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Haematological Changes Associated with Administration of Therapeutic dose of P-Alaxin in Healthy Adult Wistar Rats

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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 composed of dihydroartemisinin and piperaquine phosphate. The present study investigates the safety in-use of therapeutic dose of p-alaxin by healthy individuals.

Thirty adult wistar rats of both sexes weighing between 180 and 210g were grouped into three consisting of 5 males and 5 females 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 into heparinised tubes centrifuged at 5000rpm for 10mins and was used for haematological assay.

The result showed no significant difference *($p \geq 0.05$)* in packed cell volume (PCV), Red blood cell count and White Blood cell count of the male rats administered with P- alaxin and the recovery group when compared with the female groups. Whereas a significant increase ($p \leq 0.05$) was observed in the haemoglobin (HB) level of the male rats after treatment, there was no significant change in the Haemoglobin (HB) level of the female animals when the drug was administered as well as during the recovery period.

The results of this study indicate administration of p-alaxin in healthy individuals will neither induce haemolysis nor anaemia.
Keywords: P-alaxin, artemisinin, antimalarial, haemoglobin

INTRODUCTION

Malaria is one of the most serious health challenges facing the world today. 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).This disease results from the multiplication of *Plasmodium* parasites within red blood cells. Studies revealed that five species of *Plasmodium* can infect and be transmitted by humans (Sutherland et al., 2010).

Malaria is largely caused by *Plasmodium falciparum* while the malaria caused by *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* is generally a milder disease that is rarely fatal (Sutherland *et al.,* 2010). *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques which can also infect humans (Sibley *et al.*, 2004; Collins, 2012).

There were an estimated 225 million cases of malaria worldwide in 2009 (WHO, 2011). An estimated 655,000 people died from malaria in 2010, a decrease from the 781,000 who died in 2009 (WHO, 2011), accounting for 2.23% of deaths worldwide. However, a 2012 meta-study from the University of Washington and University of Queensland estimates that malaria deaths are significantly higher (Christopher *et al*., 2012). The study estimates that 1,238,000 people died from malaria in 2010. Ninety percent of malaria-related deaths occur in sub-Saharan Africa, with 60% of deaths being young children under the age of five (Christopher *et al*., 2012).

P-alaxin is a combination of Dihydroartemisinin (40mg) and of Piperaquine phosphate (320mg). Piperaquine is a bisquinoline, first synthesized in 1960s in China and France, which is as effective as chloroquine. The tolerability, efficacy, pharmacokinetic profile and low cost of piperaquine make it a promising combination drug for an artemisinin combined based therapy (ACT). P-alaxin is very effective in treating malaria in areas of high resistance to conventional anti-malaria drug. The drug is usually being prescribed as an alternative to other artemisinin combined therapy such as coartem (WHO, 2011). The artemisinin-derivatives, artemether, artesunate, and dihydroartemisinin, are currently the most potent anti-malarial medicines in the market. They are widely available in the different pharmaceutical dosage forms including tablets, injections, suppositories and dry powders (Olayinka and Ore, 2013).

Whereas P- alaxin is becoming popular as an antimalalrial drug with remarkable efficacy, there is paucity of information in the literature on the haematotoxic effect of its administration. This is the goal of this study

MATERIALS AND METHODS

Experimental Design

P-Alaxin tablet used for this project was obtained from Bliss GVS Pharma Limited, India. Thirty adult wistar strain rats of both sexes, weighing between 180 and 210g 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 was orally administered with normal saline. The test and the recovery groups 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.

Procedure for Sacrificing the Animals and Collection of Sample

Twenty four (24) hours after the last administration the animals were anaesthetized using diethyl ether and the blood collected by cardiac puncture into lithium heparinised bottle. The blood samples were centrifuged at 5000rpm for 5 minutes and the plasma and the packed cells separated. The plasma was kept in a clean specimen bottles placed in ice bucket and refrigerated at -4^0C until they were used for the haematological assay.

Haematological Tests

The blood samples were assayed for the following haematological parameters: Packed Cell Volume (PCV) (Dacie and Lewis, 1991); Haemoglobin Determination (Dacie and Lewis, 1991); Red Blood Count (RBC) and White Blood Count (WBC) (Dacie and Lewis, 1991).

Animal Care

The care of the animals was in accordance with the U.S. Public Health Service Guidelines and approved by the Animal Health Ethics Committee, Dept of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago- Iwoye.

Statistics

Values are expressed as Mean of 5 replicates ± SEM (Standard Error of Mean). Data were subjected to one way analysis of Variance (ANOVA) and level of significance was done using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ of SPSS version 15 software.

RESULTS

Table 1 showed the result of the effect of treatment on the PCV value. When the PCV values were compared between the female and the male rats in the control, test and recovery groups, the result showed that the values were not significant different ($p \geq 0.05$) from each other. Similarly the no significant variation was observed in the PCV values in the male and female rats when compared between the treatment groups.

The results of the treatment on the Haemoglobin levels of both experimental and control rats showed a significant increase ($p \leq 0.05$) in the level of Haemoglobin (HB) of the male rats that were treated with p-alaxin when compared with the control group. The observed HB level in the recovery group was however not different from that of test group. On the contrary, there was no significant change in the Haemoglobin (HB) level of the female rats when the drug was administered as well as during the process of recovery. When the HB levels in all the groups were compared between the male and female rats, the result showed no significant variation (Table 2). The result of the effect of treatment on the red blood cell is shown in Table 3. The result indicates that Red Blood Count (RBC) of all the groups administered with p-alaxin was not significantly different from that of the control group. When the RBC of the male and female rats was compared in all the groups of rats, no significant variations were observed in the RBC counts.

Shown in Table 4 is the result of the effect of treatment on White Blood Cell count (WBC). No significant change was observed in the WBC of the male rats when compared between the groups. A similar result was observed in the WBC of the female rats after treatment. The result also indicates that in all the groups of rats studied, the WBC count does not varied between the male and the female rats.

DISCUSSION

The haematological parameters Hb, PCV, RBC and WBC provide information on the general state of the blood of the subjects used for this study. The results of our findings suggested that P-alaxin could induce reversible

changes in some haematological parameters. Our study indicates that administration of therapeutic dose of p-alaxin to healthy individual will not significantly alter the Packed Cell Volume (PCV), Red Blood Cell Count (RBC) and White Blood Cell (WBC). The results also suggest that variation in these parameters is not sex dependent. Our result however suggest that whereas haemoglobin level increased significantly in male rats treated with the drug, the level of haemoglobin was not altered in the female rats. Both were reduced to normal in the process of recovery from the effect of the drug. These findings were similar to an earlier study reported by Obianine and Aprioku (2011) where an insignificant increase was also observed in Red Blood Count (RBC) and Haemoglobin (HB) when male guinea pigs were administered with Artesunate and Dihydroartemisinin (DHA).

The restoration of the haematological parameters after the withdrawal of the drug was similar to that observed with chloroquine, artesunate with DHA and artemether which were reported by Osonuga et al., (2009), Aprioku and Obianine (2011) and Osonuga et al., (2012) respectively.

Common side effect that has been reported to be associated with the use of ACT includes vomiting, anaemia, cough and abdominal pain (Georgewile, 2012). The changes observed in the Packed Cell Volume (PCV) indicate that administration of p- alaxin to malaria patients may not predispose to anaemia. It also suggest that the use of P-alaxin in addition to being placed on well-nourished food in malaria patients may increase the patient's Red Blood Count (RBC), White Blood Count (WBC) and Haemoglobin (HB) level which in turn will be of an advantage to the malaria patients.

The observation of a significant increase in the level of Haemoglobin (HB) in the male rats treated with p-alaxin as reported in this suggest the absence of trauma in animals administered with the drug. Increase in HB of the male animals may be due to anabolic effects brought about by their high testosterone level (Ruston et al., 2001).

CONCLUSION

P-alaxin as a form of artemisinin has been considered to have high safety margin. The result of this study suggests that the administration of therapeutic dose of p-alaxin by healthy individuals may not predispose to anaemia. Care however should be taken in administering the drug.

REFERENCES

Aprioku, J.S. and Obianime, A.W. (2011): Structure-Activity-Relationship (SAR) of Artemisinins on Some Biological Systems in Male Guinea Pigs. Insight Pharmaceutical Sciences, 1 (1): 1-10.

Christopher, J.L., Lisa C.R., Stephen S.L., Kathryn G.A., Kyle, J.M.N., Rafael, L. and Alan, D.L. (2012): Global Malaria Mortality Between 1980 and 2012: A Systematic Analysis. Lancet, 379: 413-431.

Dacie J.V. and Lewis S.M. (1991): Practical Haematology. 5th Edition. Churchill Livingstone, Edinburgh. Pp 13.

Georgewill , O.E. (2012): Artemisinin Combination Therapies; Safe Or Not Safe ?. The Internet Journal of Pharmacology. 10 (1). DOI: 10.5580/2b58

Obianime, A.W. and Aprioku, J.S. (2011): Mechanism of Action of Artemisinins on Biochemical Hematological and Reproductive Parameters in Male Guinea Pigs. International Journal of Pharmacology, 7 (1): 84-93.

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.

Osonuga, O.A., Osonuga, I.O., Akinsomisoye, O.S., Raji, Y. and Ademowo, O.G. (2006): Packed Cell Volume Changes in the Treatment of Severe Malaria Patients with Artemether and Quinine. Journal of Med. Sci., 6 (5): 853-857.

Osonuga, I.O., Ajibola, A, Osonuga, O.A., Idowu, G.O., Akindele, R.A. and Dauda, T.O. (2009): Haematotoxicity of Oral Administrtion of Chloroquine in Uninfected Male Rats. Journal of Medical and Pharmaceutical Sciences, 5 (1): 10-12.

Osonuga, I.O., Osonuga, O.A., Osonuga, A., Onadeko, A.A. and Osonuga, A.A. (2012): The Acute Hepatotoxic Effect of Halofantrine on Healthy and Uninfected Adult Wistar Rats. International Journal of Pharmacology, ISSN 1811-775: 1-3.

Osonuga, I.O., Ekun, O.A., Osonuga, O.A., Iquot, I.S. and Akinola, B.O. (2010): Effects of Coartem (Artemether and Lumenfantrine) in Liver Enzymes in Adult Wistar Rats of Both Sexes. Journal of Advancement in Medical and Pharmaceutical Sciences, 4 (1): 26-29.

Osonuga, I.O., Osonuga, O.A., Osonuga, A, Onadeko, A.A. and Osonuga, A.A. (2012): The Effect of Artemether on Hematological Parameters of Healthy and Uninfected Adult Wistar Rats. Asian Pacific Journal of Tropic Biomedicine, 1-3.

Ruston, D.H, Robin, D., Anthony, W.S., Michael, J.N., Jeremy, J.H., Lan, D.R. (2001): Why should women have lower reference limits for haemoglobin and ferritin concentration than men. BMJ, 322 (7298) :1355-1357.

Sibley, C.H., Hyde, J.E., Sims, P.F.G., Plowe, C.V., Kublin, J.G., Mberu, E.K., Cowman, A.F., Winstanley, P.A., Watkins, W.M. and Nzila, A.M. (2001): Pyrimethamine sulfadoxine Resistance in Plasmodium falciparum; What is Next. Trends in Parasitology, 17: 582-588.

Sutherland, C.L., Tanomising, N. and Nolder, D. (2010): Two Non recombining Sympatric Forms of the Human Malaria Parasite Plasmodium ovale Occur Globally, J. Infect. Dis. 201 (10): 1544-1550.

WHO (2011): National Guidelines For diagnosis and Treatment of Malaria, Pp.1-52.

Table 1 Effect of P-alaxin on Packed Cell Volume of Experimental and Control Rats

	Packed Cell Volume (PCV) (%)	
	Male	**Female**
Control (normal saline)	44.20±1.28^{a†}	42.20±1.36^{a†}
Test Group (p-alaxin)	47.60±1.40^{a†}	46.20±1.88^{a†}
Recovery Group	44.00±1.70^{a†}	45.60±1.29^{a†}

Note:
1. Results are mean ± SEM of 5 determinations
2. Values in the same column with similar superscripts are not significantly different from each other.
3. Values in the same row with the symbol † are not significantly different from each other.

Table 2 Effects of P-alaxin on Haemoglobin of Experimental and Control Rats

	Haemoglobin (HB) (g/l)	
	Male	**Female**
Control (normal saline)	133.80±3.89^{a†}	133.40±3.63^{x†}
Test Group (p-alaxin)	154.00±1.00^{b†}	145.80±5.95^{x†}
Recovery Group	135.40±4.37^{a†}	141.60±2.40^{x†}

Note:
4. Results are mean ± SEM of 5 determinations
5. Values in the same column with similar superscripts are not significantly different from each other.
6. Values in the same row with the symbol † are not significantly different from each other.

Table 3 Effect of P-alaxin on Red Blood Cell of Experimental and Control Rats

	Red Blood Cell (RBC) (x10^{12}l)	
	Male	**Female**
Control (normal saline)	4.79±0.11^{a†}	4.46±0.12^{a†}
Test Group (p-alaxin)	5.60±0.55^{a†}	5.22±0.18^{a†}
Recovery Group	4.90±0.32^{a†}	5.00±0.25^{a†}

Note:
7. Results are mean ± SEM of 5 determinations
8. Values in the same column with similar superscripts are not significantly different from each other.
9. Values in the same row with the symbol † are not significantly different from each other.

Table 4: Effect of P-alaxin on White Blood Cell Count of Experimental and Control Rats

	White Blood Cell Count (WBC) (mm^3)	
	Male	**Female**
Control (normal saline)	5480.00±381.31[a]	5700.00±404.97[x]
Test Group (p-alaxin)	5440.00±312.41[a]	6480.00±263.44[x]
Recovery Group	5440.00±618.55[a]	5720.00±382.62[x]

Note:

10. Results are mean ± SEM of 5 determinations
11. Values in the same column with similar superscripts are not significantly different from each other.
12. Values in the same row with the symbol † are not significantly different from each other.

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