Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.19, 2015

Antifertility Effects of P-Alaxin in Male Adult Wistar Rats

Kareem F. A[1] Osonuga I.O[2] Akindele R.A[2] Kukoyi B.I[2] Taiwo E.O[2] Inegbeneboh D[2].
1. Department of Science Laboratory Technology, School of Pure and Applied Sciences, Gateway Polytechnic, Saapade, Ogun State, Nigeria
2. Department of Physiology, Olabisi Onabanjo University, Remo Campus, Ikenne Remo, Ogun State

Abstract
P-alaxin, an artemisinin based combined therapy is very effective in treating malaria infection in areas of high resistance to conventional antimalarial drugs. It is a potentially promising anti-malaria drug that is composed of dihydroartemisinin and piperaquine phosphate. The present study investigates the fertility effects of therapeutic dose of P-alaxin. Fifteen male adult wistar rats of weighing between 150 and 210gm were grouped into three consisting of 5 males per group. The control group was orally administered with normal saline, the test and recovery groups were given body weight 15.4mg/kg of P-alaxin orally for three days after which the recovery group was allowed to recover from the drug's effect for another three days. The animals were sacrificed twenty four (24) hours after the experiment. The blood samples were collected through cardiac puncture.The result showed significant difference ($p \leq 0.05$) in sperm count, sperm motility, sperm viability as well as in serum testosterone level of the male rats administered with P- alaxin and the recovery group when compared with the control group.The results suggest that oral administration of P – alaxin has significant negative effects ($P < 0.05$) on sperm parameters and serum testosterone and could reduce male wistar rats fertility.
Keywords: P-alaxin, artemisinin, antimalarial, sperm parameters, serum testosterone

Introduction

Malaria is an important tropical mosquito-borne infection affecting millions of people around the world. It is a mosquito-borne infectious disease of humans and other animals caused by *Plasmodia* and are also definitely the single most destructive and dangerous infectious agent in the developing countries of the world (Olayinka and Ore, 2013). There are many health effects of this infection. Artemisinin and their derivatives have been recommended for the treatment of severe and complicated malaria. They are very active anti-malaria drugs producing up to ten thousand fold reductions in parasite biomass per a sexual cycle and they reduce malaria transmissibility (White, 1999).

P-alaxin is a film coated tablet containing 320mg of Dihydroartemisinin (DHA) and 40mg of piperaquine phosphate (PQP). The drug combination is known to be effective against *P. vivax*, *P. malariae* and the multi-resistant *P. falciparum* malaria parasites. (Song *et al.,* 2003, Olayinka and Ore 2013)

Dihydroartemisinin is the active metabolite of artemisinin and its derivatives. These derivatives have more potent blood schizonticidal activity than the parent compound. Dihydroartemisinin is the most potent antimalarial of this group of compounds but it is also the least stable. Oral dihydroartemisinin is rapidly absorbed and has a short elimination half-life (Song *et al.,* 2003).

Piperaquine phosphate, a bisquinoline, structurally related to chloroquine has been shown to exhibit cardiovascular toxicity in overdose with significant electrophysiological effects on the ear (Davis *et al.,* 2005).

The structural determinant of the activity of artemisinins is the endoperoxide bridge which is a specific feature of this type of compounds. It has been suggested that the parasiticidal activity starts with the reaction of artemisinins with haem iron, leading to the generation of activated oxygen species, such as oxygen radicals *(Mann et al., 200*). Nosten and White (Nosten and White, 2007), had reported that if there is any toxicity observed in artemisinin combination treatments, it may be due to the non-artemisinin component as artemisinin derivatives alone may have relatively low toxicological effects.

Decreased semen quality in malarial infected male has been reported (Singer et al., 1987). Many anti-malaria and antibiotic agents have been reported to have anti-fertility actions. The anti -steroidogenic and anti-fertility actions of quinine, chloroquine and artemether have been well documented (Meisel et. al., 1993; Adeeko and Dada, 1998; Raji et al., 2005). With the increased efforts in the development of more potent anti-malaria agents as a result of the challenge posed by the resistant strains of the malaria parasite, the evaluation of these anti-malaria agents for possible anti-fertility actions becomes important, this is necessary since both malaria and infertility are worldwide phenomena and the need to avoid the risk of infertility resulting from malaria chemotherapy. Whereas P- alaxin is becoming popular as an antimalalrial drug with remarkable efficacy, there is paucity of information in the literature on the possible anti- fertility effect of its administration and this is the objective of this current study.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

P-Alaxin tablet used for this project was obtained from Bliss GVS Pharma Limited, India. Fifteen adult male wistar strain rats weighing between 150 and 210gm were obtained from the Animal House of the Physiology Department, Olabisi Onabanjo University, Ikenne, Ogun State. The rats were fed with standard rat pellets (Top Feed Nigeria Ltd., Ibadan, Nigeria) and water ad libitum. They were housed in individual wire cages in a temperature and humidity controlled room, having a 12-h light and dark cycle.

The control group (5 male wistar rats) was orally administered with normal saline. The test (5 male wistar rats) and the recovery groups (5 male wistar rats) were orally administered with 15.4mg/kg body weight of P-alaxin each for three days. After the last administration, the recovery group was allowed to recover from the drug's effect for another three days.

Animal Ethics:

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Use of Animals. (American Physiological Society, 2002).

Analytical procedure

Twenty four (24) hours after the last administration the animals were anaesthetized using diethyl ether and the blood collected by cardiac puncture.

Determination of serum testosterone: Serum obtained from the blood collected via cardiac puncture was used to measure the level of testosterone using the Enzyme Immuno Assay (EIA) technique as previously described (Raji et al., 2005).

Determination of sperm viability: The sperm viability was determined using Eosin/Nigrosin stain (Raji et al, 2003).

Determination of sperm motility: A simple classification system proposed by the World Health Organization (Raji et al., 2005), which provides the best possible assessment of sperm motility, was used.

Determination of sperm count: The new improved Naubauers counting chamber was used in the determination of sperm count. Drops of semen were placed in the chamber and a cover slip was applied. This was placed under light microscope and spermatozoa counted on each of the five squares.

Sperm pH: The pH was determined by using narrow range litmus paper.

STATISTICAL ANALYSIS

The values are expressed as Mean ± S.D (standard deviation of mean). The means of the groups were compared using one way ANOVA (analysis of variance) and level of significance was done using least significant difference (LSD) and Duncan multiple range test (DMRT) at $P< 0.05$.

RESULTS

Effect of P- alaxin on Sperm parameters: The results are shown in Table 1.

Effect of P-alaxin on sperm parameters (sperm counts, viability, motility and pH):

Administration of P- alaxin at 15.4mg/kg body weight for three days significantly reduced ($p<0.05$) the progressive sperm motility, sperm count and viability when compared with the control. There was no significant change in values of **pH** of the treated rats' semen when compared with the control. Drug withdrawal resulted in gradual restoration of sperm parameters in the male rats.

Effect of P- alaxin on serum testosterone level: Serum testosterone level of P- alaxin treated rats significantly reduced ($p<0.05$) when compared to the control. However, there was an appreciable increase in serum testosterone levels of rats in recovery group (Table 2).

Table 1: Effect of P- alaxin on sperm parameters after 3 days treatments and 3 days of recovery

Groups	Sperm motility (%)	Sperm count (10^6/mL)	Sperm Viability (%)	**pH**
Control	81.00 ± 4.18	79.20 ± 5.26	86.00 ± 2.24	7.50 ± 0.16
Test	70.OO ± 5.70*	50.60 ± 7.40*	66.00 ± 5.70*	7.30 ± 0.22
Recovery	78.00 ± 3.54*	67.60 ± 12.24*	81.00 ± 3.54*	7.78 ± 0.29

*P < 0.05 (p is significant at p < 0.05)

Table 2: Effect of P – alaxin on serum testosterone levels

Groups	Testosterone (nmol/L)
Control	2.75 ± 0.03
Test	1.49 ± 0.02*
Recovery	1.67 ± 0.04*

*P < 0.05 (p is significant at p < 0.05).

Discussion

Decrease in fertility after treatment of male rats with Chloroquine and Halofantrine have been reported to be due to impairment in sperm motility (Adeeko and Dada, 1998. Orisakwe et. al., 2003; Raji et al., 2005). Treatment with such antimalarial drugs results in reducing the sperm count, motility, fertility and viability, as well as in serum testosterone level. It has been suggested that these drugs cause androgen depletion at the targets levels and particularly in the caudal epididymis, thereby affecting the physiological maturation of sperm (Adeeko and Dada, 1998).

The result of this present study revealed that P- alaxin could cause reproductive impairment in male rats. The significant reduction in the sperm motility of rat that was treated suggests that the drug was able to permeate the blood-testis barriers. The decrease in sperm motility caused by chemical agents has earlier been reported to be due to their ability to permeates the blood-testis bearer (Baldessarini, 1980) and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in its outer part (Bloom and Faweett, 1995).

Drugs that affect the testicular functions will affect the quality and quantity of spermatozoa. The mean epididymal sperm number was significantly reduced (P<0.05) in all the treated groups. There was also a significant decrease in serum levels of testosterone in all the treated rats when compared with the control. The significant difference in the sperm motility, viability and counts of the rats provides evidence for the significant reductions in the circulating androgen levels. Testosterone is required for the growth and in association with follicle stimulating hormone, acts on the seminiferous tubules to initiate and maintain spermatogenesis (Christensen, 1975).

In this study, the decreased sperm count, motility and viability, may have resulted from the alteration in the epididymal milieu, probably due to androgen deficiency (significant decrease in serum testosterone is observed) consequent to the anti-androgenic property of P- alaxin.

REFERENCES

(1).Adeeko, A. O. and Dada, O. A. (1998). Chloroquine reduces the fertility capacity of epididymal sperm in rats. Afri. J. Med. Med. Sci. 27: 63-68.

(2). American Physiological Society (2002). Guiding principles for research involving animals and human beings. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283: 281–283.

(3). Baddessarini, R.J. (1980). In drugs and treatment of psychiatric disorders. The pharmacological basis of therapeutics Ed. By Goodman and Gilman Macmillan Pub. Co. Inc. Pg 301-417.

(4). Bloom. E. and Faweett, D.W. (1995). Male reproductive system. In textbook of History (Ed.Bloom.W.Saunders company. Philadelphia).

(5).Christensen. A.C. (1975). Leydig cells. In: Handbook of physiology, edited by P.O. Greep and E. B. Astwood. Washington D.C. American Physiological Society. 165-172.

(6). Davis T.M, Hung T.Y, Sim I.K, Karunajeewa H.A, Ilett KF (2005): Piperaquine: a resurgent antimalarial drug. Drugs, 65:75-87.

(7). Mann A., Ibrahim K., Oyewale A.O, Amupitan J.O, Okogun J.I (2009). Antimycobacterial Activity of some Medicinal Plants in Niger State, Nigeria. Afri. J. Infect. Dis, 3(2): 44-48.

(8).Meisel. M.L.,Winterhoff, H.,and Jakat. F.W. (1993). Tylosin inhibits the steriodogenesis in rat leydig cells in-vitro. Life Science. 53:77-84

(9). Nosten, F. and White, N.J. (2007): Artemisinin-based Combination Treatment of Falciparum Malaria. *Am. J. Trop. Med. Hyg.,* 77: 181-192.

(10). Olayinka, E.T. and Ore, A. (2013). Alterations in Antioxidant Status and Biochemical Indices following Administration of Dihydroartemisinin-Piperaquine Phosphate (P-ALAXIN). *Journal of Pharmacy and Biological Sciences,* 5:43-53.

(11). Orisakwe, O.E., Obi, E. and Udemezue, O.O. (2003) Effect of halofantrine on testicular architecture and testosterone level in guinea pigs. European Bulletin of drug Research 11: 05-109

(12). Raji Y, Osonuga I.O, Akinsomisoye O.S, Osonuga O.A, Mewoyeka O.O. (2005)Gonadotoxicity evaluation of oral artemisinin derivative in male rats. J Med Sc 5 (4): 303 – 6.
(13). Raji, Y., Udoh. U.S., Mewoyeka, O.O., Ononye, F.C. Bolarinwa, A.F. (2003). Implication of reproductive endrocrine malfunction in male antifertility efficacy of Azadirachta indica extract in rats. Afri. J., Med., Med. Sci. 32; 159-165.
(14). Singer R, Segenreich E, Sagiv M, Shohat B, Livni E, Bartoov B. (1987) Decreased semen quality in a male infected with malaria. Int J Androl. 10:685–9.
(15). Song JP, Duong S, Suou S, Thou T, Ses S, Sim Y, Tan B, Li GQ (2003): Clinical research of dihydroartemisinin-piperaquine with uncomplicated falciparum malaria. Natl Med J China, 83:1099-1100.
(16). White, N.J. (1999). Qinghaosu in combinations. Med. Trop. Mars 58 (3 suppl.): 85-88.