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Effects of Aqueous Extracts of *Leptadenia hastata* (Pers.) Decne. (Asclepediaceae) on Male Reproductive Functions Using Castrated Immature Rats

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ABSTRACT

The present study investigated the effect of aqueous extracts of *Leptadenia hastata* on male reproductive function. For acute toxicity nine groups of 6 male mice received orally, respectively 50, 100, 200, 500, 1000, 1500, 2000, 3000 and 4000 mg kg^{-1} of *L. hastata* aqueous extracts. The results showed the non toxic effect of *L. hastata* aqueous extracts. Rat weight gain and adrenals weight did not have significantly change. Seminal vesicles, prostate, levator ani plus bulbocavernosus muscles (LABC), cowpers glands, coagulant's glands weights were significantly decreased with 200, 400, 800 mg kg^{-1} compared with controls. The serum testosterone level, the fructose and protein content of seminal vesicles and prostate were also significantly decreased with 200, 400, 800 mg kg^{-1} compared with the controls. Total protein, total cholesterol, HDL cholesterol, blood sugar and hematocrit on blood and serum did not have significantly change. Overall, these results indicate the anti-androgenic effect of *L. hastata* aqueous extract by Hershberger assay.

Key words: *Leptadenia hastata*, anti-androgenic, testosterone, immature castrated rats

INTRODUCTION

Leptadenia hastata (Pers.) Decne., is a perennial liana of the family of Asclepediaceae which pushes in cattle-breeding areas of Burkina Faso in West Africa. The breeders commonly used the leaf stems for their parasitic activity and against placental retention when animals gave birth (Kerharo and Adam, 1974; Arbonnier, 2000).

Leptadenia hastata was the subject of several studies which showed its anti-inflammatory action (Nikiéma *et al*., 2001) and its inhibitory effect on certain tumoral cells (Aquino *et al*., 1996).

Literature survey and ethnobotanic investigations with the traditional healers revealed that the consumption of the leaf stems of *L. hastata* by the donkeys, the horses and the dromedaries could have antifertility effect. In the North region of Burkina Faso, it is also arisen that the

consumption of *L. hastata* had harmful effects on fertility of the sheeps and goats. In certain areas of West Africa, breeders claimed the antifertility effect of their animals after consumption of *L. hastata* leaf stems (Berhaut, 1979; Arbonnier, 2000). But, so far no male and female antifertility has been carried out on *L. hastata* leaf stems extracts.

Hershberger assay is one of the assays in the proposed Tier I screening battery by EDSTAC (USEPA, 1998). The Hershberger assay has been used for detecting androgen receptor agonists/antagonists by organ weight measurements from sexually immature rats (Hershberger *et al*., 1953). Generally, accessory sex glands and tissues are dependent upon androgen stimulation to gain and maintain weight during or after puberty. If endogenous testicular sources of androgen are removed, exogenous sources of androgens are necessary to increase or maintain the weights of these tissues (Ashby and Lefevre, 2000).

This study was carried out to characterize potential anti-androgenic properties of the aqueous extract of leaf stems of *L. hastata* by Hershberger assay. The Hershberger assay has been widely used for evaluating compounds with Androgen Receptor (AR) mediating effects and was designed to detect potential anti-androgenic activity of the test compounds (O'Connor *et al*., 1999).

The Hershberger assay detects *in vivo* antiandrogen receptor antagonism in a castrated immature male rat model (Gray *et al*., 2004; Owens *et al*., 2006). This study aimed also to evaluate the acute toxicity of *L. hastata* aqueous extracts. Testosterone propionate and distilled water were, respectively used as positive and reference controls to confirm anti-androgenic activity.

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 of the year 2009. The plant was authenticated by the Department of Botany, of the University of Ouagadougou. Herbaria are made and their voucher specimen were deposited 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. One hundred and fifty grams of dried material were coarsely powdered and macerated in distilled water at 40°C. The obtained macerated product was then 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%, Ref. T1875, PM: 344.49) 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.

Acute toxicity experimental design: The acute toxicity study was performed on male Swiss mice (27.2±0.31 g) obtained from Charles Rivers Laboratories, France. The mice were acclimatized for 1 week before the experiment and then placed on study at about 6 weeks of age. Animals were maintained in an air-conditioned room at 22°C, 50-60% relative humidity and artificial illumination between 08:00 and 20:00 h.

For the acute toxicity assessment, we used the method of Trevan (1927) and its further modifications (Miller and Tainter, 1944; Litchfield and Wilcoxon, 1949; Prieur, 1973; Descotes, 1985). For the evaluation of the median Lethal Dose (LD50), we used nine (09) groups made of six

(06) mice each. Each group received a specific dose of the extract to be tested as follows: 50, 100, 200, 500, 1000, 1500, 2000, 3000, 4000 mg kg^{-1}. All doses were given by oral gavage. The animals were observed daily for abnormalities of condition or behaviour.

Animal model: Wistar male rats from Charles River Laboratories (France) were checked without 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 cycle/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 commenced 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: The experiment was carried out for the assessment of anti-androgenic activity of the aqueous extract of leaf stems of *L. hastata* (50, 100, 200, 400, 800 mg kg^{-1}) administered by oral gavage to castrated male rats for 10 days and then testosterone (0.4 mg kg^{-1}) was administered subcutaneously as a positive control. Distilled water was administered orally for 10 consecutive days as a reference control. The total volume of administration per rat was 4 mL kg^{-1} per day for *L. hastata* and 0.5 mL kg^{-1} per day for testosterone. All test materials were prepared daily prior to injection. Testosterone propionate was dissolved in a minimal amount of 95% ethanol and diluted to the working concentration with corn oil (the final concentration of absolute ethanol was 2.5%).

Measurement of organ weights: Approximately 24 h after the last administration of test substances, the rats were killed by decapitation. After necropsy, the accessory sex organs were removed and weighed without blotting (to the nearest 0.1 mg). The excised tissues were trimmed of any fat. The excision procedures used were reproducible over time and paid particular attention 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 following accessory sex tissues was measured: seminal vesicles, prostate, cowper's glands, coagulant's glands, levator anis and bulbocavernous muscles (LABC). The adrenals were also removed and weighted.

Tissue biochemistry: Tissues were kept at -20°C until assayed for protein (Lowry *et al*., 1951) and fructose was estimated in seminal vesicles and prostate (Mann, 1964).

Blood and serum biochemistry: Serum protein (Lowry *et al*., 1951), total cholesterol (Zlatkis *et al*., 1953), blood sugar, HDL-cholesterol (Burnstein *et al*., 1970) and haematocrit were assayed.

Hormonal assay: Serum testosterone levels were assayed from samples using radio immuno assay method (Belanger *et al*., 1980). The sensitivity of the assay was 10 pg mL^{-1}.

Statistical analysis: Data were analyzed using the statistical package SYSTAT (Version 10). Data are presented as Mean±standard error (n = 6) of the mean (SEM). 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 AND DISCUSSION

Acute toxicity: All mice survived at the end of the 72 h study with the gavage of different doses of aqueous extracts of *L. hastata* (50, 100, 200, 500, 1000, 1500, 2000, 3000, 4000 mg kg^{-1}). No abnormalities of condition or behaviour were detected.

Body and organ weights: The oral administration of *L. hastata* extract to immature castrated male rats for 10 days did not cause any significant ($p>0.05$) change in the body and adrenals weights of treated rats compared to control animals (Fig. 1).

However, the weights of seminal vesicles, prostate, LABC, Cowper's glands, coagulant's glands were significantly reduced ($p<0.05$) with the doses of 200, 400 and 800 mg kg^{-1} of *L. hastata* when compared to control values (Table 1).

Tissue biochemistry: The protein and fructose contents of seminal vesicles and prostate of rats treated with 200, 400 and 800 mg kg^{-1} of *L. hastata* were reduced significantly ($p<0.05$) in comparison to positive and reference controls. The dose of 50 and 100 mg kg^{-1} did not show any significant ($p>0.05$) decrease of protein and fructose (Table 2).

Blood and serum biochemistry: Blood variables haematocrit and sugar were within the normal range and did not show any significant change compared to positive and reference controls. Serum protein, cholesterol, HDL-cholesterol did not change significantly after *L. hastata* extract treatment to rats compared to control groups (Table 3).

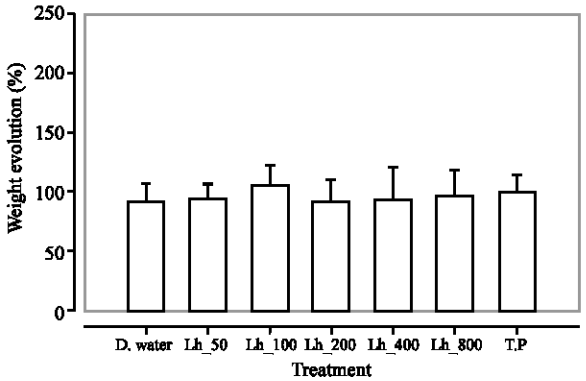

Fig. 1: Effect of *Leptadenia hastata* aqueous extract on weight evolution of immature castrated rats during 10 days consecutive treatments. Weight evolution of immature castrated rats treated with Testosterone Propionate (TP), distilled water (D. water) and different doses (50, 100, 200, 400, 800 mg kg^{-1}) of aqueous extracts of *Leptadenia hastate* (Lh). Castrated male rats were treated daily for 10 consecutive days. Before the beginning of the test, the initial body weight was took and 24 h after the last dosing, the rats were weighed. Data are expressed as mean SE (six animals per group). Significantly different from vehicle control. Weight evolution (%) = ((final weight - initial weight)/10)x100

Table 1: Effect of *Leptadenia hastata* aqueous extract on accessory sex glands and adrenals weights of immature castrated rats after 10 days consecutive treatments

Treatments	No.	Dose (mg kg^{-1})	Seminal vesicles (mg)	Prostate (combined)[a] (mg)	LABC (mg)	Cowper's glands (mg)	Coagulante's glands (mg)	Adrenals (mg)
T. propionate	6	0.4	143.66±6.32	93.16±17.31	275.83±20.17	15.05±1.36	29.01±1.57	40.11±3.45
Distilled water	6	DW	19.98±5.85	14.53±1.10	158.16±9.38	4.11±0.23	5.48±0.28	38.16±2.15
Lh_50	6	50[b]	12.96±3.21	8.50±2.32	116.16±16.21	2.98±0.29	4.86±0.32	37.53±1.54
Lh_100	6	100	9.95±1.62*	5.56±1.21	120.36±11.82	2.73±0.44	3.25±0.26	35.98±3.37
Lh_200	6	200	9.61±1.47*	4.28±1.74**	99.21±5.96*	1.73±0.19*	2.46±0.19*	35.55±3.78
Lh_400	6	400	9.33±1.21*	4.75±0.91**	96.05±5.42*	1.80±0.17**	1.76±0.10**	34.51±2.21
Lh_800	6	800	6.00±0.83**	3.86±1.09**	86.85±11.91**	1.43±0.14**	1.48±0.14**	37.26±1.72

Castrated immature rats were administered with testosterone propionate (0.4 mg/kg/day) by subcutaneous injection, distilled water and different doses of *Leptadenia hastata* (50, 100, 200, 400, 800 mg kg^{-1}) by oral gavage for 10 days. One day after the final treatment, the rats were weighed and the accessory sex glands were removed carefully and weighed separately. Mean+SE, n = 6. Significantly different from castrated group **p<0.01; *p<0.05. [a]: Ventral and dorsal prostate; [b]: DW: Distilled water; [c]: LABC: Levator ani/Bulbocanernous muscles

Table 2: Effect of *Leptadenia hastata* aqueous extract on tissues biochemical parameters of immature castrated rats after 10 days consecutive treatments

					Protein (mg g^{-1})		Fructose (mg g^{-1})	
Treatments	No.	Age (week) of castration	Dosage (mg kg^{-1})	Route of injection	Seminal vesicles	Prostate	Seminal vesicles	Prostate
T. propionate	6	3	0.4	s.c	121.41±4.21	111.16±5.87	2.34±0.92	1.89±0.39
Distilled water	6	3	DW[a]	p.o	99.21±3.12	87.44±5.09	1.81±0.76	1.18±0.06
Lh[b]_50	6	3	50	p.o	78.13±2.23	70.14±4.76	1.37±0.19	0.81±0.02
Lh_100	6	3	100	p.o	69.24±3.45	54.13±3.67	1.29±0.30	0.77±0.08
Lh_200	6	3	200	p.o	51.17±2.14*	36.76±2.65*	0.89±0.07*	0.69±0.04*
Lh_400	6	3	400	p.o	47.14±1.54**	30.53±1.98**	0.76±0.05*	0.51±0.08**
Lh_800	6	3	800	p.o	49.76±4.76**	41.19±4.32**	0.56±0.09*	0.49±0.07**

Castrated immature rats were administered with testosterone propionate (0.4 mg/kg/day) by subcutaneous (s.c) injection, distilled water and different doses of *Leptadenia hastata*(Lh) (50, 100, 200, 400, 800 mg kg^{-1}) by oral gavage for 10 days. One day after the final treatment, the seminal vesicles and prostate were used for the dosage of total protein and fructose. Mean±SE, n = 6. Significantly different from castrated group (**p<0.01; *p<0.05); [a]: Distilled water; [b]: *Leptadenia hastata*

Hormonal assay: Serum testosterone level of rats treated with 100, 200, 400 and 800 mg kg^{-1} of *L. hastata* extract was decreased significantly (p<0.01) in comparison to control groups (Fig. 2).

For the evaluation of acute toxicity, after 72 h of observation, the various doses of *L. hastata* did not cause any mortality. This result confirmed other published work which already showed the non toxicity of *L. hastata* extracts by another routes administration (Nikiema, 1997; Tamboura *et al*., 2005). The non toxic effect of extracts was confirmed by the fact that some African populations commonly used the leaves of *L. hastata* as food (Hutchinson and Dalziel, 1937; Freiberger *et al*., 1998). The normal development of weight gain of all rats treated with different doses of *L. hastata* aqueous extracts could also be explained by the lack of toxicity of extract.

Seminal vesicles, prostate, LABC, cowper's glands, coagulant's glands are all androgen-dependent (Lund *et al*., 2004). We have demonstrated that the weight of all these accessory sex organs was decreased significantly. Stroheker *et al*. (2003) studies with dietary

Table 3: Effect of *Leptadenia hastata* aqueous extract on serum biochemical parameters of immature castrated rats after 10 days consecutive treatments

Treatments	No.	Dosage (mg kg^{-1})	Protein	Total cholesterol	Blood sugar	HDL cholesterol	Hematocrit
			----------(mg dL^{-1})----------				(%)
T. propionate	6	0.4	840.32±98.4	60.13±6.40	51.20±2.12	18.13±1.14	35.21±1.40
Distilled water	6	D.W	820.40±79.42	73.40±5.12	48.13±3.20	19.14±1.12	37.40±1.17
Lh_50	6	50	810.6±60.30	75.73±5.21	46.12±5.13	20.13±0.98	36.40±1.29
Lh_100	6	100	860.23±50.14	69.40±7.23	43.20±3.21	21.14±0.62	35.40±1.54
Lh_200	6	200	890.56±48.21	68.60±4.76	40.13±2.14	19.02±1.98	36.24±2.20
Lh_400	6	400	892.79±62.13	71.51±5.87	49.12±2.15	21.14±0.98	35.12±2.40
Lh_800	6	800	843.38±80.60	67.21±4.12	47.31±12.20	21.12±1.98	35.45±1.02

Castrated immature rats were administered with testosterone propionate (0.4 mg/kg/day) by subcutaneous injection, distilled water and different doses of Leptadenia hastata (Lh) (50, 100, 200, 400, 800 mg kg^{-1}) by oral gavage for 10 days. One day after the final treatment, the serum was use for the dosage of some biochemical's parameters. Mean±SE, n = 6

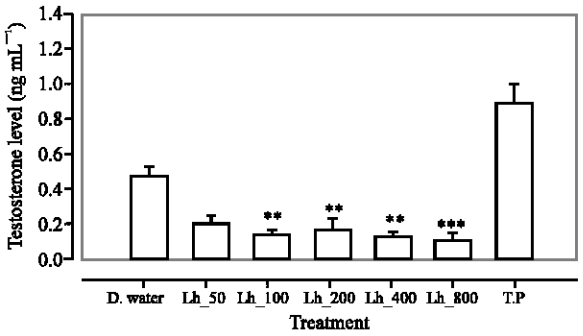

Fig. 2: Effect of *Leptadenia hastata* aqueous extract on testosterone level of immature castrated rats after 10 days consecutive treatments. Serum testosterone levels in immature castrated rats treated with testosterone propionate (TP), distilled water (D.W) and different doses of aqueous extracts of Leptadenia hastata. Castrated male rats were treated daily for 10 consecutive days. Twenty-four hours after the last dosing, serum testosterone levels were measured using an RIA kit. Data are expressed as Mean±SE. (six animals per group). Significantly different from vehicle control (*: $p<0.05$ and **: $p<0.01$)

isoflavones have demonstrated that seminal vesicles were the most sensitive organ to testosterone stimulation. The biological activity of *L. hastata* may be due to one or more of the phytochemicals present in the extract. The reduction in the weight of accessory sex organs reflects interference on testosterone output and antiandrogenic nature of plant extract (Nijar *et al*., 1995). Decreased weights of accessory sex glands indicate the atrophy of glandular tissue, diminished secretary ability and low level of testosterone as these organs are androgen-dependent (Reiter *et al*., 1995).

The protein and fructose concentration in the seminal vesicles and prostate are androgen dependent (Swathy *et al*., 2006; Gonzales and Villena, 2001; Gonzales, 2002). Administration of *L. hastata* extracts caused a significant decrease in the protein and fructose concentration. The present study also showed that *L. hastata* aqueous extracts did not affect the weight of adrenals and the level of total cholesterol, blood sugar, HDL cholesterol and haematocrit. A significant reduction in serum testosterone levels was observed. This low value was correlated with low values of accessory sex organs weight and the protein and fructose concentration in seminal vesicles and

prostate. These results suggested that *L. hastata* aqueous extracts contain some substances with potential antiandrogenic properties which can interfere with androgen signalling by two mechanisms. Firstly the action of substances can be the inhibition of androgen binding to androgen receptor (Andersen *et al*., 2002; Long *et al*., 2003; Mason *et al*., 1987) and secondly the inhibition of enzymes involved in the production of sex hormones, such as, 5α-reductase and aromatase (OECD, 2001).

In conclusion, *L. hastata* aqueous extracts proved to have anti-androgenic activity by Hershberger assay in immature castrated rats. Our studies showed clear effects of *L. hastata* aqueous extracts on accessory sex organs, protein and fructose contents of seminal vesicle and prostate. The serum testosterone level was also decreased after *L. hastata* aqueous extracts treatment. Further studies will be required to clarify the competition between the extracts and TP. These results confirmed the claims of breeders about the infertility of their animals after consumption of leaves and stems of *L. hastata*.

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