THE EFFECTS OF P-ALAXIN ON FERTILITY AND HEMATOLOGICAL PARAMETERS IN MALE ADULT WISTAR RATS

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ABSTRACT

P-Alaxin, is an anti-malarial drug that is highly effective in treating malaria all forms of malarial infection including multi drug resistance malaria in areas of high resistance especially in Africa. It is composed of dihydroartemisinin and piperaquine phosphate. This study investigates the effects of therapeutic dose of P-alaxin on fertility and hematological indices in rats. Fifteen male adult Wistar rats weighing between 150 and 220g were divided into three consisting of 5 rats per group. Normal saline was administered to the control group while the test and recovery groups were given 15.4mg/kg body weight of P-Alaxin orally for three days. The recovery group was allowed to recover for three days from the drug's effect. The animals were anaesthetized using diethyl ether. Blood samples were collected through cardiac puncture. Oral administration of P-alaxin (15.4mg/kg body weight) for three days reduced ($p<0.05$) significantly the sperm motility, sperm count, viability and testosterone as well as the PCV, Hb, WBC and RBC when compared to the control and there was no significant change in values of pH of the tests rats' semen. Allowing the rats to recover from the effects of the drug resulted in gradual restoration of sperm parameters and hematological indices under investigation. From the study it can be safely concluded that oral administration of P-Alaxin caused reduction in the hematological and sperm parameters and serum testosterone and could reduce male Wistar rats' fertility and also lead to anemia in rats

Keywords: antimalarial, sperm parameters, serum testosterone, hematological parameters.

Accepted Date: 9 June 2020

INTRODUCTION

Malaria 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). It is an important tropical mosquito-borne infection affecting millions of people around the world and 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, 2003). P-alaxin is a film coated tablet containing 40mg of Dihydroartemisinin (DHA) and 320mg 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; Carbello *et al*., 2011; 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, Carbello *et al,.*2011). 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 of the activity of artemisinins is determined by the endo-

peroxide 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.,* 2005; Aprioku, 2013). 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 hematological parameters and semen quality in malarial infected male has been reported (Singer *et al.,* 1987; Onuche and Mohammed, 2015). Many anti-malaria and antibiotic agents have been reported to have anti-fertility actions. The anti -steroidogenic and antifertility actions of quinine, chloroquine and artemether have been well documented (Meisel *et al.,* 1993; Adeeko and Dada, 1998; Raji *et al.,* 2005; Salman *et al.,* 2010; Akinsomisoye and Raji, 2011). 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 antimalarial drug with remarkable efficacy, there is paucity and conflicting information in the literature on the possible hematological as well as anti-fertility effects 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 in a Pharmaceutical store in Sagamu, Ogun State, Nigeria and manufactured by Bliss GVS Pharma Limited, India. Fifteen adult male Wistar rats weighing between 150 and 220g were obtained from the Animal House of the Department of Physiology, Olabisi Onabanjo University, Ikenne, Ogun State. The rats were given standard rat pellets (Top Feed Nigeria Ltd., Ibadan, Nigeria) and water ad libitum. They were housed in wire cages and under a 12-h light and dark cycle. The control group (5 rats) was given normal saline orally. The test (5 rats) and the recovery groups (5 rats) were orally administered with 15.4mg/kg body weight of drug each for three days. After the last administration, the test group were sacrificed by administration of diethyl ether and the recovery group was allowed to recover from the drug's effect for another three days before sacrifice.

Animal Ethics:

Ethical clearance was obtained from the departmental ethical committee and 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 (American Physiological Society, 2002) in the Use of Animals.

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; WHO, 2010). Determination of sperm motility: A simple classification system proposed by the World Health Organization (WHO, 2010), 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 (Oehninger *et al*., 2014). Sperm pH: The pH was determined by using narrow range litmus paper. Hematological parameters: Hematology was done according to standard methods (Dacie and Lewis, 1991; Weix *et al*., 2014).

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.

RESULTS

Effect of P-alaxin on sperm parameters is shown in Table 1.

Effect of P-alaxin on sperm parameters (sperm counts, viability, motility and pH): Oral **a**dministration 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 and there was no significant change in the of pH of the test rats' semen. Allowing the rats to recover from the effects of the drug resulted in gradual restoration of sperm parameters in the male rats.

Effect of P-alaxin on serum testosterone level: there was a significant reduction ($p<0.05$) in serum testosterone level of treated rats when compared to the control. However, the serum testosterone levels appreciably increase 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	83.00±4.18	77.20±5.26	89.00±2.24	7.50±0.16
Test	73.00±5.70*	55.60±7.40*	68.00±5.70*	7.30±0.22
Recovery	77.00±3.54*	65.60±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 after 3 days' treatments and 3days of recovery

Groups	Testosterone (nmol/L)
Control	2.85±0.03
Test	1.59±0.02*
Recovery	1.77±0.04*

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

Table 3: Effect of P-alaxin on hematological parameters after 3 days' treatments and 3days of recovery

Groups	**PCV (%)**	**Hb (x 10g/L)**	**WBC (10^9/L)**	**RBC (10^{12}/L)**
Control	43.07 ± 0.35	14.00 ± 0.20	6.50± 0.18	7.0± 0.30
Test	36.00 ± 1.80*	7.00 ± 1.60*	3.80 ±1.20*	4.70 ± 0.60*
Recovery	38.45 ± 0.20*	9.70 ± 0.86*	4.96 ±1.80*	5.52 ± 0.45*

*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 and this may be due to impairment in sperm motility (Adeeko and Dada, 1998; Orisakwe *et al.,* 2003; Raji *et al.,* 2005; Onuche and Mohammed, 2015). 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 advocated that these drugs caused androgen depletion at the targets levels and particularly in the caudal epididymis, thereby affecting the physiological maturation of sperm (Adeeko and Dada, 1998).This present study revealed that P- alaxin could cause 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 (Orisakwe *et al.,* 2003; Aprioku, 2013) and thus, creating a different micro environment 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 might 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 androgen levels. Testosterone 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 changes in the epididymal milieu; probably due to androgen deficiency (significant decrease in serum testosterone was observed) consequent to the anti-androgenic property of P- alaxin. Efforts were also made in this study to investigate the effect of P-alaxin on hematological parameters in male Wistar rats. We demonstrated that administration of P-alaxin significantly ($P<0.05$) reduced these measured parameters. Similar results were obtained by previous workers (Salako, 1984; Nontprasert *et al.,* 2000; Shuhua *et al.,* 2002; Osonuga *et al*., 2012)onpathological effects on body organs caused by oral administration of artemether and some other antimalarial agents. The changes observed in the Packed cell volume (PCV) and red blood cell count indicate that administration of P-alaxin to malaria patients may predispose them to anemia. In conclusion, P-alaxin is a form of artemisinin-based antimalarial medication that has been considered to have high margin of safety, but the result of this study suggests that the administration of therapeutic dose of P-alaxin may predispose to male reproductive toxicity and anemia in rats. Hence caution should be taken in administering the drug.

Conflict of interest: The authors declare that there is no conflict of interest between them.

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