

Inductive Effect of the leaf extract, ADHJ, on Ovarian Activity And LH Secretion in Anoestrus Adult Ewe

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ABSTRACT

In the course of the characterisation of the biologic activity of ADHJ (leaf mixture extract of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus*, *Justicia insularis* locally used to regulate the menstrual cycle and to treat cases of infertility in women); 24 Ile de France non-cyclic adult ewes were used for 60 days into two successive experiments. During the first experiment, animals received for 15 days subcutaneous injection of various doses of the aqueous extract of ADHJ (0.0, 0.2, 2 and 20 mg/kg). At the end of the treatment, they were ovariectomised and ovulations detected. Their ovaries were then weighted and dissected for follicles count and size evaluation. The second experiment started four weeks later with previously ovariectomised ewe. They were homogeneously distributed in two blocks of 12 ewes each. Animals in one block were implanted with estradiol. After 15 days of subcutaneous injection of the same previous doses of ADHJ, implants of estradiol were changed between blocks and extract injection resumed 2 weeks later for another 15 consecutive days. During the two phases of treatment, blood samples were collected into heparinised test tubes every 15 minutes for 6 hours (on experimental days: D0, D1, D8 and D15) for plasmatic LH assay. Small follicles (2 – 3 mm) number increased in animals treated with 0.2 mg/kg ADHJ comparatively to control ewes (16.8 ± 4.3 vs. 7.2 ± 1.9 ; $p < 0.05$). Preovulatory follicles (> 6 mm) were only found in ovaries of ewes treated with ADHJ. 25% and 50% of the ewes respectively injected with doses of 2 and 20 mg/kg of ADHJ ovulated (against 0% in Control group). The inductive effect of ADHJ on LH secretion started on the 8th day of treatment whatever the dose of ADHJ administered. These data clearly attest to the indirect effect of ADHJ on follicular growth of non-cyclic ewe via increase on LH production.

Key words: *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus*, *Justicia insularis*, ewe, follicle, LH.

RÉSUMÉ

Dans le cadre de la caractérisation de l'activité biologique d'ADHJ (extrait aqueux du mélange de feuilles d'*Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus*, *Justicia insularis* localement utilisé pour régulariser le cycle menstruel et traiter certaines formes de stérilité chez la femme) ; 24 brebis adultes Ile de France, non cycliques, ont été soumises pendant soixante jours à deux séries d'expérimentation. Dans un premier temps, les animaux ont reçu par voie sous-cutané 4 doses d'extrait (0, 0.2, 2 et 20 mg/kg). A l'issue du traitement, ils ont été ovariectomisés, les ovulations détectées. Leurs ovaires ont ensuite été pesées puis disséquées en vue du dénombrements des follicules. La seconde phase expérimentale a débuté quatre semaines plus tard et a porté sur les brebis précédemment ovariectomisées qui ont été réparties de façon homogène en deux lots de 12 brebis chacun. Les animaux d'un des lots ont reçu un implant d'estradiol. Les doses d'ADHJ utilisées lors de la première série expérimentale ont été maintenues dans chaque lot. A la fin de la première vague de traitement, les implants d'estradiol ont été échangés entre les lots et les injections reprises dans les mêmes conditions 15 jours plus tard. Durant les deux vagues de traitement, des prises sanguins sériés ont eu lieu toutes les 15 minutes durant 6 heures aux jours J0, J1, J8 et J15 en vue du dosage de la LH plasmaticque. Le nombre de petits follicules (2 – 3 mm) est augmenté chez les brebis traitées avec 0.2 mg/kg d'ADHJ ($16,8 \pm 4,3$ vs $7,2 \pm 1,9$; $p < 0,05$). Les follicules préovulatoires (> 6mm) sont uniquement présents dans les ovaires des animaux traité à l'ADHJ. 25% et 50% des brebis traitées respectivement aux doses de 2 et 20 mg/kg d'ADHJ ovulent contre 0% chez le groupe témoin. L'effet stimulateur d'ADHJ sur la sécrétion de LH s'observe à partir du 8^e jour de traitement quelle soit la dose administrée. Ces observations attestent l'effet stimulateur indirect d'ADHJ sur l'activité folliculaire de la brebis non cyclique via l'augmentation de la sécrétion de LH.

Mots clés : *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus*, *Justicia insularis*, brebis, follicules, LH.

INTRODUCTION

The leaf mixture aqueous extract (ADHJ) of *Aloe buettneri* (A), *Dicliptera verticillata* (D), *Hibiscus macranthus* (H), *Justicia insularis* (J) is used by traditional healer of the Western Province of Cameroon to regulate the menstrual cycle and treat cases of infertility in women.

Preliminary studies on immature rats demonstrated its stimulating effect on ovarian folliculogenesis and steroidogenesis (Telefo et al., 1998; 2002). This stimulation may result from a direct effect of chemical components of ADHJ on ovarian cells and/or an indirect effect via gonadotrophin secreted by the pituitary and hypothalamus.

In the present work, a confirmation of the ovarian activity of ADHJ using a new animal model (Ile de France anoestrus ewe) will first be conducted. Then, the indirect implication or not of the hypothalamo-pituitary axis assessed by evaluating the inductive or inhibitory effect of ADHJ on LH secretion in ovariectomised adult ewe.

METHODOLOGY

Animals

Two set of experiments were conducted at the unit for "Physiologie de la Reproduction et du Comportement" experimental unit (INRA – Tours, France) on 50 Ile de France adult ewe aged 2 to 7 years and weighing 40 to 85 Kg. They were housed in standard environmental conditions and were allowed free access to tap water and food.

Preparation of the extract.

The fresh leaves of the four medicinal plants *A. buettneri* A. Berger (Liliaceae), *D. verticillata* G.J.H. Amshoff (Acanthaceae) *H. macranthus* Hochst ex A. Rich (Malvaceae) and *J. insularis* T. Anders (Acanthaceae) were collected in August 2002 in Batoufam and Fontsa - Toula villages (Western Province of Cameroon). They were mixed as described previously (Telefo et al., 2001), washed and dried at 50°C in a ventilated oven for 48 h. The dried mixture was ground in a mortar and 50 g of the leaf mixture powder was used to prepare the aqueous crude extract by decoction, using 1 l of distilled water for 30 min. The extract was then vacuum-filtered, clarified by centrifugation at 3000 rpm for 20 minutes, lyophilised and stored at 4°C. The yield of the lyophilised powder was 25% w/w.

Experimental design

Experiment 1: Animals were first submitted during

two weeks to a cyclicity test. Blood samples were collected 3 times/week into heparinised tubes and assayed for progesterone. Twenty four animals presenting plasmatic progesterone levels inferior or equal to 0.5 ng/ml for 12 consecutive days were selected and distributed into 4 experimental groups of 6 ewes each. Their ovaries were first cauterised to homogenise their ovarian activity. They then received during 14 days through subcutaneous injections various doses of ADHJ (0, 0.2, 2 and 20 mg/kg). At the end of the period of extract administration, ewes were ovariectomised, ovulations detected, ovaries weighted then dissected for follicles count and size measurement.

Experiment 2: It started four weeks later with previously ovariectomised ewe. They were homogeneously distributed in two blocks of 12 ewes each. Animals in one block were implanted with estradiol. After 15 days of subcutaneous injection of the same previous doses of ADHJ, implants of estradiol were changed between blocks and extract injection resumed 2 weeks later for another 15 consecutive days. During the two phases of treatment, blood samples were collected into heparinised test tubes every 15 minutes for 6 hours (on experimental days: D0, D1, D8 and D15) for plasmatic LH assay.

Analytical techniques

Plasma LH concentration was assayed by the radioimmuno assay (RIA) double-antibody method, using antiovine-LH and antirabbit-gammaglobulin antisera and ovine LH standard. The assay sensitivity was 0.1 ng/tube and the intra- and interassay coefficient of variation were 12.6 and 13.0% respectively. Progesterone concentration was assayed by a direct RIA method routinely used in the INRA laboratory of hormonal dosage.

Statistical analysis

Data were analysed using Students' t-test, P-value of 0.05 or less was considered to be significant.

RESULTS AND DISCUSSION

Daily subcutaneous injection of ADHJ aqueous extract for 14 days to anoestrus adult ewes resulted in an increase of 25, 35 and 21% in ovarian weight of those respectively treated with doses of 0.2, 2 and 20 mg/kg comparatively to that of control ewes (Figure 1). This increase in ovarian weight of treated ewes may result in the number of preantral and antral follicles during the process of folliculogenesis.

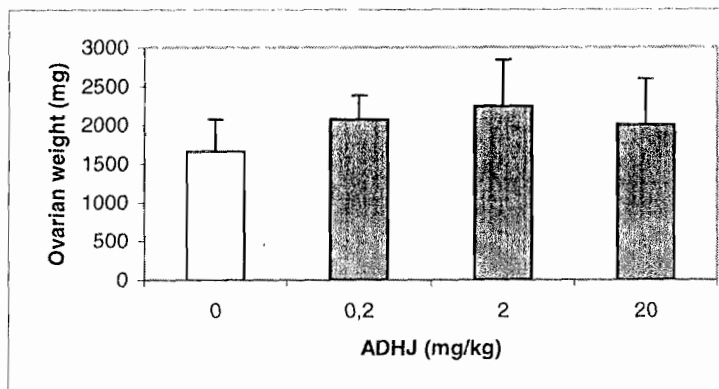


Figure 1: Ovarian weight (mg) of adult anoestrus ewe treated for 14 days with various doses of ADHJ.

This observation is confirmed by data presented in Table 1. There is a significant increased in the number of small follicles in ewes treated with 0.2 mg/kg of ADHJ as compare to that of control group (16.8 ± 4.3 vs. 7.2 ± 1.9 ; $p < 0.05$). More, preovulatory follicles (diameter > 6mm) are only found in ovaries of ewes treated with ADHJ. It is well established that during follicular growth, the selection and preservation of follicles to ovulate is under the direct regulation of gonadotrophins (FSH and LH). FSH induces follicular growth, aromatise activity and prepares follicles for ovulation (Hsueh et al., 1984). Ovulation occurs only after LH peak induction (Erickson et al., 1985).

25% and 50% of the ewes respectively injected with doses of 2 and 20 mg/kg of ADHJ ovulated (against 0% in Control group). Ovulations obtained with this animal model which ovarian cyclic activity is completely arrested during the anoestrus season indicate a probable resumption of pulsatile GnRH and/or LH secretion in treated ewes. The confirmation of this assumption is verified with figure 2 data where pulsatile secretion of LH is observed on the 8th and 15th days of treatment in ewes subcutaneously injected with the various doses of ADHJ while LH plas-matic secretion of the control remained low throughout the experimental period.

Table 1: Number of ovarian follicles, corpus luteum and percentage of ovulation after 14 days of subcutaneous injection of various doses of ADHJ to anoestrus Ile de France adult ewe.

Dose ADHJ (mg/kg)	N	Follicules size					
		2-3 mm	4-5 mm	Preov.foll. (> 6mm)	Total foll .	C.L	% ovulation
0	4	7,50	3,50	0,00	12,25	0,00	0 %
		± 1,91	± 1,73	± 0,00	± 1,89	± 0,00	
0,2	5	16,80*	6,40*	0,60*	24,40***	0,00	0 %
		± 4,27	± 1,51	± 0,54	± 3,91	± 0,00	
2	4	12,25	3,25	0,25	17,75	0,50	25 %
		± 6,85	± 1,26	± 0,50	± 7,36	± 1,00	
20	6	8,17	2,83	0,50*	12,00	1,17	50 %
		± 9,51	± 2,32	± 0,84	± 8,44	± 1,33	

Each data represents Mean ± s.d of 4 to 6 animals per group. Data significantly different to that of control at: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Students' t test)

N represents the number of animals in each experimental group.

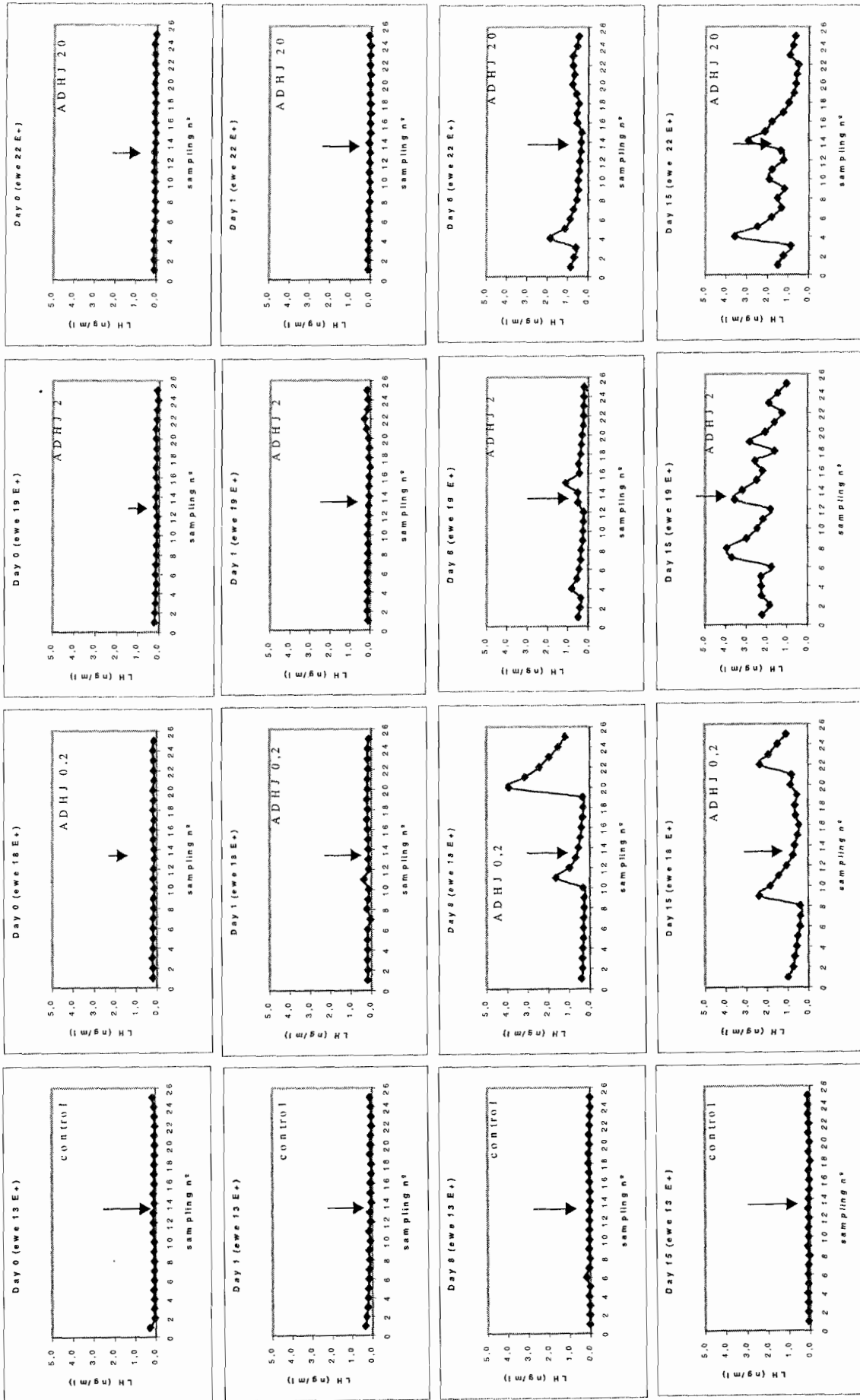


Figure 2: Plasmatic LH concentration (ng/ml) of anoestrus adult ewe ovariectomised during non breeding season and bearing 0.5 cm estradiol implant. Animals receive subcutaneous injections of NaCl 0.9% (control) as well as increasing doses of ADHJ (0.2, 2, 20 mg/kg). On days 0 (during which all animals receive NaCl 0.9%), 1, 8 and 15 of treatment, serial blood samples are collected every 15 minutes for 6 hours (sampling n° 1 to 25). Product injections takes place just after the 13th sampling (vertical arrow on top of each graph).

CONCLUSION

Besides infertile woman and immature female rat (Telefo, 1998), results of this study on the characterisation of ADHJ ovarian activity in anoestrus adult ewe clearly attest its inductive effect on ovarian folliculogenesis in mammals. This induction may include the hypothalamo-pituitary axis through increase of plasmatic LH level.

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REFERENCES

- Erickson G.F., Magofin D.A., Dyer C.A. and Hofeditz C.**(1985). The ovarian androgen producing cells: A review of structure/function relationship. *Endocrinology Review*, 6: 317 – 399.
- Hsueh A.J.W., Adashi E.Y., Jones P.B.C. and Welsh T.H.**(1984) Hormonal regulation of differentiation of cultured ovarian granulosa cells. *Endocrinology Review*, 5: 76 – 127.
- Telefo P.B., Moundipa F.P., Tchouanguép M.F., Tchana A.** (1998). Effect of aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera verticillata* on some physiological and biochemical parameters of reproduction in immature female rats. *Journal of Ethnopharmacology* Vol 63(3): 193-200.
- Telefo P.B.** (1998). Contribution à l'étude des plantes médicinales du Cameroun : Influence de l'extrait aqueux du mélange de feuilles d'*Aloe buettneri* A. Berger (LILIACEES), *Dicliptera verticillata* G.J.H. Amshoff (ACANTHACEES), *Hibiscus macranthus* Hoscht ex. A. Rich (MALVACEES) et *Justicia insularis* T. Anders (ACANTHACEES) sur certains paramètres biochimiques et physiologiques de la fonction de la reproduction chez la ratte. Thèse de Doctorat 3^e Cycle-Biochimie, Université de Yaoundé I.. 159p.
- Telefo P.B., Moundipa F.P., Tchouanguép M.F.** (2001). Influence de l'extrait aqueux de feuilles d'*Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, et *Dicliptera verticillata* sur la fertilité et quelques paramètres biochimiques de la reproduction chez la rate. *J. Cam. Acad. Sc.* 1(3) : 144 – 150.
- Telefo P.B., Moundipa F.P., Tchouanguép M.F.** (2002). Oestrogenicity and effect on hepatic metabolism of the aqueous extract of the leaf mixture of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis*. *Fitoterapia*, 73 : 472 – 478.