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Restorative effect of *Justicia insularis* aqueous extract on Testosterone propionate induced infertility in female wistar strain rats

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Abstract:

Aqueous extract of *Justicia insularis* (AEJi), an herbaceous and perennial plant widely distributed in tropical Africa stimulates folliculogenesis in immature female rats, exhibits FSH-like and/or estrogenic effects and improves *in vitro* follicular survival and activation of ovine primordial follicles. This study was designed to evaluate the potentiality of AEJi on fertility restoration in testosterone propionate (TP)-induced infertile female albinowistar rats. Hyperandrogenic infertility were induced in 30 days-old female albino wistar rats by daily subcutaneous injection of TP (10 mg/kg) for 21 days. From the 16th day, distilled water, Cyproterone acetate or AEJi (50 mg/kg and 100 mg/kg) were added by oral route for 20 days. TP-treated rats exhibited delayed vaginal openings, irregularity in the estrous cycle phases and infertility. Upon treatment with AEJi, remarkable changes were found in animals through normalizing the estrous cycle and restoring fertility and mostly in animals receiving 50 mg/kg of AEJi in which the values were quite similar to the vehicle control group through statistical

analysis ($p<0.05$). These effects prove that AEJi is a potent antidote for restoring fertility in TP-induced infertile female rats. Thus could be a good candidate for the treatment of infertility due to hyperandrogenism.

Key words: AEJi, estrous cycle, infertility, restorative effect, testosterone propionate.

1. Introduction

Infertility is a common problem experienced by many couples. Approximately 15 to 20% of couples at the reproductive age are infertile. Indeed, the responsibility in the situation can equally be attributed to both male and female factors (Agarwal and Allamaneni, 2004); though 25% of responsibility is attributed to female (Larsen, 2000). Those female factors occur at different levels of the reproductive process, from ovulation to implantation. As a result, female infertility can be due to anatomic, immunological, and/or genetic problems or endocrine disorders (Daar and Merali, 2002). Hyperandrogenemia in women is an endocrine disorder leading to abnormal menstrual cycles and ovulation disorders characterized by amenorrhea (several months) and/or out of menses bleedings (Taieb and Lachgar, 2012). It also impedes oocyte maturation and release and consequently reduces fertility.

Recently, the research is more focusing on medicinal plants all over the world and there are many evidential results showing immense potentials of medicinal plants used in various traditional systems (Akindahunsi and Olaleye, 2003). Ethnopharmacological studies have suggested that medicinal plants produce secondary metabolites that may affect reproduction in humans and various mammals (Mbemya *et al.* 2017[a]). *Justicia insularis* is one of these potent plants which have already been revealed by researchers. Previous studies carried out by Telefo *et al.* (2004, 2002, 2001, 1998) on the aqueous extract of leaves mixture of *Aloe buettneri, Dicliptera verticillata, Hibiscus macranthus* and *Justiciainsularis* demonstrated its inductive effects on ovarian steroidogenesis and folliculogenesis. On the other hand, it has been demonstrated that the administration of aqueous extract of *Justicia insularis* for 20 days to immature female rats stimulates folliculogenesis (Telefo *et al.,* 2012). Besides, *Justicia insularis* has FSH-like and/or estrogenic effect (Goka *et al.,* 2017) and improves *in vitro* follicular survival and activation of ovine primordial follicles (Mbemya *et al.,* 2017[b]). This implies that certain chemical constituents of *Justicia insularis* might act either at the central level on the synthesis and secretion of GnRH or directly on its pituitary receptors or finally, at the ovarian level on the gonadotrophins receptors and may present a restoring effect on certain natural or induced forms of infertility.

With regard to the effects of *Justicia insularis* on the estrous cycle regulation and folliculogenesis, this plant may be effective for managing infertilities associated to sexual cycle disorders and ovarian dysfunctions from diverse origins such as hyperandrogeny.

Despite the aforementioned properties of AEJi, to the best of our knowledge, there is no study investigating its effects on fertility restoration in hyperandrogeny-induced infertility in wistar female rats. Thus, this study was conducted to investigate the restoring effect of AEJi in testosterone propionate-induced infertile female rats to contribute to its valorization in the treatment of female infertility.

2. Materials and methods

Experimental protocols used in this study strictly is conformed to the internationally accepted standard ethical guidelines for laboratory animals' use and care as described in the European Community guidelines, EEC directive 86/609/EEC of the 24th November 1986 (EEC, 1986).

2.1.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 the experiment, animals were 30 days old, weighing 55 to 60 g. They were housed under natural and uniform husbandry conditions of light (12h cycle) and temperature (22 ± 2 °C) and fed with standard laboratory diet and tap water *ad libitum.*

2.2.Plant material to extract preparation

Fresh leaves of *Justicia insularis* previously identified at the national herbarium of Cameroon under voucher specimen code 34997 (Telefo *et al.,* 1998) were collected in Batoufam village (western region of Cameroon) in June 2019. These leaves were washed, dried for 2 weeks at room temperature in the shade and finely crushed using electric grinder. One hundred (100) grams of the plant powder were infused in 1 L of hot water (95°C) and subsequently boiled for 30 minutes according to the protocol of Telefo *et al.* (2012). After cooling, the extract was filtered and evaporated in a ventilated oven at 45°C for 48 hours and the extract obtained was diluted in distilled water to the appropriate concentrations corresponding to doses of 50 mg/kg and 100 mg/kg.

The doses of *Justicia insularis* used through the study were defined according to previous studies performed by Goka *et al.* 2017 and Telefo *et al.* 2012.

2.3.Testosterone propionate and Cyproterone acetate preparation

One (1) ml of Testosterone propionate (100 mg/ml) (Medi Tech Laboratory) was diluted with sesame oil to obtain the working solution used (10 mg/ml). Also, a tablet of 50 mg of cyproterone acetate ("androcur 50 mg", Delpharm), an antiandrogen, was dissolved in 5 ml of distilled water to be used in the experiments.

2.4.Experimental design

The hyperandrogeny induction followed the method described by Beloosesky *et al.* (2004) and involved subcutaneous daily injection of immature female rats with testosterone propionate in sesame oil at 10 mg/kg for 21 days. Afterwards, the second part of the treatment consisting to Cyproterone acetate or Plant extract administration as from the sixtieth day was traced from the method described by Abdulghani *et al.* (2012). So,fifty (50) immature female rats (55 – 60 g, 30 days old) were divided into 5 groups of 10 animals each. The vehicle control group received 1 ml/kg of sesame oil by subcutaneous injection from day 1 to day 21 of treatment and 10 ml/kg of distilled water by oral route from day 16 to day 35. The 4 Testosterone propionate treated groups received 10 mg/kg of testosterone propionate from the 1st to the 21st day of treatment by subcutaneous injection and respectively 10 ml/kg of distilled water (negative control), cyproterone acetate (10 mg/kg) (positive control) or AEJi 50 mg/kg and 100 mg/kg (test groups) by oral route from day 16 to day 35 of treatment.

Throughout the experimental period, vaginal opening was checked in all animals and when it occurred, vaginal smear was collected daily between 8:00 and 10:00 am (Yener *et al.* 2007) to microscopically monitor the cytological changes in the vagina allowing to follow the different phases of the cycle in rats of all groups (Goldman *et al.*, 2007; Westwood 2008). The percentage of rats displaying regular estrous cycle was calculated (number of animals with regular cycle over total number x 100) and the number of regular estrous cycle noted.

At the 36th day (day after the experiment period duration), the animals were mated with males of proven fertility and the vaginal smears were collected on daily basis in order to assess the presence of spermatozoa and then confirm the gestation or fertility restoration since male acceptance in rodents

occurs only during ovulation period (Mingoti *et al*, 2003) and determine the conception term at the end of the treatment as well as the fertility restoration rate.

2.5.Statistical analysis

Parametrical data from biological assays were registered as mean ± SE. The statistical difference between the values was analyzed by ANOVA (analysis of variance) test. Student-Newman-Keuls test was used for comparison between means whenever experimental factors were significant through ANOVA. The analysis of percentages was performed using the Chi-square test. Kruskal-wallis test was used for non-parametrical data and Mann-Whitney test when their differences were significant (Campbell and Swinscow, 2009)

3. Results

3.1. Effects of AEJi on vaginal opening and sexual cycle in testosterone propionate-induced infertile female albino rats

Table 1: Effects of AEJi administration on certain reproduction parameters in testosterone propionate-induced infertile female rats.

		Vehicle Control	TP + distilled water (negative control)	TP + cyproterone acetate (positive control)	TP + AEJi 50mg/kg	TP + AEJi 100mg/kg
Vaginal opening age (days)		35.80 ± 0.84^{a}	42.20 ± 0.84^{b}	42.00 ± 0.71^{b}	42.00 ± 0.71^{b}	42.20 ± 0.84^{b}
Percentage of rats with regular estrous cycle (%)	Before treatment	90^{b}	0^{***a}	0^{***a}	0^{***a}	0^{***a}
	After treatment	100^{d}	20^{***a}	40^{**b}	90^{d}	60^{**c}
Sexual cycle resumption term after different treatment (days)		0.00 ± 0.00^{a}	16.24 ± 2.63^{d}	12.37 ± 1.29^{c}	8.03 ± 0.97^{b}	7.58 ± 1.47^{b}

Each value represents the mean ± SE for 10 rats. In the same line, values carrying the same letter are not significantly different (Student- Newman- keuls $p<0.05$). For percentages, values significantly different at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ from those of the vehicle control (Chi square and Kruskall-Wallis tests). TP= testosterone propionate, AEJi= Aqueous extract of Justicia insularis.

Table 1 presents the mean age of treated animals at vaginal opening, the percentage of animals with regular estrous cycle (before and after extract administration) and the sexual cycle resumption and

normalization after infertility induction. Vaginal opening occurred significantly earlier (35 days old) in vehicle control animals as compared to those treated with testosterone propionate (42 days old).

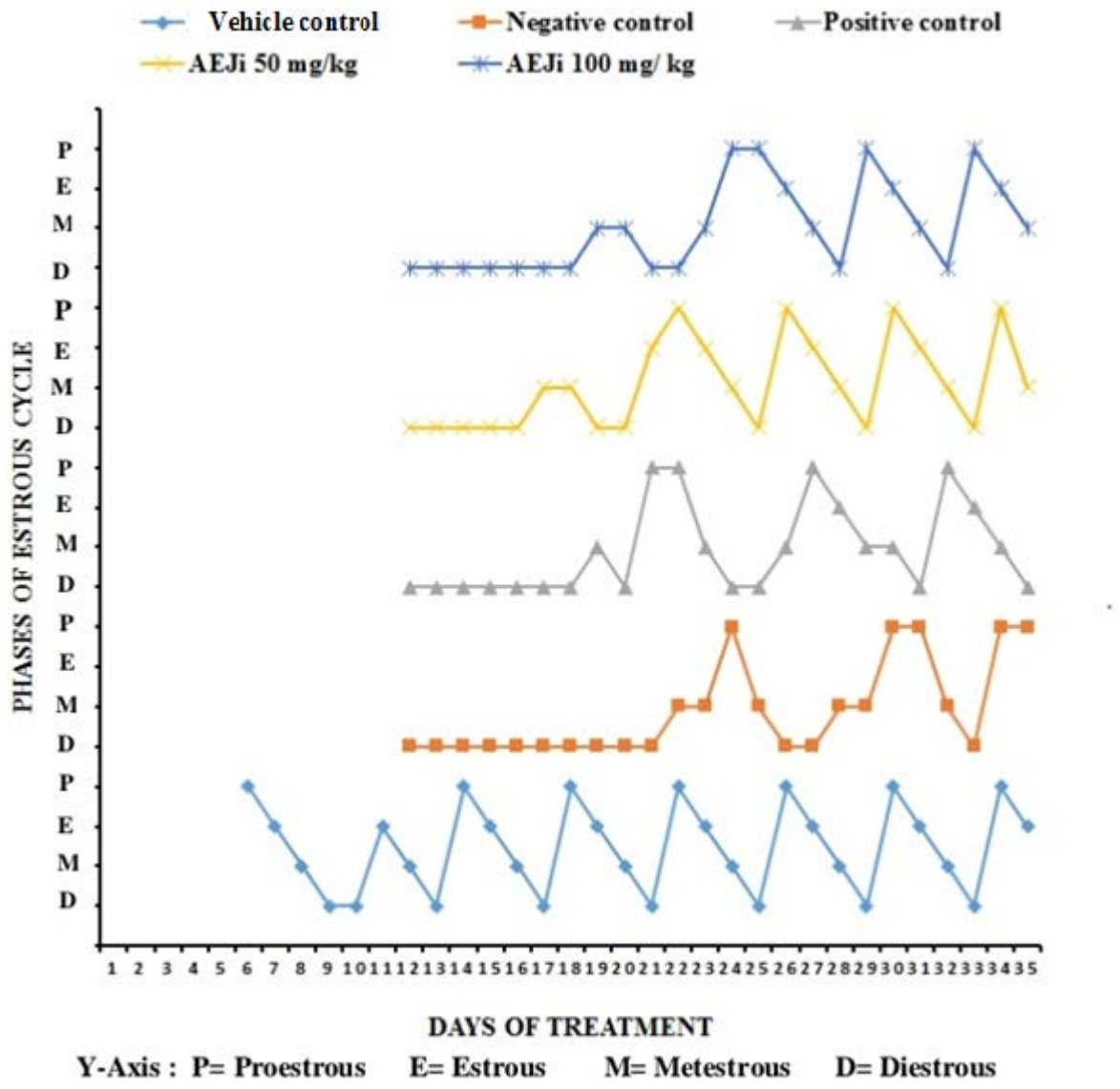

Figure 1: representative estrous cycle of different groups during the experimental period

Each graph represents the female estrous cycle variation per group from vaginal opening to the end of treatment.

AEJi=Aqueous extract of *Justicia insularis*

After vaginal opening, estrous cycles in testosterone propionate treated animals were blocked at diestrous phase as shown in figure 1 while in vehicle control animals it remained continuous and regular. After administration of Cyproterone acetate and AEJi at the 16th day of infertility induction, estrous cycles previously blocked at diestrous resumed in all treated animals but at different periods. In animals treated with cyproterone acetate and AEJi (50 mg/kg and 100 mg/Kg), the resumption occurred

respectively after 12, 8 and 7 days while in those receiving distilled water (negative control), it was after 16 days thus more than a week after the animals treated with AEJi. The percentage of female rats with regular sexual cycles varied from one group to another. The vehicle control group displayed 90% of female with regular sexual cycles against 20% and 40% respectively for negative and positive control groups. On the other hand, animals treated with AEJi (50 and 100 mg/kg) displayed respectively 90% and 60% of female with regular sexual cycles through the experimental period after sexual cycle resumption. These percentages were significantly ($p<0.05$) increased in all AEJi treated groups as compared to negative control group.

Moreover, it clearly appears on figure 1 that the estrous cycle variation curve after its resumption in 50 mg/kgAEJi - treated group is close to that of the vehicle control group whereas in the other groups, the appearance is still disordered.

3.2.Effects of AEJi on fertility rate and conception term

Table 2: Effects of AEJi administration on fertility rate and conception term in testosterone propionate-induced infertile female rats.

	Vehicle control	TP + distilled water (negative control)	TP + cyproterone acetate (positive control)	TP+ AEJi 50mg/kg	TP + AEJi 100mg/kg
Fertility rate (%)	100^{d}	20^{***a}	60^{**b}	80^{*c}	$70^{**b,c}$
Conception term (days)	1.90 ± 0.87^{a}	11.00 ± 1.41^{d}	3.50 ± 0.50^{b}	2.50 ± $0.52^{a,b}$	6.14 ± 0.89^{c}

Each value represents the mean ± SE for 10 rats. In the same line, values carrying the same letter are not significantly different (Student- Newman- keuls $p<0.05$). For percentages, values significantly different at $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ from those of the vehicle control (Chi square and Kruskall-Wallis tests). TP= testosterone propionate, AEJi= Aqueous extract of Justicia insularis, ***Fertility rate****=number of animals with pregnancy over total number of mated rats x 100*

Table 2 presents the fertility rate and the conception term in female rats treated with cyproterone acetate and AEJi after infertility induction with testosterone propionate. It has been noticed that only 20% of animals treated with testosterone propionate-alone recovered fertility until up to 2 post-treatment weeks while 60%, 80% and 70% recovery were respectively noticed with cyproterone acetate, 50 mg/kg and 100 mg/kg of AEJi-treated groups respectively. Moreover, only 20% of animals in the negative control group got pregnant about 11 days ($p<0.001$) after the end of treatment against 2 days in vehicle control group. This pregnancy term was significantly increased in cyproterone acetate ($p<0.05$) and 100 mg/kg AEJi-treated groups ($p<0.01$) while it was not significantly different in animals treated with the 50 mg/kg dosage of the extract as compared to the vehicle control group.

4. Discussion

The present study was undertaken to evaluate the potentiality of AEJi in the restoration of fertility in testosterone propionate-induced infertile female wistar rats. The subcutaneous injection of testosterone propionate significantly delayed the occurrence of vaginal opening in immature rats through the study. Testosterone propionate is a synthetic androgen and anabolic steroid with strong androgenic and moderate anabolic effects (Kicman, 2008); hence it is an agonist of the androgen receptor, the biological target of testosterone and dihydrotestosterone. Its prolonged administration leads to ovary activity suppression (Beloosesky *et al.*, 2004) associated to a delayed occurrence of vaginal opening in young female rats. It is known that vaginal opening during the rat pubertal age results from an increase in estradiol release by ovarian follicles (Nicolino and Forest, 2001). This enable us to establish a correlation between ovary activity suppression, absence of increase in estradiol release by ovarian follicles and consequently a delayed occurrence of vaginal opening in Testosterone propionate-induced hyperandrogenic young female rats.

Testosterone propionate administration also altered estrous cycle by extending the length of the diestrous phase in a significant manner (figure 1), leading to irregular estrous cycle in all treated animals. These changes in rats' estrous cycle are probably linked to alterations in the circulating concentrations of the sex hormones and gonadotrophins (Westwood 2008) which control the ovarian function including follicular maturation (Goldmann *et al*, 2007). At the same time, a hormonal imbalance may lead to irregular estrous cycle thereby affecting ovarian function. Similar results associated with cystic follicles and significantly lower number of healthy follicles were obtained by Abdulghani *et al.* (2012) and confirmed later by Bhuvaneshwari *et al.* (2015) who observed cystic condition in antral follicular regions in immature rats after testosterone propionate administration, leading to polycystic ovary syndrome (PCOS). After cyproterone acetate or AEJi administration, as from the 16^{th} day of infertility induction, all the treated groups showed a significant increase in the percentages of rats with regular estrous cycle as compared to the values of the same groups before the treatment. This is evidence that both treatments present a regulative effect on the estrous cycle. However, these percentages, as well as the sexual cycle resumption period, varied from one group to another. It has been noticed that 20% of animals of the negative control group resumed estrous regular cyclicality, value significantly lesser ($p<0.001$) than the 100% presented in the vehicle control group and significantly higher than the 0% observed in the same group before; this happened about 16 days after the beginning of the extract administration and 11 days after the last dose of testosterone propionate. This lesser percentage on one hand precise with self-conceit the extend of dysfunctions due to

hyperandrogenism in female rats and on the other hand may suggest the progressive elimination of testosterone propionate in rats' organism since it has a short half-life of about 4.5 days (Rastrelli *et al* 2018) leading to the reduction of its effects on the reproductive system. Forty percent (40%) of rats of the positive control group displayed regular estrous cycle after about 12 days of administration thus testifying the antiandrogenic effect of Cyproterone acetate. In fact, cyproterone acetate is an androgen antagonist which acts by blocking androgen receptors, preventing androgens fixation and by its progestagenic action, it exerts a negative feedback on hypothalamo-pituitary axis which reduces LH secretion resulting in testosterone levels decrease (Nader, 2008). This reduction of LH secretion can stimulate FSH production, folliculogenesis and estrogen production (Greenwald and Roy, 1994); this can lead to estrous cycle normalization and fertility restoration in hyperandrogenic-induced infertile rats. Over animals which received AEJi (50 and 100 mg/kg), 90% and 60% of rats respectively displayed regular estrous cycles. This percentage value of the group receiving 50 mg/kg dose was not significantly different from the vehicle controls' result and may suggest a restorative activity of the extract on dysfunctions led by testosterone propionate even though the sexual cycle resumption occurred about 8 days after extract administration (3 days after the last dose of testosterone propionate). Moreover, these percentages were significantly higher than the one obtained with the positive control group and implies the most pronounced progestagenic and/or antiandrogenic effect of AEJi on testosterone propionate induced dysfunctions.

Mating the treated rats with males of proven fertility is a test used to assess the post-treatment fertility of female rats in order to ensure that the sexual cycle resumption is combined with fertility restoration in testosterone propionate-induced infertile rats. Therefore, 20% of rats got pregnant in the negative control group after about 11 post-treatment days. The value is significantly weak ($p<0.001$) as compared to the vehicle control animals and may express the great extent of the disturbances caused by testosterone propionate still acting in those animals after the observation period (2 weeks). Regarding the positive control group and the AEJi 100 mg/kg-treated group, 60% and 70% of pregnancy were respectively registered; these values are significantly weak ($p<0.01$) when compared to the vehicle control value (100%) and significantly higher than the 20% obtained with negative control animals. This points-up both the antiandrogenic effect of cyproterone acetate and the ameliorative effect of AEJi on testosterone propionate-induced infertility. However, these results suggest that the above mentioned effects may have brought down the androgen level. Yet low androgen levels may be associated with abnormalities of follicular growth, low functional ovarian reserve and primary ovarian insufficiency, thereby negatively impacting female fertility (Prizant *et al.,* 2014*)*. Moreover, 80% of fertility rate was

obtained with animals that received 50 mg/kg of AEJi. This result is significantly lesser ($p<0.05$) than the 100% obtained with the vehicle control group. Nevertheless, the conception term of this group was not significantly different from that of the vehicle control group, showing the restorative effect of AEJi on testosterone propionate-induced infertility at its 50 mg/kg dosage.

Indeed, previous studies carried out by Telefo *et al.* (2012) and Goka *et al.* (2017) respectively demonstrated that administration of AEJi to immature rats stimulates folliculogenesis through FSH-like and/or estrogenic effect. Moreover, androgens are aromatized into estrogens (estradiol and estrone) in granulose cells by P_{450} aromatase whose activity is stimulated by FSH (Taieb and Lachgar, 2012). The restorative effect of AEJi on both estrous cycle irregularity and infertility may therefore be due to the FSH-like and/or estrogenic activity of the extract that may have lowered the androgen action.

Conclusion

AEJi regulates estrous cycle and restores fertility in hyperandrogenic albino wistar female rats. Better than cyproterone acetate, its administration at 50 mg/kg to testosterone propionate-induced infertile female rats ensures sexual cycle resumption and reestablishes the estrous cycle regularity after a week. It also enhances fertility rate in the same animals making this study a hope for women suffering from infertility due to hyperandrogenism and lead to a foreseeable solution for the management of polycystic ovaries syndrome. Further experiments will focus on sexual hormones and gonadotrophins dosage in testosterone propionate-induced infertile females in order to provide a better understanding of the obtained results.

References

1. M. Abdulghani, A.H. Hussin, S.A. Sulaiman, and K.L. Chan, “The ameliorative effects of *Eurycoma longifolia* Jack on testosterone-induced reproductive disorders in female rats”. *Reproductive biology*, vol. 12, no 2, pp. 247-255, 2012.
2. A. Agarwal, and S.S.R. Allamaneni, ‘‘Rôle of free radicals in female reproductive diseases and assisted reproduction’’. *Reproductive BioMedecine Online*, vol. 9, no 3, pp. 338-347, 2004.
3. A.A. Akindahunsi, and M.T. Olaleye, “Toxicological investigation of aqueous extract of calyces of *Hibiscus sabdariffa L*”. *Journal of ethnopharmacology*, vol 89, no 1, pp. 161-164, 2003.
4. R. Beloosesky, R. Gold, B. Almog, R. Sasson, A. Dantes, A. Land-Bracha, L. Hirsh, J. Itskovitz-Eldor, J.B. Lessing, R. Homburg and A. Amsterdam, “Induction of polycystic ovary by testosterone in immature female rats: Modulation of apoptosis and attenuation of glucose/insulin ratio”. *International Journal of Molecular Medicine*, vol. 14**,** pp. 207-215, 2004.

5. S. Bhuvaneshwari, R. Poornima, H.I. Averal, "Effects of *Purgulara daemia* in polycystic ovary syndrome induced rats' ovaries and thyroid gland". *International Journal of recent scientific Research.* Vol. 6, no. 1, pp. 2390-2394, 2015.
6. M.J. Campbell and T.D.V. Swinscow, "Statistics at Square one". Wiley-Blackwell: *BMJ Books* 11, 2009.
7. A.S. Daar and Z. Merali, "Infertility and social suffering: the case of ART in developing Countries". InVayena E - Rowe PJ -Griffin PD (editors): *Current practices and controversies in assisted reproduction. Report of a meeting on "Medical, ethical and social aspects of assisted reproduction"*. World Health Organization. Geneva, Switzerland, 17-21 September 2001, pp. 15-21, 2002.
8. EEC, "Council directive 86/609/EEC of 24 november 1986 on the approximation of laws, regulations and administrative provisions of the member States regarding the protection of animals used for experimental and other scientific purposes". *Official Journal of European Community,* L358, pp. 1-29, 1986.
9. C.M.S. Goka, L.L. Lienou, N.S. Njina, B.F. Fekam and P.B. Telefo, "Effect of four Cameroonian medicinal plants on sexual maturation of immature female rats". *African Journal of Integrated Health,* vol.7, no. 1, pp. 54-60, 2017.
10. J.M. Goldman, A.S. Murr and R.L. Cooper, "The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies". *Birth Defects Research Part B Developmental and Reproductive Toxicology,* vol. 80, pp. 84-97, 2007.
11. A.G. Greenwald and S.K. Roy, "Follicular development and its control". In E. Knobil and J.D. Neil (Eds): *The physiology of reproduction,* 2nd edition. Raven Press Ltd New York. pp. 629-724, 1994.
12. A.T. Kicman, "Pharmacology of anabolic steroids". *British Journal of Pharmacology,* vol. 154 no. 3, 2008.
13. U. Larsen, "Primary and secondary infertility in sub-Saharan Africa". *International Journal of Epidemiology*, vol. 29, pp. 439-451, 2000.
14. T.G. Mbemya, A.L. Vieira, G.F. Canafistula, O.D.L. Pessoa and A.P.R. Rodrigues,"Reports on *in vivo* and *in vitro* contribution of medecinal plants to improve the female reproductive function". *Reproducâo and Climatéro,* vol. 32, no. 2, pp. 109-119, 2017.
15. T.G. Mbemya, D.D. Guerreiro, J.N. Donfack, M.L. Silva, A.L. Vieira, G.F. Canafistula de Sousa, B.G. Alves, P.A. Izaguirry, F.W. Santos, P.B. Telefo, O.D.L. Pessoa, J. Smitz, J. Ricardo de

Figueiredo and A.P.R. Rodrigues, "*Justicia insularis* improves the *in vitro* Survival and Development of Ovine Preantral Follicles Enclosed in Ovarian Tissue". *Journal of Pharmacy and Pharmacology*, Vol. 5, pp. 668-680, 2017.

16. G.Z. Mingoti, R.N. Pereira and C.M.R. Monteiro, "Fertility of male adult rats submitted to forced swimming stress". *Brazilian journal of Medical and Biological Research.* Vol. 36, no. 5, 2003.
17. S. Nader, "Treatment for Polycystic Ovary Syndrome: A Critical Apraisal of treatment options". *Expert Review of Endocrinology and Metabolism*, vol.3, no. 3, pp. 349-359, 2008.
18. M. Nicolino and G.M. Forest, G.M. (2001). La puberté. In Thibault, C. & Levasseur, M.C. (Eds):*La reproduction chez les mammifères et l'homme.*I NRA, Ellipses paris chap 29: 655-679.
19. H. Prizant, N. Gleicher, and A. Sen, "Androgen actions in the ovary: balance is the key". *Journal of endocrinology*, Vol. 222, no. 3, pp. 141-151, 2014.
20. G. Rastrelli, Y. Reisman, S. Ferri, O. Prontera, A. Sforza, M. Maggi, and G. Corona, "Testosterone replacement therapy". In K. Gunasekaran and S. Khan (eds) *Sexual Medicine*. Springer, Singapore, pp 79-93, 2018.
21. J. Taieb and M. Lachgar, ''Exploration biologique de la fonction androgénique chez la femme : problèmes liés aux immunodosages des androgènes''. *Mt Medecine de la reproduction, Gynécologie Endocrinologie* ; vol 14, no. 3, pp. 196-203, 2012.
22. P.B. Telefo, F.P Moundipa and M.F. Tchouanguep, "Influence de l'extrait aqueux d'*Aloe buettneri, Justicia insularis, Hibiscus macarathus* et *Dicliptera verticillata* sur la fertilité et quelques paramètres biochimiques de la reproduction chez la ratte''. *Revue de l'académie des sciences du Cameroun*, vol 1, no. 3, pp.144-150, 2001.
23. P.B. Telefo, F.P. Moundipa and F.B. Tchouanguep, "Oestrogenicity and effects on hepatic metabolism of *Aloe buettneri, Dicliptera verticillata, Hibiscus macranthus and Justicia insularis"*. *Fitotherapia.* Vol. 73, pp. 472-478, 2002.
24. P.B. Telefo, P.F. Moundipa, A.F. Tchana, D.C. Tchouanguep, and F.T. Mbiapo, "Effects of an aqueous extract of *Aloe buettneri, Dicliptera verticillata*, *Hibiscus macranthus,* and *Justicia insularis* on some biochemical and physiological parameters of reproduction in immature female rat". *Journal of Ethnopharmacology*, vol.63, pp. 193-200, 1998.
25. P.B. Telefo, S.R. Tagne, O.E.S. Koona, D.M. Yemele, and F.M. Tchouanguep, "Effect of the Aqueous Extract of *JusticiaInsularis*T. Anders (Acanthaceae) on Ovarian Folliculogenesis and Fertility of Female Rats". *Afr. J. Tradit. Complement. Altern. Med. Vol.* 9, pp.197-203, 2012.

26. S.R. Westwood,"The female rat reproductive cycle: a practical histological guide to staging". *Toxicologic Pathology*, vol. 36**,** pp. 375-384, 2012.
27. T. Yener, T.A. Turkkani, H. Aslan, H. Aytan and C.A. Cantug, "Determination of oestrous cycle of the rats by direct examination: How reliable? *Anatomy Histology Embryology,* vol. 36, pp. 75-77, 2007.