Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 41 (2011) 6032-6036

ISSN: 2229-712X

Evaluation of the therapeutic potentials of *enantia chlorantha* (oliv.): an antimarial herb

Ikpeme E.V[1]., O. Udensi[2], U.B.Ekaluo[3], A.I.Nta[4] and I.ATakon[5]

[1,2,3]Department of Genetics and Biotechnology, University of Calabar-Calabar, Nigeria

[4]Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria

[5]Department of Microbiology, University of Calabar, Calabar, Nigeria.

ARTICLE INFO

Article history:

Received: 20 October 2011;

Received in revised form:

17 December 2011;

Accepted: 29 December 2011;

Keywords

Enantia chlorantha,

Anti-malarial,

Sperm quality,

Hormonal profile,

Fertility.

ABSTRACT

The devastation caused by malaria in the sub-Saharan African countries has led to alarming and unprecedented search for cure, especially herbals. This has prompted the evaluation of bark extract of *E. chlorantha* though a known anti-malarial agent for other therapeutic effects. Thirty-two mature male albino rats weighing between 90 – 120g were randomly grouped into four with eight rats per group and administered 0, 200, 400, and 600mg/kg of the extract per body weight for 30 days through oral gavage. Results showed that there were significant effects ($P < 0.05$) of *E. chlorantha* treatment on sperm quality but weight of epididymes and testes revealed no significant difference ($P > 0.05$). Hormonal profile also showed significant differences ($P < 0.05$) on treatment but the dosage administered should be monitored. Correlation result revealed that there were significant positive relationships between sperm count and follicle stimulating hormone (0.686*), sperm viability and prolactin level (0.958**), follicle stimulating hormone and testosterone (0.687*) while a negative correlation were observed between weight of testis and luteinizing hormone (-0.740*), luteinizing hormone and progesterone (-0.814*) and between follicle stimulating hormone and progesterone (-0.704*). Succinctly, our results are suggestive of the fact that *E. chlorantha* is a multipurpose medicinal herb as it can be used as an anti-malarial agent and fertility booster, especially when administered at lower dosages.

Introduction

Malaria appears to be one of the greatest sources of misery on earth today. The World Health Organization reported that an average of 3,000 people is infected by malaria every minutes and approximately one million die of the disease yearly. Worldwide prevalence of the disease causes an estimated 500 million clinical episodes each year, resulting in over one million deaths annually (Snow et al., 2005). Dishearteningly, however, about 90% of malaria–elected modify usually occurs amongst children less than five years of age and pregnant women in sub-Saharan Africa (Rinaki, 2004), where a large part of the population has no access to health services or relies on herbal remedies which are often not effective. According to Mueller *et al*. (2004) malaria has substantial negative impact on the economic development of Africa nations where the disease is endemic. Unfortunately, the emergence through mutation, of multi-drug resistant plasmodium species which has recently sparked a global concern, has rendered traditional and affordable anti-malaria drugs such as chloroquine and sulphadoxine pyrimethamine and recently some Artemisinin Combined Therapies (ACTs) ineffective (Brisibe *et al*., 2008). Consequently, consumers in developed countries are becoming disillusioned with modern health care and are thus seeking for alternatives (Ikpeme *et al*., 2007; Ikpeme *et al*., 2010).

This paradigm shift has refocused attention to herbal remedies. This recent resurgence of plant remedies, however, has resulted from several factors–the effectiveness of plant medicine; the side effect of most modern drugs and the development of science and technology. Though Artemisinin from *Artemisia annua* has been found highly potent and efficacious against resistant strains of malarial parasites, other anti-malarial herbs development of science and technology. Though Artemisinin from Artemisia annua has been found highly potent and efficacious against resistant strains of malarial parasites, other anti-malarial herbs needed to be explored and exploited. The global "Roll back malaria" initiative that set up medicine for malaria venture to states and accelerates research into innovative drugs with anti-malarial properties has motivated the search for plants with potential pharmacological and therapeutic uses such as *Enantia chlorantha*. There are several reports on the potential of *E. chlorantha* (Salman and Adesokan, 2008; Adesokan *et al*., 2007; Atata *et al*., 2003; Akanji and Adesokan, 2005; Siminialayi, 2004) Like other therapeutic agents, *E. chlorantha* may not be devoid of side effects or toxicities in both human and animals studies. Nowadays, the linking of the indigenous knowledge of medicinal plants to modern research activities provides a new approach, which makes the rate of discovery of drugs much more effective than with random collection.

Due to the prevalence of malaria in tropical Africa, plants that demonstrate potency against the disease are usually over-exploited (Gbadamosi and Oni, 2005). The contention at this point is not over-exploitation, which could lead to extinction as biotechnological techniques offer help in the domestication and reintroduction of the species to agro-ecosystem, rather its holistic scientific evaluation, which is pivotal in elucidating the

Tele:

E-mail addresses: princeuou4u@yahoo.com

effects, its ability to cure malaria notwithstanding. Though the main aim of this study is as stated above, x-raying the relationship between sperm quality and hormonal parameters becomes also crucial.

Materials and methods

Collection and preparation of plant material

The bark of *E. chlorantha* was obtained from Mr. Otu Arikpo in the department of forestry and wild-life conservation, University of Calabar, Calabar, Nigeria. The bark was sun-dried and ground into powder using an electric blender (Christison 37BL1B, Model 240 CB6) and soxhlet extracted.

Experimental animals and procedure

Thirty-two matured male albino rats, weighing between 90 – 120g were purchased from the animal house, Pharmacology Department, University of Calabar, Calabar. The rats were maintained under standard condition of humidity, temperature and a constant 12 hours light and 12 hours dark lighting schedule. The cages were lined with wood shavings, which were changed as the need arose. The rats were fed with standard commercial feed and clean water *ad libitum*. Acclimatization was done for one week. The rats were divided into four groups according to their body weight. Group A served as the control, which was giving 1ml of normal saline. Groups B, C, and D received 200mg/kg, 400mg/kg and 600mg/kg BW of *E. chloratha* bark extract, respectively through oral gavage for 60 days. After the treatment regimen, they were anaesthetized with chloroform. Blood samples were collected through cardiac puncture for hormonal analysis while semen was collected for sperm quality analysis from the epididymes.

Hormonal analysis

Blood samples were centrifuged immediately to obtain the sera which were assayed for the following hormones: Testosterone, Luteinizing Hormone/Intertestial Cell Stimulating Hormone (LH/ICSH), Follicle Stimulating Hormone (FSH), Estradiol and Prolactin in Chemical Pathology Laboratory, University of Calabar Teaching Hospital, Calabar. The method adopted was micro-well Enzyme-Linked Immunoassay (ELISA) using analytical grade reagents (Syntron Bioresearch Inc. USA).

Evaluation of sperm motility

The sperm cell suspension was diluted in 2ml of physiological saline and dropped on glass slides. This was viewed under light microscope as to determine the motile and non – motile sperm cells by their movement (WHO, 1992).

Estimation of sperm count

This was carried out according to the method of Abd El-Rahman *et al*. (1999) as modified by Ekaluo *et al*. (2005). The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymis in a glass petridish containing physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. The sperm cells were counted by cytometry. Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

Sperm viability determination

This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin – nigrosin stain and air dried smears were prepared on glass slides for each samples according to Bjorndahl *et al*., (2003). The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin – nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

Data analysis

Data obtained were subjected to analysis of variance (ANOVA) and correlation analysis using statistical software PASW Ver. 18.0.

Results

Sperm quality

The results on sperm quality parameters revealed that *E. chlorantia* extract had significant effect ($P < 0.05$), which was dose-dependent, especially on the sperm count, sperm motility and sperm viability. Mean Sperm count and percentage sperm viability were increased as the dose of administration increases while there was a reducing effect on sperm motility. However, weight of epididymes and testes showed no significant differences ($P > 0.05$) upon treatment (Table 1).

Hormonal parameters

Figure 1 shows the effect of the crude extract of *E. chlorantha* on male albino rats. There are obvious significant differences ($P < 0.05$) among the treatment groups. The result revealed that the extract caused significant increase on the luteinizing hormone (LH), Follicle stimulating hormone (FSH), prolactin and testosterone while estradiol decreased significantly ($P > 0.5$). This effect was dose-dependent. For FSH, there was no significant difference between rats treated with 400mg/Kg and those treated with 600mg/Kg while in prolactin, rats in the control and those treated with 200mg/Kg and 400mg/Kg showed no significant differences.

Correlation analysis

Table 2 shows the correlation matrix of sperm parameters and hormonal profile of albino rats treated with crude bark extract of *E. chlorantha*. Result revealed that there was significant positive relationship between sperm count and follicle stimulating hormone (0.686*), sperm viability and prolactin level (0.958**), follicle stimulating hormone and testosterone (0.687*) while a negative correlation were observed between weight of testis and luteinizing hormone (-0.740*), luteinizing hormone and progesterone (-0.814*) and between follicle stimulating hormone and progesterone (-0.704*). However, other parameters studied showed that there were correlations, which were not significant ($P > 0.05$; 0.01).

Discussion

It has been reported worldwide that malaria prevalence causes an estimated 500 million clinical episodes each year resulting in over one million deaths annually (Snow *et al*., 2005). This scenario has led to the sourcing of malaria drugs preferably, herbs. This is fundamentally the case of the teeming population in the suburbs of developing countries. As fantastic this development might appear, little or no attention is paid to the will-be side-effects.

Our result showed that the administration of *E. chlorantha* bark extract caused the reduction in the motility of the sperm cells, which is contrary to the submission of Salman and Adesokan (2008) who reported that *E. chlorantha* caused significant effect on the motility and viability of sperm cells while sperm count was not affected significantly. Though the concentration used was different, their report showed that the herb is more effective at low dosage in enhancing fertility, which corroborates with our present report. Simple sugars were involved in the motility and viability of sperm cells. This is the

case because the metabolism of simple sugars such as glucose usually leads to the production of pyruvate, which is known to be the preferred substrate essential for the activity and survival of sperm cells. It then becomes confusing why sperm motility reduced significantly while increasing sperm viability. This condition is suggestive of the fact that the motility and viability of sperm cells do not depend solely on the presence of simple sugars alone, rather there are other intrinsic factors working in synergy. According to Abu-Sharka (1994), Olajide *et al*. (1999) anti-malarial agents that reduce sperm motility and viability such as quinine, chloroquine and *Morinda lucida* are known to have hypoglycemic property. It thus mean that E. *chlorantha* might have a slight hypoglycemic potential which needs to be studied.

Sex hormones, particularly estradiol and progesterone in females and testosterone in males are produced primarily in the gonads under the influence of FSH and LH. This however, implies that any factor(s) affecting FSH and LH concentration in the animal will exert a corresponding and proportional effect on the other reproductive hormones. The increase in the concentrations of sex hormones is known to exert positive feedback influence at the level of the pituitary gland where they regulate the secretion of gonadotrophins. The results from this research revealed that *E. Chlorantha,* which is a known anti-malarial herb affected the hormonal profile of albino rats. The increase in the levels of testosterone in our result might have been enhanced by the increase in the levels of LH and FSH. It has been reported that FSH and LH influence testosterone concentration, the positive correlations between sperm count and FSH and between FSH and testosterone points to the fact that there is a seeming proportional relationship between FSH, testosterone and sperm count. Expectedly, there should have been positive correlation between LH and FSH with other reproductive hormones. An inverse relationship observed between them and estradiol is worrisome. It might be that there are other factors militating against the relationship. Additionally, the effect of the herbal therapy on the estradiol level of the rat suggests that the treatment did not significantly increase it, which might have been as a result of specific bioactive compounds altering the synthetic pathways of the hormone. Testosterone being the main male reproductive hormone, though it needs others to function properly, was significantly affected by the treatment. This implies that increasing the concentration/quantity of the herb will cause a proportional increase in the testosterone level. According to Lunenfeld (2003) endocrine changes and decline in endocrine function involve tissue responsiveness, reduced secretory output from peripheral glands and alterations in the central mechanisms controlling the temporal organization of hormonal release.

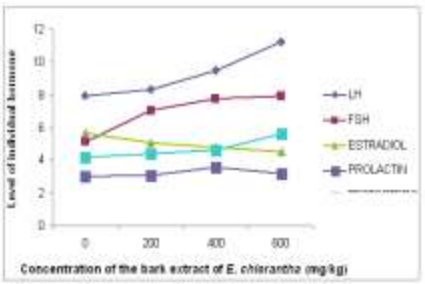
This might be the mechanism underlying the potency of the herb. Worthy to note is the fact that *E. chlorantha* contains important bioactive components, whose effect singly and synergistically might have either enhanced or altered the biosynthetic processes underlying hormone production. The results suggest probably that different and specific bioactive components in the plant might be responsible for the effect on specific reproductive hormones. It is also possible that the enhancing effect of the treatment occurs when a particular quantity of the herbal components is available. It therefore becomes relevant to submit that the administration of *E. chlorantha* as an anti-malarial herb should be done with caution as suggested by the results as there will be a threshold where further treatment could lead to a reverse condition.

Adeeko and Dada (1998); Raji *et al*. (2003); Nwanjo *et al*. (2007), Ekaluo *et al*. (2008) reported that anti-malarial agents posseses anti-fertility effects. From our results, *E. chlorantha* being an anti-malarial agent cannot be said to have anti-fertility property. This is premised on the fact that any herb or drug that has the potential of enhancing the sperm count, sperm viability, LH, FSH, prolactin and testosterone is obviously a fertility booster, which *E. chlorantha* is. According to McGarvey *et al*. (2001), Weber *et al*. (2001), Pastuszewska *et al*. (2006) plants with high alkaloid content were responsible for increase in serum concentration of estradiol and prolactin, that are capable of inhibiting gonadothrophic action of the testes and subsequently the fertility of male animals. This therefore implies that comparatively, *E. chlorantha* bark extract has lower alkaloid content than other anti-malarial herbs where fertility has been reported.

Ekaluo *et al*. (2010) reported that aqueous leaf extract of neem is capable of disrupting the normal hormonal milieu of male rats and may increase the risk of infertility as a result of malaria chemotherapy. Though *E. chlorantha* is anti-malarial agent, it does not affect the fertility of the animal, instead there was a boosting effect, which depends on the dosage administered. This is a total diverge from earlier reports of Adeeko and Dada (1998); Raji *et al*. (2003); Nwanjo *et al*. (2007), Ekaluo *et al*. (2008); Ekaluo *et al*. (2010) on anti-malarial agents.

Conclusion

Succinctly, our results are suggestive of the fact that *E. chlorantha* is a multipurpose medicinal herb as it can be used as an anti-malarial agent and fertility booster, especially when administered apropriately.



References

Abd El-Rahman, HA., El-Badry, AA., Mahmoud, OM. & Harraz, FA. The effect of the aqueous extract of *Cynomorium coccineum* on the epididymal sperm pattern of the rats. *Phytotherapy Research*. 1999; 13:248-250.

Abu-Sharka, MN. Hypolycemia. C*linical Experiment of pharmacology*, 1994; 12:19-25.

Adeeko, AO. & Dada, AO. Chloroquine reduces the Fertility Capacity of epididymal sperm in rats. *African Journal of Medical Science*, 1998; *27*:63-68.

Adesokan, AA., MA. Akanji and MT. Yakubu. Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark. Afr. J. Biotechnology, 2007; 6(22): 2502-2505

Akanji, MA., Adesokan, AA. Effects of repeated administration of aqueous extract of *Enantia chlorantha* stem bark on some selected enzyme activities of rat liver. *Biochemistry*, 2005; 17 (1)23-26

Atata, RF., A. Sanni and SM. Adewole. Effect of stem bark extracts of *Enantia chlorantha* on some clinical isolates. Biokemistri, 2003; 15(2): 84-92.

Bjórndahl, L., Sódourlund, I., & Kvist, U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Human Reproduction*. 2003; *18*:813-816.

Brisibe, EA., EA. Uyoh, F. Brisibe, PM. Magalhaes and JFS. Ferreira. Building a golden triangle for the production and use of artemisinin derivatives against falciparum malaria in Africa. Afr. J. Biotechnology., 2008; 7:4884-4896.

Ekaluo, UB., Ikpeme, EV., Udensi, O., Markson, AA., Madunagu, BE., Omoun, G. and Umana, EJ. Effect of aqueous leaf extract of neem (*Azadirachta indica*) on the hormonal milieu of male rats. International Journal of Current Research, 2010; 4: 1-3

Ekaluo, U. B., Udokpoh, A. E., Ikpeme, E. V. and Peter, E. U. Effect of chloroquine treatment on sperm count and weight of testes in male rats. Global Journal of Pure and Applied Sciences, 2008; 14: 175-177

Ekaluo, UB., Udokpoh, EA., Udofia, UU. & Ajang, OR. Comparative toxicology of five commonly used analgesics and rats sperm count and sperm morphology. *Global Journal of Pure and Applied Science*. 2005; 11:81-84.

Gbadamosi, AE and O. Oni. Macro-propagation of an endangered medicinal plant, *Enantia chlorantha*. International Society of Arboriculture, 2005.

Ikpeme, EV., Udensi, O., Ekaluo, UB., Uyoh, EA., Asuquo, BO., Udoh, FV & Udoh, PB. Effect of Crude extract of *Cariza papaya* seeds on the reproductive efficiency of male albino rats. *Global Journal of Pure and Applied sciences*, 2007; *13*: 365-368.

Ikpeme, EV., Udensi, O., Ekaluo, UB. and Efieneokwu, N. Biological response of male wistar rats to crude extract of *Ficus exasperata* (VAHL). International Journal of Current Research, 2010; 7: 9-13.

Lunenfeld, B. Androgen therapy in the aging male. World J. Urol., 2003; 21:292-305.

McGarvey, C., Cates, PA., Brooks, A., Swanson, IA., Milligan, SR., Coen, CW and O'byrne, KT. Phytoestrogens and gonadotropin releasing hormone pulse generator activity and pituitary luteinizing hormone release in the rat. Endocrinology, 2001; 142: 1202-1208.

Mueller MS., Rungambo N, Wagner I, Borr-Mann, S, Dietz K, Heide L. Randomized controlled trial of a traditional preparation of *Artemisia annua* L. (annual wormwood) in the treatment of malaria. *Transactions of the Royal society of Tropical Medicine and Hygiene*, 2004; 98:818-321.

Nwanjo, HU., Iroagba, II., Nnatuanya, IN., and Eze, NA. Antifertility activity of dihydroartemisinin in male albino rats. The Internet Journal of Endocrinology, 2007; 4(1)

Olajide, OA., Awe, OA., and Makinde, JM. Pharmacological studies on the leaf of *P. guajava*. Fitoterapia, 1999; 70: 25-31.

Pastuszewska, B., Taciak, P., Ochabiniska, A., Tusnio, A., Misztal, T., Romanowicz, K. and Morawski, A. Nutritional value and physiological effects of soya-free diets fed to rats during growth and reproduction. Journal of Animal Physiology and Animal Nutrition, 2006; 10: 1439-1496

Raji, Y., Mewoyeka, US & Onoye, OO. Implication of reproduction endocrine malfunction in male antifertility of *Azadirachta indica* extract in rats. *African Journal of Medical Science*. 2003; *32*:159-165.

Rinalde, A. Fighting malaria at a cross road: the tools to battle the disease exist, but the lack of political wills are developed nations jeopardizing their success. *Embo Reports*, 2004; 5:847 – 851.

Salman, TM and AA. Adesokan. Sperm quality of male rats treated with aqueous extract of *Enantia chlorantha* stem bark. Afr. J. Biotechnology, 2008; 7(7): 865-867

Siminialayi, IM. Gastroprotective effects of the ethanolic extract of *Enantia chlorantha* in rats. *West African Journal of Pharmacological Drug Resistance*. 2004; *20*:35-38.

Snow RW, Guerra CA, Noor AM., Myint HY and Hay SI. The Global distribution of clinical episode of *Plasmodium falciparum* malaria. *Nature*, 2005; 434:214 – 217.

Weber, KS., Setchell, KD., Stocco, DM and Lephart, ED. Dietary soy-phytoestrogen decrease testosterone levels and prostate weight without altering LH, prostate 5 alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. Journal of Endocrinology, 2001; 170:591-599.

World Health Organization. WHO *Laboratory Manual for the Examination of Human Semen and sperm cervical mucus interaction*. Cambridge: Cambridge University Press, 1992.

Table 1: Effect of E. chlorantha (Oliv.) on sperm quality and weights of organs

Parameters	Concentration (Mg/Kg)			
	0	200	400	600
Sperm count 10^6/ml	35.3±2.4a	44.3±1.2b	39.3±1.5a	43.0±1.2ab
Sperm motility (%)	65.0±2.9b	55±5.4b	36.7±8.8a	61.0±1.3b
Sperm Viability (%)	35.8±5.7a	36.8 ± 0.8a	55.1±3.8b	43.5±5.2a
Weight of Epdidymes (g)	0.2±0.03a	0.18± 0.06a	0.17±0.04a	0.17±0.05a
Weight of testes (g)	1.18±0.39a	0.97±0.16a	1.18±0.05a	1.27±0.07a

* Mean followed with same case letter in a given horizontal array as superscript indicates no significant difference at P > 0.05.

Table 2: Correlation analysis of sperm parameters and hormonal profile of albino rats treated with bark extract of E. chlorantha (OLIV)

	Sperm Count	Sperm Motility	Sperm Viability	Wt. of epididymis	Wt. of testes	LH	FSH	Prog.	Prol.	Test.
Sperm count	1	-0.037	0.149	-0.386	-0.304	0.462	0.686*	-0.651	0.142	0.454
Sperm motility		1	-0.440	0.182	0.206	-0.310	-0.535	0.616	-0.321	0.014
Sperm viability			1	-0.135	0.348	0.129	0.473	-0.165	0.958**	0.278
Weight of epididymis				1	-0.244	0.405	-0.439	0.050	-0.057	-0.036
Weight of testis					1	-0.740*	0.115	0.584	0.296	0.455
LH						1	0.277	-0.814*	0.240	-0.033
FSH							1	-0.704*	0.390	0.687*
Progesterone								1	-0.173	-0.213
Prolactin									1	0.175
Testosterone										1

* Significant at P =0.05; ** Significant at P =0.01

LH = Luteinizing hormone; FSH = Follicle stimulating hormone; Prog. = progesterone; Prol. = prolactin; Test. = testosterone