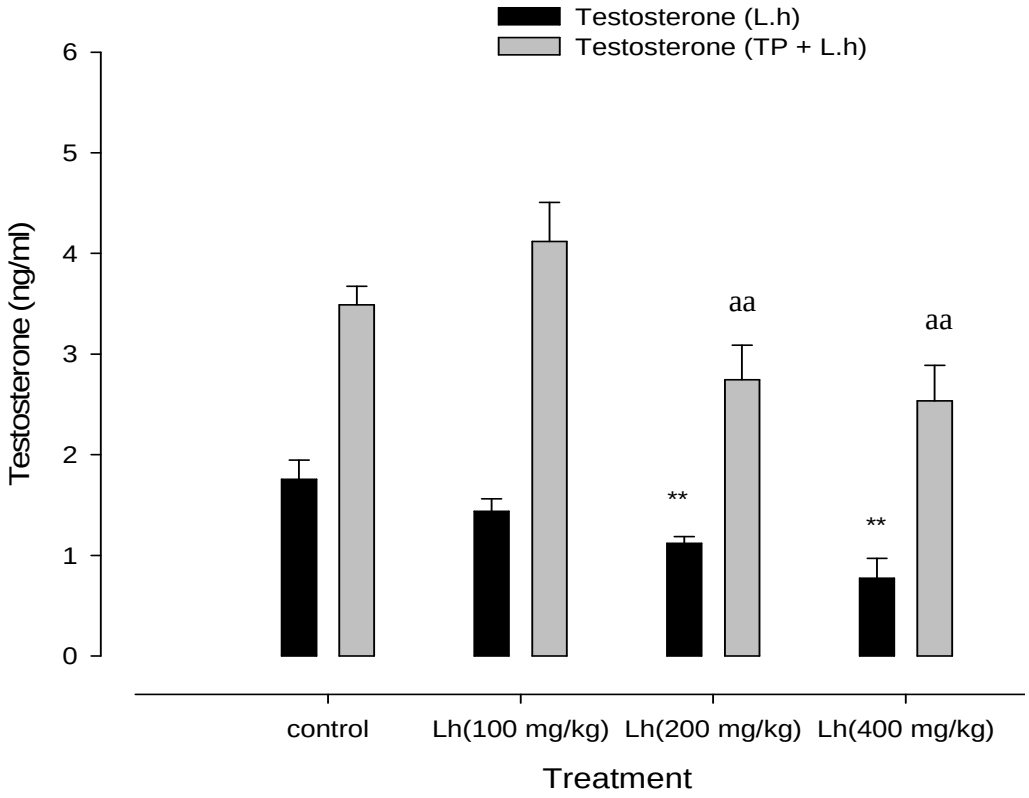


**Figure 5.** Testosterone level from immature castrated Wistar male rats given 10 consecutive daily treatments of *L. hastata* (100, 200, 400 mg/kg per day) and the same doses of *L. hastata* combined to testosterone propionate (0.4 mg/kg per day), commencing 8 days after castration. *$P$ < 0.05, **$P$ < 0.01. a: P < 0.05; aa: P < 0.01.

when rats were treated only with *L. hastata* aqueous extracts. When rats were treated simultaneously with testosterone propionate and *L. hastata* extracts, the fructose level significantly ($p < 0.05$) decreased with 200 and 400 mg/kg of *L. hastata* aqueous extracts. The dose of 100 mg/kg of *L. hastata* was non significant ($p > 0.05$). Same observations were made with PAP level.

The level of fructose and PAP in rats treated simultaneously with TP and *L. hastata* were significantly higher ($p < 0.01$) than those of rats treated only with *L. hastata* extracts (Table 1).

### Effect of *L. hastata* extracts and TP on serum testosterone level

Serum testosterone level significantly ($p < 0.01$) reduced in the rats treated with the different doses of *L. hastata* compared to control. In the rats treated simultaneously with TP and *L. hastata,* serum testosterone level significantly ($p < 0.01$) decreased with the doses of 200 and 400 mg/kg of *L. hastata* compared to control. The dose of 100 mg/kg of *L. hastata* aqueous extracts increased non-significantly ($p > 0.05$) the level of TP.

The level of serum testosterone in rats treated simultaneously with TP, and *L. hastata* were higher *($p <$* 0.01) than those of rats treated only with *L. hastata* extracts (Figure 5).

## DISCUSSION

In agreement with previous studies (Bayala et al., 2011), *L. hastata* aqueous extracts were found to reduce significantly the weight of androgen dependant sex glands, the level of PAP and fructose in seminal vesicles and prostate and the serum testosterone level. When TP was administered simultaneously with 100, 200 and 400 mg/kg of *L. hastata* aqueous extracts, we observed a potentiate action with 100 mg/kg of *L. hastata* by the increase of androgen-dependant sex glands weights, fructose and PAP levels in seminal vesicles and prostate and the serum testosterone level. The anti-androgenic effect of *L. hastata* appeared with the doses of 200 and 400 mg/kg which reduced significantly the weight of androgen-dependant sex glands, the levels of fructose and PAP in seminal vesicles and prostate, and serum testosterone. These results showed that at low doses of

**Table 1.** Fructose and prostatic acid phosphatase (PAP) in prostate and seminal vesicles of controls and treated rats for 10 days.

| Treatment | Doses (mg/kg) | n | Seminal vesicles | Prostate | |
|---|---|---|---|---|---|
| | | | Fructose (mg/g) | Fructose (mg/g) | PAP |
| Control (TP) | 0.4 | 6 | 2.872 ± 0.421 | 2.191 ± 0.213 | 0.213 ± 0.021 |
| Control (distilled water) | Distilled water | 6 | 1.631 ± 0.263 | 1.685 ± 0.021 | 0.175 ± 0.011 |
| Lh_100 | 100 | 6 | 1.136 ± 0.192 | 1.092 ± 0.017 | 0.101 ± 0.021 |
| Lh_200 | 200 | 6 | 0.983 ± 0.303***** | 0.673 ± 0.081***** | 0.082 ± 0.002***** |
| Lh_400 | 400 | 6 | 0.792 ± 0.070***** | 0.495 ± 0.025****** | 0.056 ± 0.003****** |
| TP + Lh_100 | 0.4 + 100 | 6 | 3.321 ± 1.234 | 2.987 ± 0.076 | 0.298 ± 0.045 |
| TP + Lh_200 | 0.4 + 200 | 6 | 2.023 ± 0.765***** | 1.517 ± 0.032***** | 0.177 ± 0.008 |
| TP + Lh_400 | 0.4 + 400 | 6 | 1.765 ± 0.876***** | 1.231 ± 0.043****** | 0.097 ± 0.003****** |

PAP: phosphatase acid prostatic; TP: Testosterone propionate; Lh: *Leptadenia hastata*. * $p < 0.05$; ** $p < 0.01$.

*L. hastata*, TP effects were potentiated and with higher doses of *L. hastata* effects were inhibited. The potentiation of TP may be due to the weakness of *L. hastata* aqueous extracts dose. This low dose of extract of *L hastata* would increase the effect of TP by a significant recognition of the androgen receptors. This recognition requires the presence of the testosterone propionate which sensitizes its own receptors (Kelce et al., 1998; Gray et al., 1999).

Circulating levels of testosterone are required for the maintenance of accessory sex organ functions, but the threshold levels of required hormone might be different for different functions. The reduced weights of seminal vesicle and prostate further support the suppressed concentration of testosterone in the circulation (Gupta et al., 1993; Lohiya and Ansari, 1999).

*L. hastata* aqueous extracts could inhibited androgen function through multiple mechanisms (Lambright et al., 2000; Wilson et al., 2004), antagonism of androgen receptors (Vinggaard et al., 2002) and inhibition of testosterone production by increasing testosterone metabolism through induction of metabolic enzymes. Recently, a metabolite of the aromatic glucosinolates was described as a specific antagonist of the androgen receptor; therefore, it is possible that the effects of *L. hastata* are due, at least in part, to interactions between glucosinolates and the androgen receptor (Le et al., 2003). Also, it is conceivable that *L. hastata* extracts contains phytoestrogens: the potential role of phyto-estrogens on male fertility has been attributed to both estrogenic or anti-estrogenic activities (Rochira et al., 2001).

These results could explain the loss of fertility of animals consuming the leaf stems of *L. hastata* by the presence of non steroid anti-androgens in the plant. Anti-androgens had many applications in the regulation of animal reproduction (Navarro-Martin et al., 2009) and could have, for human prostate cancer, an important therapeutic value. All current anti-androgen therapies reduced ligand accessed to androgen receptors, whether by competitive antagonism or inhibition of androgen production, but are limited by acquired resistance and serious side-effects (Jones and Diamond, 2008; Taplin, 2008).

The increase of body and glands weights, biochemical and hormonal parameters of group co-treated with testosterone propionate and *L. hastata* extracts compared to group treated only with *L. hastata* extracts could be due to the anabolic effect of testosterone propionate (Prakasam et al., 1999; Parrilla-Carreroa et al., 2009). Testosterone improved total protein level, bone mineral density and muscle fiber and strength (Snyder et al., 1999; Gregory et al., 2003). Some studies reported that testosterone replacement produced a substantial increase in lower body strength (Ferrando et al., 2002).

## Conclusion

This study showed that the low doses of *L. hastata* potentiated the action of TP and the high doses inhibited its action. These results confirmed the anti-androgenic effects of *L. hastata* aqueous extracts and can explained the loss of fertility of animals consuming its leaf stems.

## ACKNOWLEDGEMENTS

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