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Research Article
Potentialisation of Pregnant Mare Serum Gonadotropin Inducing Effect on Ovarian Follicles Growth by the Aqueous Extract of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis* Leaves in Immature Rats

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Abstract

Background and Objective: *Aloe buettneri* (A), *Dicliptera verticillata* (D), *Hibiscus macranthus* (H) and *Justicia insularis* (J) are medicinal plants whose leaf mixture aqueous extract (ADHJ) is locally used to regulate the menstrual cycle and to treat infertility in women. This study was designed to evaluate the ovarian follicles growth inducing potential of ADHJ in Pregnant Mare Serum Gonadotropin (PMSG)-primed immature female rats. **Methodology:** Various dose (0.0004-1.6 IU per rat) of pregnant mare serum gonadotropin was subcutaneously administered to immature female rats for 5 consecutive days in view to selecting the highest dose, which doesn't induce significant increase of ovarian and uterine weights. The selected PMSG dose was then co-administered with various doses (0, 12.5, 50 and 100 mg kg^{-1}) of ADHJ for 3, 5, 7 and 10 days. Half of animals in each subgroup was further injected, the day after each experimental period duration with 10 IU of human chorionic gonadotropin (hCG) and the remaining was sacrificed and some parameters of ovarian function were recorded. The animals injected with hCG were subjected to the same treatment 48 h later. **Results:** The selected PMSG dose was 0. 01 IU per rat. The ovarian weight of control animals (PMSG) started to decrease significantly on the 7th day of treatment, while the one of animals co-treated with PMSG and the doses of 12.5 and 100 mg kg^{-1} of ADHJ started 2 days earlier. An increase ($p<0.05$) in the percentage of vaginal opening and the number of haemorrhagic points (12.5 mg kg^{-1}) was noticed after 5 days of treatment. A significant increase ($p<0.01$) in the numbers of tertiary follicles was observed after 3, 5 and 7 days of treatment in PMSG/ADHJ-treated rats comparatively to the PMSG control groups. **Conclusion:** These results provide evidence that ADHJ potentialises the PMSG-inducing effect on folliculogenesis in PMSG-primed immature rats which could be a suitable experimental model for the evaluation of gonadotropin-like compounds.

Key words: Medicinal plants, extract, potentialisation effect, ovaries, follicle, infertility

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In developing countries, up to 90% of the population rely on the use of medicinal plants for their primary health care[1,2]. Unfortunately this precious resource is threatened in most African countries due to their rapid urbanization, accompanied by severe economic decline and worsening health conditions (malaria, HIV/AIDS and infertility)[3]. One of the measures that should immediately be taken to counter this trend would be to make health care affordable for all through the valorisation of our traditional knowledge on medicinal plants. *Aloe buettneri* (Liliaceae), *Dicliptera verticillata* (Acanthaceae), *Hibiscus macranthus* (Malvaceae) and *Justicia insularis* (Acanthaceae) are herbaceous plants widely distributed in tropical area of Africa[4]. Ethnopharmacological studies have demonstrated their implication for the treatment of dysmenorhea, regulation of menstrual cycle and functional sterility in women[5-7]. Previous studies on the aqueous extract of the leaf mixture of these plants revealed its increasing effect, after 20 days of oral administration on uterine and ovarian weights as well as serum estradiol[6]. Others studies showed the inducing potential of this extract on *in vitro* production of oestradiol and on enhancement of ovulation rate in anoestrus Ile-de-France ewes after 15 days of sub cutaneous administration[8,9]. These results may be related to the gonadotropin-like compounds present in the plant mixture. Gonadotropin play a pivotal role in ovarian follicles growth and ovulation[10,11]. Induction of the precocious puberty in immature female rats by exogenous gonadotropin treatment has been the subject of many studies[12,13]. Oestrogens play an important role in this induction by exerting a stimulatory feedback action on gonadotropin-releasing hormone production. This precocity in sexual maturation of female rat is clearly dose dependent at a certain age (16-23 days) and takes place within 1-5 days after gonadotropin injection[14-16]. Given the gonadotropin-like effect of the plant mixture, treatment of immature animals initially primed with a dose of PMSG with no significant effect on their ovarian weight would conduct either to an increase in the ovarian weight or no change. The first alternative could only be the result of gonadotropin-like compounds present in the extract. Moreover, the priming of the immature rat with PMSG would advance their maturating age to a stage where only short-duration of treatment (3, 5, 7 or 10 days) with any exogenous stimulating compound would be needed to attend the puberty onset. This approach could be useful for the confirmation of the FSH-like effect of the ADHJ extract. As *in vivo* experimental protocol for the study of medicinal plants used to cure some forms of infertility take long time[6,17,18], this opproach will also help in future analysis of several other medicinal plants containing gonatotropin-like compounds knowing that the usual duration of the experiment will be shortened.

MATERIALS AND METHODS

Preparation of PMSG and hCG solutions: The PMSG lyophilisat (Prospec protein specialist, CAT: HOR-272) was diluted in NaCl 0.9% to obtain an initial solution (200 IU mL^{-1}). Successive dilutions of this solution was done to obtain the different solutions (8, 4, 1, 0.5, 0.1, 0.05, 0.01 and 0.002 IU mL^{-1}) used in this study. The hCG lyophilisat (Prospec protein specialist, CAT: HOR-250) was diluted in NaCl 0.9% to obtain a solution of 50 IU mL^{-1} used in this study.

Plant materials and preparation of extract: The aqueous extract used in this study was prepared from fresh leaves of *Aloe buettneri* (A), *Dicliptera verticillata* (D), *Hibiscus macranthus* (H) and *Justicia insularis* (J). The fresh leaves were collected in Batoufam village (Western region of Cameroon) in May, 2010. The plants were identified at the Cameroon National Herbarium (Yaounde) by comparing this present study samples with available deposited specimen having voucher numbers 52232, 34997, 41881 and 20387, respectively. They were washed, dried for 2 weeks at room temperature in the shade and finely crushed using an electric grinder. The mixture (ADHJ) was prepared by mixing 23, 20, 15 and 42 g of leaf powder from *A. buettneri*, *D. verticillata*, *H. macranthus* and *J. insularis*, respectively. The mixed powder (100 g) was infused in 1 L of hot water (95°C) and subsequently boiled for 30 min (as described by the traditional healer). The ADHJ extract at 22 mg mL^{-1} (initial concentration) was obtained after filtration of the solution prepared above and evaporation of 1 mL of the filtrate in a ventilated oven. Concentrations of 20, 10 and 2.5 mg mL^{-1} were prepared from the above extract for this study.

Phytochemical analysis: Phytochemical tests for detection of alkaloids, flavonoids, glycosides, coumarins, polyphenols, triterpenoids, steroids and quinones (Table 1) were separately carried out on the dried leaf extract of *A. buettneri*, *D. verticillata*, *H. macranthus*, *J. insularis* and their mixture[19,20].

Experimental animals: Female immature rats of the Wistar strain, bred in the animal house of Biochemistry Department (University of Dschang, Cameroon) were used. At the beginning of each experiment, the animals were 21-23 days old, weighing 30-35 g. They were housed under uniform

Table 1: Phytochemical analysis of leaf extracts of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus*, *Justicia insularis* and their mixture (ADHJ)

Medicinal plants	Alkaloids	Coumarins	Glycosides	Flavonoids	Anthraquinones	Polyphenols or tanins	Triterpenoids/steroids
Aloe buettneri	-	-	+	-	+	+	±/±
Dicliptera verticillata	+	-	+	+	+	+	±/±
Hibiscus macranthus	+	-	+	+	-	+	±/±
Justicia insularis	+	-	+	+	-	+	±/±
Mixture (ADHJ)	+	-	+	+	±	+	±/±

+: Present, ±: More or less and −: Absent

husbandry conditions of light (12 h cycle) and temperature (22±2°C) and fed with standard laboratory diet and tap water *ad libitum*. Experimental protocols used in this study strictly conformed with the internationally accepted standard ethical guideliness for laboratory animal use and care as described in the European community guideliness, EEC directive 86/609/EEC of the 24th November, 1986[21].

PMSG dose-response effects on ovarian and uterine weights: During the first phase of this study, the highest dose of PMSG without effect on ovarian and uterine weights was determined. Thus, 54 animals were subdivided into 9 experimental groups of 6 animals each and subcutaneously injected for 5 consecutive days with the PMSG doses of 0, 0.0004, 0.002, 0.01, 0.02, 0.1, 0.2, 0.8 and 1.6 IU/rat/day. The various amount of PMSG was dissolved in 0.2 mL of solvent prior to their injection. The animals were weighed daily and check for vaginal opening. Twenty four hours after the last injection, the animals were sacrificed by intra-abdominal injection of thiopental sodium (6 mg mL^{-1}, 30 mg kg^{-1}). Their ovaries and uteri were carefully removed from the body cavity, cleaned of adherent tissue, weighed to the nearest 0.1 mg and the number of haemorrhagic points and/or corpora lutea on the surface of the ovaries were counted.

Potentialisation of PMSG-inducing effect on ovarian follicles growth by ADHJ: To evaluate the minimal duration of treatment necessary for ADHJ to potentialise the inducing effect of PMSG on ovarian function, 160 animals were subdivided into four main experimental groups of 40 animals each. This distribution correspond to different period of treatment (3, 5, 7 and 10 days). Each main group was equally subdivided into 4 subgroups and treated with the highest dose of PMSG without effect on both ovarian and uterine weights (selected during the first phase of the study) and then with various doses of ADHJ (0, 12.5, 50 and 100 mg kg^{-1}). The pregnant mare serum gonadotropin solution was injected, as previously indicated prior to the oral administration of ADHJ. In the morning of the day following each last period of treatment, half of the animal in each subgroup was injected with 10 IU of hCG, the remaining half was sacrificed and the same reproductive organs were excised and treated as previously indicated. In addition, the right ovary of each rat was fixed in Bouin's liquid and conserved for 2 days for histological analysis and counting of the follicles at different growing stages. The animals injected with hCG were sacrificed 48 h later and the number of haemorrhagic points and/or corpora lutea on the surface of their ovaries were counted.

Ovarian histology: The right ovary of each rat was removed from Bouin's liquid and successively dehydrated with ethanol (70, 80, 90 and 100%) and after with xylene (100%). Each tissue block was then embedded in paraffin wax, serially sectioned at 7 µm thickness every 60 µm using Leica rotary microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Deutschland) and strips of sections were gently lowered into the surface of a warm water bath at 40°C. The floated sections were mounted on microscopic slides and put in an oven maintained at 60°C for 30-40 min to fix the tissue firmly on the slide. They were colored with haematoxylin and eosin. Following the staining stage, all sections were examined microscopically at 40x magnification and the mean number of primary, secondary and tertiary follicles in the ovarian cortex was calculated for each specimen.

Statistical analysis: The analysis of variance was used to determine the sources of variation (or to detect the significance of treatment) in mean values of number of follicles, ovarian and uterine weights. Student- Newman-Keuls and Fisher LSD tests were used for comparison between means whenever experimental factors were significant for ANOVA. The percentages of rats showing vaginal opening were compared using the chi-square and Fisher's exact probability tests. The number of haemorrhagic points and corpus luteum were analysed with Kruskall-Wallis (for treatment effects) and Mann-Withney (for mean comparisons) tests.

RESULTS

PMSG dose-response effect on relative ovarian and uterine weights: The variations of ovarian and uterine weights of immature rats treated with increasing doses of PMSG are shown in Fig. 1. There were no significant variation of these parameters at doses below 0.02 IU per rat comparatively to the control group. However, PMSG injection at 0.02 IU per rat and doses above led to a significant increase ($p<0.01$) of these parameters.

Effect of PMSG on vaginal opening: Concerning vaginal opening (Table 2), 2 rats injected with 1.6 IU of PMSG per day showed vaginal opening 4 days after the beginning of the treatment. At the end of the treatment period, all the animals injected with 0.1 IU of PMSG and the doses above showed vaginal opening, while those treated with the doses of PMSG less than 0.1 IU showed any vaginal opening.

Potentialisation of PMSG-inducing effect on ovarian follicles growth by ADHJ

Effect on relative ovarian and uterine weights: Figure 2 illustrates the variation of ovarian and uterine weights after the various treatment administrated to immature female rats. The rats treated with 50 and 100 mg kg^{-1} of extract showed an irregular variation of the ovarian weight as the treatment period was prolonged. Nevertheless with the control and the 12.5 mg kg^{-1} treated groups, a linear decrease of the ovarian weight was noticed as from the 7th and 5th days of treatment, respectively. Relative to the dose-response effect, significant decrease ($p<0.05$) of the ovarian weight of ADHJ-treated animals was noted after 5 days of experimetation, irrespective of the administered dose (Fig 2a). There was a linear increase of the relative uterine weight of control animal group. However, this trend was not observed in ADHJ-treated animals irrespective of the administered dose. A general decreasing dose-response effect of the extract was observed on uterine weight from day 3-7 of treatment. This decrease was pronounced ($p<0.05$) on the 5th day of treatment with all ADHJ-treated animals (Fig 2b).

Effect on the percentage of vaginal opening: Vaginal opening in control animal was observed only after 7 days of treatment, while in all ADHJ-treated groups it occurred 2 days earlier (Fig. 3). The dose-response effect was observed from day 5-10 of treatment with significantly increasing effect ($p<0.05$) after 5 days.

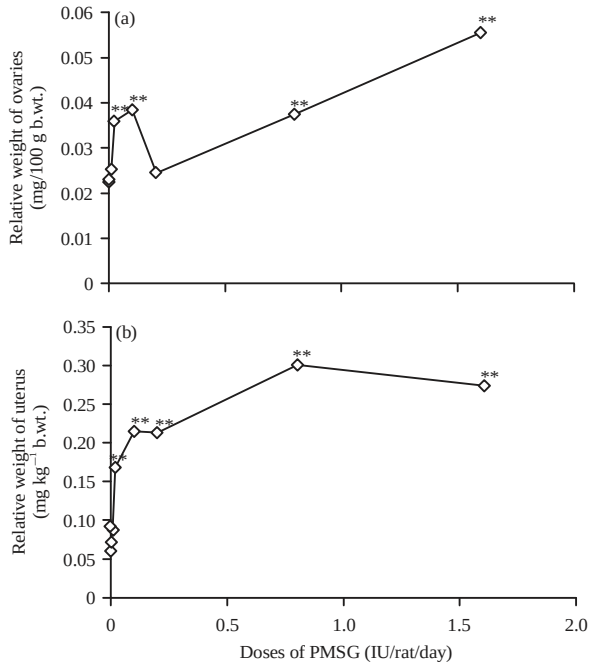

Fig. 1(a-b): Variation of the relative (a) Ovarian and (b) Uterine weights with different doses of PMSG, each point represents Mean±SEM of 6 animals per group, **Values statistically different from that of the control group at $p<0.01$ (ANOVA and Fisher LSD)

Table 2: Percentages of animal showing vaginal opening after treatment with different doses of PMSG

	Dose of PMSG (IU/rat/ day)																										
	0			0.0004			0.002			0.01			0.02			0.1			0.2			0.8			1.6		
Treatment duration (days)	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%	Na	Nb	%
1	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0
2	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0
3	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0
4	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	2	33.33
5	6	0	0	6	0	0	6	0	0	6	0	0	6	0	0	6	6	100	6	6	100	6	6	100	6	6	100

Na: No. of rats per group, Nb: No. of rats showing vaginal opening and %: Percentage of rats showing vaginal opening

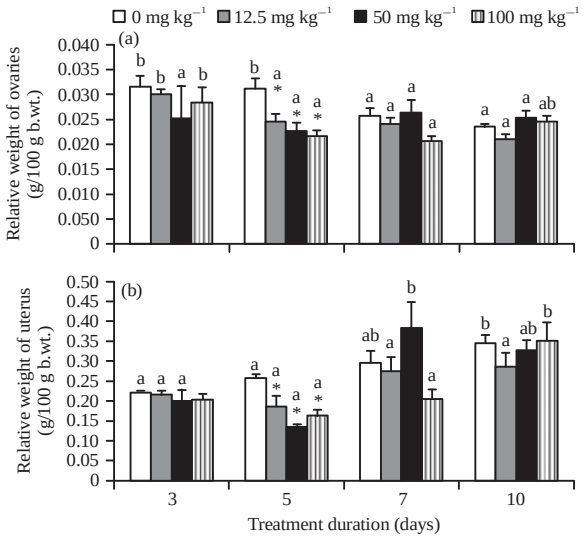

Fig. 2(a-b): Effect of ADHJ on the relative weight of the (a) Ovaries and (b) Uteri for a given dose, histograms carrying the same letter are not significantly different (Student-Newman-Keuls test at $p \leq 0.05$), *Values significantly different at $p<0.05$ (ANOVA and Fisher LSD) from those of the control group, each histogram represents the Mean±SEM of the values for 5 animals

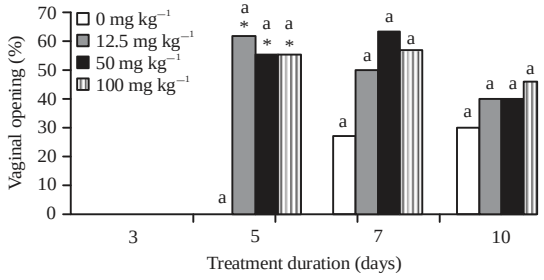

Fig. 3: Effect of oral administration of ADHJ on the vaginal opening, for a given dose, histograms carrying the same letter are not significantly different (Student-Newman-Keuls test at $p \leq 0.05$), *Values significantly different at $p<0.05$ from those of the control (Chi-square and Fisher exact tests), each data represents the mean of 5 animals

Effect on the follicle count and the number of haemorrhagic points: Whatever the dose and the treatment duration period, the follicles at different growing stages (primary, secondary and tertiary) are present in a variable numbers. The control as well as ADHJ-treated animals showed an irregular variation in their number of primary and secondary follicles as the treatment period was prolonged

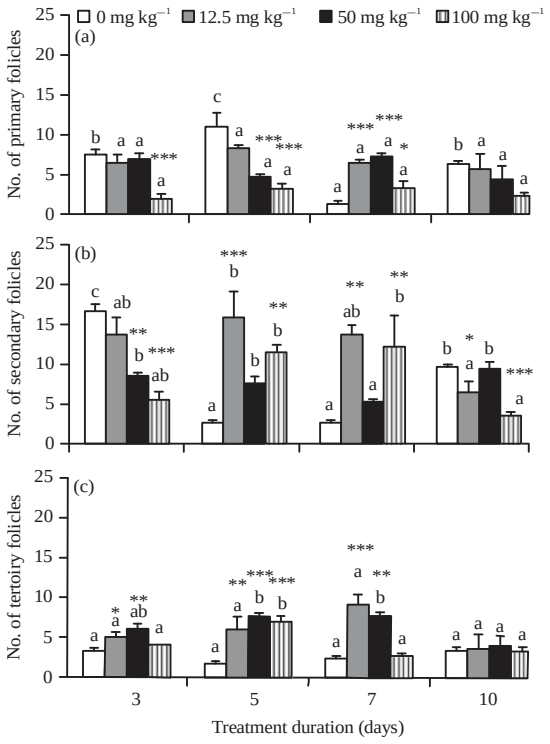

Fig. 4(a-c): Effect of ADHJ administration on number of (a) Primary follicles, (b) Secondary follicles and (c) Tertiary follicles, for a given dose, histograms carrying the same letter are not significantly different (Student-Newman-Keuls test at $p \leq 0.05$), *,**,***Values significantly different at $p<0.05$, $p<0.01$, $p<0.001$ (Kruskall-Wallis and Mann-Withney tests) from those of control group respectively, each histogram represents the Mean±SEM of the values for 5 animals

(Fig. 4a, b). Nevertheless, this trend was not followed with the number of tertiary follicles. Indeed, no change was obtained in the control and the 12.5 mg kg^{-1} treated groups, while a significant increase ($p<0.05$) was noticed on the 5th and 7th days for the 50 mg kg^{-1} treated groups and on the 5th day for the 100 mg kg^{-1} one (Fig. 4c). With the exception of animals treated during 7 days with increasing doses of ADHJ for which the number of primary follicles significantly increased ($p<0.001$), the opposite effect with the other treatment duration was observed. This decreasing dose-response effect was more pronounced ($p<0.001$) in animals treated for 5 days with 50 and 100 mg kg^{-1} of plants extract comparatively to the control (Fig. 4a). This downward trend was also noted in the number of secondary follicles of animals treated for 3 and 10 days, while a significant ($p<0.01$) increase was obtained after 5 and 7 days with the

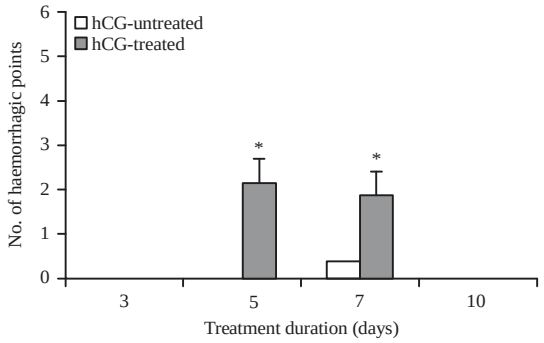

Fig. 5: Effect of hCG administration on number of haemorrhagic points, *Values significantly different at p<0.05 (Kruskall-Wallis and Mann-Withney tests) from those of control group (hCG-untreated groups), each histogram represents the Mean±SEM of the values for 5 animals

12.5 and 100 mg kg^{-1} ADHJ-treated animals (Fig. 4b). A significant (p<0.01, p<0.001) increase in the number of tertiary follicles was observed after 3, 5 and 7 days of treatment with doses of 12. 5 and 50 mg kg^{-1} of ADHJ. The extension of the treatment period to 10 days was without effect (Fig. 4c).

Irrespective of the administered dose of hCG (Fig. 5) or ADHJ (Fig. 6), no haemorrhagic points was noticed after 3 or 10 days of treatment. Independently of the dose of ADHJ administered, the number of haemorrhagic points was significantly increased (p<0.05) in hCG-treated animals as compared to the hCG-untreated ones. Moreover, hCG-untreated animals presenting haemorrhagic points were observed in all ADHJ-treated groups after 7 days of treatment (Fig. 6a). The injection of hCG induced the appearance of haemorrhagic points on the 5th day of treatment and increased their number in all ADHJ-treated animals. In 12.5 mg kg^{-1} treated group, a significant increase of this parameter was observed after 5 days of treatment (Fig. 6b).

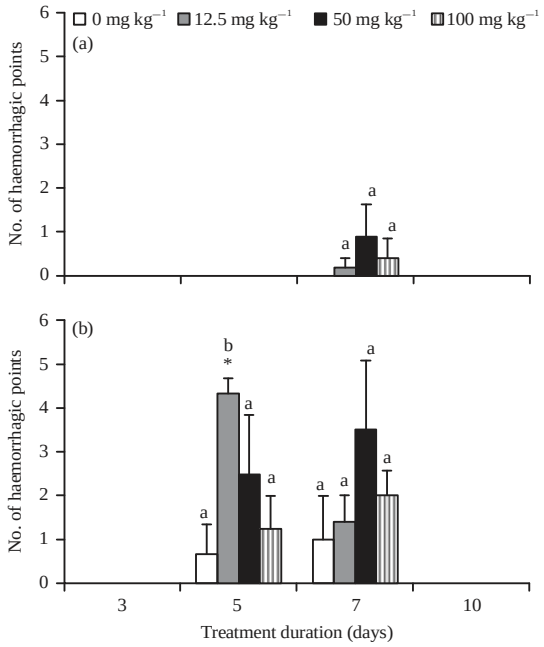

Fig. 6(a-b): Effect of ADHJ administration on number of haemorrhagic points of (a) hCG-untreated rats and (b) hCG-treated rats, for a given dose, histograms carrying the same letter are not significantly different (Student-Newman-Keuls test at p≤0.05), *Values significantly different at p<0.05 (Kruskall-Wallis and Mann-Withney tests) from those of control group, each histogram represents the Mean±SEM of the values for 5 animals

DISCUSSION

The present study was undertaken to find the potentialisation of Pregnant Mare Serum Gonadotropin (PMSG) inducing effect on ovarian follicles growth by the extract from the leaves mixture of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis* in immature rats. Subcutaneous administration of PMSG to immature female rats during 5 days led to a significant increase in ovarian and uterine weights and stimulation of vaginal opening of animals treated with doses greater than 0.01 IU per rat. The PMSG is a natural glycoprotein, it mainly has a Follicle Stimulating Hormone (FSH)-like and a weak Luteinising Hormone (LH)-like activities[22]. In response to its FSH-like activity, several ovarian follicles in immature female rat model mature through the fixation of FSH to its granulasa cell receptors, thus regulating the expression of some genes related to proliferation and steroidogenesis of ovarian cells[23]. The resulting increase of oestrogen level in circulation stimulates the hypothalamus to produce gonadotropin-releasing hormone which in turn activates the anterior pituitary to release FSH and LH. The overall hormonal signalization culminates in the opening and cornification of the vagina and to the increase in ovarian and uterine weights[24]. This mechanism may clearly explain the increase of the ovarian and uterine weights observed at the doses of PMSG above 0.01 IU per rat. Given the gonadotropin-like effect of the plant mixture[8,9], treatment for a short duration of immature animals initially primed with a dose of PMSG with no significant effect on their ovarian and uterine weights would also conduct to an increase of these parameters. The co-administration of 0.01 IU per rat of PMSG (chosen as the

highest dose of PMSG without effect on animal's ovarian and uterine weights) and the extract (12.5 and 100 mg kg^{-1}) to immature female rats led to a decrease of their ovarian weights. These results are contradictory not only to our hypothesis but also to the results obtained by Telefo *et al.*[6]. Indeed, these researcher found that ovarian weight of immature rats treated during different experimental periods by various doses of ADHJ extract alone were significantly increased at different rates depending on the treatment period. This difference between the 2 results may be imputed to PMSG since, ovarian weight of the control group of the present study (PMSG alone) also decreases, but later on the 7th day of treatment. However, ovarian weights of the animals treated with the ADHJ extract in addition to the PMSG decrease early on the 5th day. This suggests the presence of molecules acting as FSH in ADHJ extract. Additional amount of PMSG injected to control animals after the 5th day on the one hand and the cumulated effect of PMSG and FSH-like compounds present in ADHJ extract on the other hand probably affected the FSH receptors at the level of the granulosa cells through a down regulation mechanism. *In vitro* studies have shown that high concentrations of FSH (>10 ng mL^{-1}) in rats caused down regulation of its own receptors[25,26].

Concomitantly, uterine weights of control and ADHJ-treated rats (50 and 100 mg kg^{-1}) increased as treatment period was prolonged. It is well known that stimulation of uterine growth is under the control of oestrogen, whose synthesis depends on gonadotropin secretion[27]. Thus, this uterine weight increase may be either related to the combined effect of PMSG and FSH-like compounds or to the direct effect of phytoestrogens present in ADHJ. Phytoestrogens are a diverse group of polyphenolic non-steroidal plant compounds that can bind to human oestrogen receptors and exert the characteristics of endogenous steroidal oestrogens[28]. The phytochemical screening of the individual plants and the mixture revealed the presence of polyphenolic compounds. The significant decrease in uterine weight of treated rats for 5 days may be linked to the action of both oestrogens produced in response to gonadotropin activity and oestrogenic compounds present in the extract. The increase in serum estradiol level induces the down-regulation effect at the level of the pituitary gland, leading to a decrease in FSH and LH secretions. Indeed, oestrogens at high dosage can fix to their receptors located on the hypothalamic tissue and reduce the pulsatory release of GnRH leading to a decrease in LH and FSH synthesis and release by the anterior pituitary[24,29]. The decrease of ovarian weight noticed with all ADHJ doses might also be a consequence of these down regulation effects. The FSH-like activity of the plants aqueous extract could be completed with its effect on vaginal opening observed early on day 5 with all ADHJ doses. The opening of the vagina on attainment of the pubertal age of rats results from the increase in the secretion of oestrogens by ovarian follicles[30]. Ovarian folliculogenesis is a complex series of events in which a recruited primordial follicles grow and develop into a specialized graafian follicles with the potential to release their oocytes into the oviduct. The second interval of follicullar growth (from preantral to antral preovulatory follicle) is strictly dependent on the presence of the follicle stimulating hormone[31]. The ovulation occurs prior to the Luteneising Hormone (LH) peak and leads to the lysis of graafian follicle membrane and the release of oocytes. The lysis is followed by a stratum transformation of broken follicles into haemorrhagic points[32], visible on the surface of the ovaries. The presence of haemorrhagic points on the ovarian surface gives evidence that the follicles attained the terminal stage of growth, while their absence reveals the non-attainment of the terminal maturation of the follicles (absence of graafian follicles). In this study, 10 IU hCG were injected to animals treated with PMSG and increasing doses of ADHJ aqueous extract in order to simulate the LH peak required for ovulation. Human chorionic gonadotropin (hCG) have biological effects which are similar to those of LH and single injection of low dose of this hormone after completed maturation of follicles leads to ovulation between 34 and 46 h[33]. Haemorrhagic points are noticed exclusively after 5 and 7 days of treatment (Fig. 6b). Their presences in control groups (PMSG alone) attest the FSH activity of this hormone which may induce follicle maturation (Fig. 7a). The slight increase in the number of haemorrhagic points in control animals on day 7 is probably due to the additive effect of FSH in response to more PMSG injection. The significant increase in the number of tertiary follicles (Fig. 4c, 7b) and haemorrhagic points in PMSG/ADHJ treated rats may result from the additive FSH-like effect of some compounds of the ADHJ extract and may clearly show the potentialisation of PMSG inductive effect of the extract of these plants on ovarian folliculogenesis.

The absence of haemorrhagic points on the 3rd and 10th day of treatment reveals on the one hand, the insufficient follicular growth (absence of graafian follicles) and on the other hand, a precocious follicular growth leading to ovulation early before the injection of 10 IU hCG. This suggestion is not only supported by the 2 corpora lutea

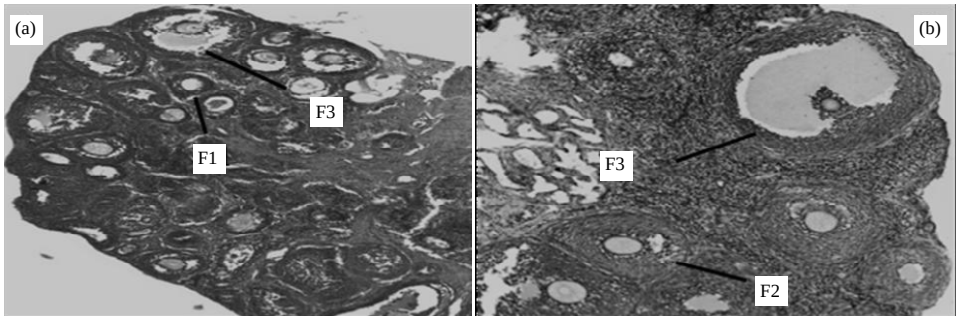

Fig. 7(a-b): Photography of some ovarian cortical regions showing the different stages of growing follicles in (a) Control and (b) ADHJ-treated rats, F1: Primary follicle, F2: Secondary follicle, F3: Antral follicle, Magnification: 40x

recorded in hCG-treated rats for 10 days at 100 mg kg^{-1} of extract, but also by the appearance of haemorrhagic points on the 7th day of treatment in ADHJ-untreated animals (Fig. 6a).

CONCLUSION

Globally, this study has confirmed the presence of gonadodotropin-like compounds in ADHJ, acting in synergy with PMSG to allow a precocious puberty after 5 days of treatment. Thus, the PMSG-primed immature rats could be a suitable experimental model to evaluate gonadotropin-like compounds. However, since a decrease (propably due to the down regulation effect) rather than an increase in reproductive organ's weight was observed, further studies with doses of PMSG and/or plants extract less than those used during this study will be necessary. Moreover, the dosage of serum and ovarian FSH and estradiol would also be required for the clarification of this mechanism.

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