

Comparative Effect of Three Antimalarials (Quinine, Artemether and Fansider) on Some Reproductive Organs and Serum Testosterone Level in Male Wistar Rats

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Abstract: The aim of this study was to investigate the effect of some commonly used ant malarial agents in Nigeria on sperm concentration, epididymal, seminal vesicle, testicular weights and serum testosterone level on adult male albino rats. Malaria and its complications pose serious health challenges in sub-saharan Africa and the possible side effects of anti-malarials on reproductive functions should be of serious consideration. Twenty one adult rats were randomly divided into five groups (Group A, B₁, B₂, C and D) of five rats each with group B₁ and B₂ having 3 rats each. Group A serve as the control and was administered 0.9% normal saline; Group B₁ was administered quinine dihydrochloride (5 mg/kg); Group B₂ was administered quinine dihydrochloride (10 mg/kg); Group C was administered Artemether (20 mg/kg) and Group D was administered sulfadoxine/pyrimethamine (22.5 mg/kg). The ant malarial agents were administered via intraperitoneal route for 4 days. Testicular, seminal vesicle and epididymal body weight ratio; sperm count and serum testosterone level were evaluated using standard methods. Results of the experiment showed a dose dependent significant decrease ($p < 0.05$) in the epididymal weight by Artemether and higher dose of quinine (10 mg/kg bw), but there were no significant differences in seminal vesicle and testicular weights; sperm concentration and serum testosterone levels between the experimental and control animals. The results suggest that the administration of the three antimalarial agents at recommended doses and duration employed in this study had no adverse effects on testicular function and serum testosterone level.

Keywords: Artemether, epididymis, fansidar (sulfadoxine/pyrimethamine), quinine, seminal vesicle, sperm concentration, testes, testosterone

INTRODUCTION

Malaria is one of the most deadly diseases in Africa, causing high morbidity and mortality with attendant adverse economic consequences (WHO, 1998; Sachs and Malaney, 2002). Effective treatment of malaria has been a great challenge due to resistance of the malaria parasite to antimalaria drugs (Olliaro and Taylor, 2004; White, 2004). Currently, artemisinin compounds are advocated for the treatment of the disease in Africa due to their high efficacies (Greenwood *et al.*, 2005; Maiteki-Sebuguzi *et al.*, 2008).

The most widely used artemisinin derivatives are artesunate, artemether and Dihydroartemisinin (artemimol, DHA). Others include arteether (artemotil) and artelinic acid. Although artemisinin derivatives are effective against the plasmodium parasite (as monotherapies), combination therapies consisting of artemisinin and other standard antimalaria drugs have been demonstrated to have better parasite clearance and efficacies (Olliaro and Taylor, 2004; Adjuik *et al.*, 2004; Nosten and White, 2007). These Artemisinin based Combination Therapies (ACTs) include

artesunate + amodiaquine, artemether + lumenfantrine, artesunate + sulfadoxine + pyrimethamine and artesunate + mefloquine (WHO, 1995; Nosten and White, 2007).

Some antimalaria agents have been associated with male reproductive dysfunction in laboratory animals (Joshi *et al.*, 1996; Parveen *et al.*, 2003). Studies with mice and albino rats had shown that some artemisinin derivatives have adverse effects on sperm parameters (Dapper *et al.*, 2002; Nwanjo *et al.*, 2007). The aim of this study therefore was to investigate the effect of some commonly used antimalarial agents in Nigeria on sperm concentration, epididymal, seminal vesicle, testicular weights and serum testosterone level in male albino rats.

METHODOLOGY

Laboratory animals, laboratory conditions and ethical considerations: Twenty one adult male wistar rats were purchased from National Institute of Trypanosomiasis Research (NITR), Vom, Jos, Plateau State, NIGERIA. The weight ranged from 110 to 226 g. They were randomly divided into five groups (A, B₁,

B₂, C, D) of five rats each with group B having 6 rats, they were housed in suspended cages placed in a well ventilated animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were fed pelleted growers' feed (Vital Feed, Jos, Nigeria) before and during the experimental period, had free access to water. The temperature, relative humidity and photoperiods (12 h Light/12 h dark cycle) were maintained during the period of acclimatization and throughout the study.

Drinking water was changed daily and alternate day clearing and replacement of sawdust and droppings were carried out.

Laboratory animals were humanely handled according to Ahmadu Bello University Ethical Committee on Research guidelines on handling of laboratory animals.

Experimental procedure: The animals were divided into 5 groups A, B₁, B₂, C, D of five animals each:

Group A : (Control) received physiologic (normal) saline

Group B₁ : Received Quinine dihydrochloride (5 mg/kg b.w.)

Group B₂ : Received Quinine dihydrochloride (10 mg/kg b.w.)

Group C : Received Artemether (20 mg/kg b.w.)

Group D : Received Fansidar (22.5 mg/kg b.w.)

Route of drug administration: The antimalarial agents were administered Intraperitoneally (IP) for a period of 4 days before the sacrifice.

Anaesthesia and blood collection: At the termination of drug administration, the animals were anaesthetized by light chloroform inhalation. The thorax of each anaesthetized animal was cut open and with the aid of 5 mL syringe and 23 G needle, the pulsating heart of the rat was pierced and blood was aspirated and immediately centrifuged to obtain serum which was stored at 4°C until used for the determination of serum testosterone level.

Tissue collection: Under anaesthesia, the scrotal sacs were opened to expose the testes and epididymis. The right testis, right epididymis and seminal vesicle of each rat were cleaned of fats, weighed and the right testes and epididymis were fixed in Bouin's fluid and seminal vesicles in 10% formalin for histological examinations while the left epididymis of each rat was used for determination of epididymal sperm concentration.

Determination of epididymal sperm concentration: Sperm analysis was done on sample derived from the caudal epididymis. The epididymis was opened using a

pair of scissors and semen expressed into a petri dish. 1 mL of normal saline was added to semen and mixed thoroughly using a syringe to draw and release the mixture continuously (Verma and Chinoy, 2002).

The semen mixture was then sucked up into red blood cell pipette to the 0.5 mark then normal saline was sucked up to 101 marks, the normal saline in the stem of the pipette was discarded and the content of the bulb of the pipette was mixed thoroughly. A drop of the mixture was placed in the Neubaer counting chamber which then spreads under the cover slip by capillary action. A grid system divides the counting chamber into 5 major squares each containing 16 smaller boxes. The count included all the sperm cells within the 5 major squares using the top and right system of counting (Verma and Chinoy, 2002; Zaveneid and Polakoski, 1977).

The epididymal sperm concentration for each rat was calculated as $n \times 1 \times 10^{-6} / \text{mL}$ of semen.

Hormonal assay: Blood samples were centrifuged at 2500 rpm for 10 min in a BTL bench centrifuge. Serum samples obtained were assayed for testosterone using the tube-based Enzyme Immunoassay (EIA) method. The EIA kits were manufactured by Immunometrics (UK) Ltd.

STATISTICAL ANALYSIS

Data were expressed as mean \pm standard errors of mean. Comparisons between control and treated groups of wistar rats were performed with One-Way Analysis of Variance (ANOVA). Statistical significance was set at $p < 0.05$.

RESULTS

From the results obtained, administration of fansidar, quinine and Artemether caused no significant change in the weights of the testis and seminal vesicles after four days of administration when compared with the control. However, epididymis somatic weight ratio of $0.16 \pm 0.00\%$ and $0.16 \pm 0.0\%$ in animals treated with quinine at a dose of 10 mg/kg and Artemether (20 mg/kg), respectively were significantly lower ($p < 0.05$) than values obtained from the control animals (Table 1).

Sperm concentration of 12.6 ± 3.18 million/mL in animals treated with 5 mg/kg body weight of Quinine was significantly lower ($p < 0.05$) than values obtained in the control and other experimental groups as shown in Table 1.

Administration of the various anti-malarial drugs to the experimental animals as shown in Table 2 did not significantly alter the serum testosterone levels when compared to the serum testosterone levels in the control rats ($p > 0.05$).

Table 1: Effect of antimalarial agents on epididymal, testicular, seminal vesicle body weight ratios and sperm concentration of albino rats

Parameters	Control	Quinine 5 mg/kg	Quinine 10 mg/kg	Atmt 20 mg/kg	Fans 22.5 mg/kg
Epididymal wt body ratio (%)	0.28±0.03	0.26±0.4	0.016±0.0*	0.016±0.0*	0.020±0.02
Testicular body wt ratio (%)	0.68±0.10	0.72±0.5	0.80±0.55	0.59±0.02	0.63±0.02
Seminal vesicle body wt ratio (%)	0.46±0.07	0.25±0.0	0.26±0.15	0.29±0.05	0.47±0.06
Sperm concentration (million/mL)	22.40±1.03	12.6±3.18*	22.00±9.61	23.00±4.51	21.40±1.25

Values are expressed as mean±SEM, n = 5; *: Significantly lower than control at p<0.05; Atmt = Artemether., Fans = Fansidar (Pyremethamine/sulphadoxime)

Table 2: Effect of antimalarial agents on serum testosterone level of male rats

Groups	Mean±SEM	Serum testosterone (mg/mL)
Group A	Control	1.06±0.50
Group B ₁	Quinine 5 mg/kg	1.23±0.58
Group B ₂	Quinine 10 mg/kg	1.43±0.30
Group C	Artemether 20 mg/kg	1.98±0.69
Group D	Fansidar 22.5 mg/kg	1.38±0.37

Values are expressed as mean±SEM

DISCUSSION

The study revealed an insignificant reduction in epididymal sperm concentration in the Artemether, quinine dihydrochloride and sulfadoxine/pyrimethamine administered groups as compared with control. This result agrees with the findings of Akinlolu *et al.* (2007) who reported that rats exposed to recommend doses of Artemether for 7 days showed normal morphological appearance of the testis with evidence of spermatogenesis. A contrary report by Raji *et al.* (2005) indicates that exposure to Artemether for a period of 3 days at a very high dose level cause impairment to reproductive activity exhibited by reduce sperm count, motility, normal morphology; testosterone level and fertility index. It therefore, appears that effect of Artemether on reproductive functions in the rat is both dose and duration dependent with the dose factor being more pronounced. It is reported that sperm production, development and maturation are processes that are vulnerable to interferences in the internal environment of the reproductive organs (Orgebin-Christ *et al.*, 1975).

The weight of the male reproductive organ usually provides a useful reproductive risk assessment in experimental studies (Raji *et al.*, 2005). This observation may be due to the short duration of exposure of the male genital organs to the antimalarials (Dapper *et al.*, 2002). It is therefore plausible to note that the weight of the testis and these accessory reproductive organs may be associated with degenerative changes and reduced tubular size as reported by Ukenya *et al.* (2010). The observed decrease weight in higher doses of quinine and Artemether administered rats may be associated to the sensitivity of the organ to the agents. This reflects the extent of degeneration caused by the drugs and might have resulted from reduced tubular size (Ukwenya *et al.*, 2010).

The serum testosterone level in this study remained normal (Adeeko and Dada, 1998).

CONCLUSION

In conclusion the short term administration of quinine, Artemether and fansidar at the doses and durations employed in this study had no adverse effects on testicular functions. However, there is indication that high doses of quinine and arthemeter with prolonged treatment cause a decrease in epididymal body ratio and could possibly cause some degenerative changes in the seminiferous tubules.

REFERENCES

- Adeeko, A.O. and O.A. Dada, 1998. Chloroquine reduces the fertility capacity of epididymal sperm in rats. *Afr. J. Med. Sci.*, 27: 63-68.
- Adjuik, M., A. Babinker, P. Garner, P. Olliaro, W. Taylor and N. White, 2004. Atesunate combination for treatment of malaria: Meta-analysis. *Lancet*, 363: 9-17.
- Akinlolu, A.A., J. Ootulana, O. Olatunde, B.E. Adebayo and O.B. Akinlola, 2007. The antispermatic and antifertility effects of artemether on the testis of adult wistar rats. *Pak. J. Pathol.*, 18(2): 64-67.
- Dapper, D.V., B. Didian and C. Gbigbi, 2002. Preliminary study on the effect of Halofantrine Hydrochloride on the testes of mature Wistar Rats. *J. Appl. Sci. Environ. Manage.*, 6(1): 45-48.
- Greenwood, B.M., K. Bojang, C.J. Whitty and G.A. Targett, 2005. Malaria. *Lancet*, 365: 1487-1498.
- Joshi, A.R., R.N. Ahamed, K.N. Pathan and B. Manivannah, 1996. Effects of Azadirachtaindica leaves on the testis and its recovery in albino rats. *Ind. J. Exp. Biol.*, 34: 1091-1094.
- Maiteki-Sebuguzi, C., P. Jagannathan, V.M. Yau, T.D. Clark, D. Njama-Meya *et al.*, 2008. Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children. *Malaria J.*, 7: 106-109.
- Nosten, F. and N.J. White, 2007. Artemisinin-based combination treatment of Falciparum malaria. *Am. J. Trop. Med. Hyg.*, 77(6): 181-192.
- Nwanjo, H.U., I.I. Iroagba, I.N. Nnatuanya and N.A. Eze, 2007. Antifertility activity of dihydroartemisinin in male Albino rats. *Internet J. Endocrinol.*, 4(1).
- Olliaro, P.L. and W.R. Taylor, 2004. Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria: A review. *J. Postgrad. Med.*, 50: 40-44.

- Orgebin-Christ, M.C., B.J. Denzo and J. Davier, 1975. Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: Halmiton, D.W. and R.O. Greeps (Eds.), *Handbook of Physiology. Section 7, Vol. 5.* Am. Physiol. Soc. Washington, DC, pp: 319-338.
- Parveen, S., S. Das, C.P. Kundra and B.M.I. Pereira, 2003. A comprehensive evaluation of the reproductive toxicity of Quassiaamara in male rats. *Reprod. Toxicol., 17:* 45-50.
- Raji, Y., S.O. Ifabunmi, O.S. Akinsomisoye, A.O. Morakiyo and A.K. Oloyo, 2005. Gonadal responses to antipsychotic drugs: Chlorpromazine and thioridazine reversibly suppress testicular functions in albino rats. *Int. J. Pharmacol., 1(3):* 287-292.
- Sachs, J. and P. Malaney, 2002. The economic and social burden of malaria. *Nature, 415:* 680-685.
- Ukwenya, V., G.A. Sodunke, A.S. Tijani and J.B. Falunle. 2010. Acute administration of co-artesiane induces oxidative stress in the testis of adult male Wistar rats. *Biosci. Res. Commun., 22(5):* 259-264.
- Verma, R.J. and N.J. Chinoy, 2002. Effects of carica papaya seeds extract on male albino rats. *Asian J. Androl., 4(1):* 77-78.
- White, N.J., 2004. Antimalarial drug resistance. *J. Clin. Invest., 113(8):* 1084-92.
- WHO, 1995. *The Role of Artemisinin and its Derivatives in the Current Treatment of Malaria.* World Health Organization, Geneva.
- WHO, 1998. *Malaria Chemotherapy.* World Health Organization, Geneva.
- Zaveneid, L.D.J. and K.L. Polakoski, 1977. Collection and the Physical Examination. In: Hafez, E.S.E. (Ed.), *Techniques of Human Andrology.* Holland Biomedical Press, Amsterdam, pp: 147-156.