British Journal of Pharmaceutical Research
7(1): 22-33, 2015, Article no.BJPR.2015.088
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Assessment of Mutagenic Effects of Sulfadoxine-Pyrimethamine (SP) on Animal Model

N. J. Chukwurah[1*], O. O. Aina[2] and O. A. Otubanjo[1]

[1]Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria.
[2]Division of Biochemistry and Nutrition, Nigerian Institution of Medical Research Yaba, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author NJC designed the study, performed the statistical analysis and wrote the protocol as well as the first draft of the manuscript. Author OAO managed the literature searches. Author OOA managed the study design, carried out the experiment and managed the analysis of the study. All authors read and approved the final draft.

Article Information

DOI: 10.9734/BJPR/2015/18164
Editor(s):
(1) Elena G. Zavyalova, Chemistry Department, Moscow State University, Russia.
Reviewers:
(1) Taratisio Ndwiga, Department of Environmental Health, Moi University, Eldoret, Kenya.
(2) Zoraima Neto, Institute of Hygiene & Tropical Medicine (IHMT), New University of Lisbon (UNL), Portugal.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1176&id=14&aid=9358

Original Research Article

Received 8th April 2015
Accepted 9th May 2015
Published 23rd May 2015

ABSTRACT

Introduction: The use of medicines in pregnancy has always been a source of concern because of adverse effects on the foetus.
Aim: The study therefore investigated the mutagenic potential of sulfadoxine-pyrimethamine (SP) in Swiss albino mice.
Study Design: Comparative study.
Place and duration of the study: The study was carried out at Nigerian Institute of Medical Research, Yaba within 4weeks.
Methodology: Four groups of pregnant female laboratory bred virgin mice were used. Group 1 received the normal human therapeutic dose (HTD) of SP. Group 2 received 1½ X HTD group while group 3 received 2X HTD. Group 4 received only water and served as the control. Assessment of sperm head abnormality was conducted using four groups of laboratory bred male Swiss albino mice. Group 1 received the HTD; group 2, 1½ while group 3 received 2 X HTD. The fourth group received only water and served as the control.
Results: The result showed no abnormality when the fetuses were examined and there was no significant association between the test mice and the control mice, P>0.05. Analysis of the sperm

**Corresponding author: Email: ekachu2006@yahoo.com;*

head abnormality showed no significant association between the test and control male mice.
Conclusion: Inference can be made from this study that that SP may not be adjudged to induce sperm head abnormalities and may not be mutagenic. It can therefore be concluded that SP is safe for use in pregnancy after quickening.

Keywords: SP; mutagenic effect; mice; pregnancy.

1. INTRODUCTION

Effective treatment of malaria had been a great challenge to medicine and this has impacted enormously on man's health and economy [1,2]. Treatment failures in malaria have been linked majorly to the development of resistance of the malaria parasite to standard antimalarial agents particularly to chloroquine and to some extentsulfadoxine-pyrimethamine [3-5]. Consequently, different antimalarial agents and regimens have been developed over the period to cope with this challenging problem [3]. The present choices of drugs are the artemisinin and its derivatives [6,7]. Also, better cure rates and clearance of the malaria parasite from the blood are achieved with the artesmisin-based combination therapies (ACTs), which include-artesunate / amodiaquine, artesunate / sulfadoxine / pyrimethamine, artesunate / mefloquine and artemether / lumefantrine [8,9].

Quinine, as a component of the bark of the cinchona (quina-quina) tree, was used to treat malaria from as early as the 1600s, when it was referred to as the "Jesuits' bark," "cardinal's bark," or "sacred bark [10]. Quinine was widely used until the discovery of chloroquine during the Second World War, [10]. With the development of parasite resistance to quinine, the newer more effective drugs like chloroquine and amodiaquine were used. Quinine however has been found to be useful in treating malaria in pregnancy [11]. Quinine continues to play a critical role in the management of malaria in pregnancy, especially in the first trimester. In areas where multidrug resistance (MDR-*PF*) is prevalent, only quinine is safe and effective in pregnant women. [11,12] Quinine is the only recommended drug by Word Health Organization for the treatment of uncomplicated malaria in pregnancy [13] and it will remain a mainstay of treatment until safer alternatives become available.

In recent times there has been an increasing awareness of the genotoxic potential of a wide variety of drugs, plants and chemicals to which the human population is exposed [14]. Many anti-malarial drugs have not been licensed for use in pregnancy because their risk has not been assessed in controlled trials and some are potentially embryotoxic [9]. In most African countries, including Nigeria, Sulphadoxine-pyrimethamine (SP) is recommended during the second and third trimesters of pregnancy for intermittent preventive treatment (IPT) in areas of moderate to high malaria transmission [15]. Fortunately, SP use in IPTp programmes in Africa, with 2-4 treatment doses over 6 months has been well tolerated in multiple IPTp trials [16]. There has been no evidence that sulfa drugs like pyrimethamine cause abortion or still births when administered in the second and third trimester [17]. Studies have shown no adverse effect of SP [18]. SP has a favourable safety profile during pregnancy despite some concerns about severe cutaneous reactions and teratogenesis, although it is not recommended for HIV-positive women on cotrimoxazole prophylaxis for whom there is a higher risk of severe adverse reactions [16]. Use of anti-folate agents like SP is also contra-indicated in the first trimester because of the risk of neural tube defects. A postulated association between SP and kernicterus has not been observed in practice. The risks of malaria to mother and foetus are judged to outweigh potential risks of SP toxicity, and policies and protocols governing its use are intended to protect pregnant women and their babies from IPTp exposure in the first trimester [18]. There has been no report of clinical association between SP use and kernicterus. This is in spite of the extensive use of SP and related compounds to treat maternal malaria and congenital toxoplasmosis in near-term pregnant women and newborns. SP can be considered completely safe and has a favourable safety margin when delivered as IPTp–SP [16]. Pharmacovigilance programmes throughout Africa are now needed to confirm its safety as access to IPTp-SP increases, [19].

The awareness of the potential danger of drugs and chemicals led to the recent development of appropriate, sensitive and practical methods for detecting and estimating the effects of these drugs and chemicals. Animal studies have been conducted by giving Pyrimethamine alone and in combination with Sulfadoxine to hamsters, rats

and other animals. Complete embryo resorption and embrotoxicity in Wistar rats were observed when SP was given in early gestation [20]. An *In vivo* study was conducted in which abnormal sperm induction was evaluated in mice using Sulfamethoxypyridazine-pyrimethamine [21]. The study showed that 0.5X the human therapeutic dose of Sulfamethoxypyridazine-pyrimethamine (Metakelfin) gave a statistically significant increase over the negative control, but concluded that the medicine was not teratogenic. Assessments of sperm abnormality have also been conducted by exposing mice to chemicals. This was followed by visual scoring of the percentage sperm with abnormal head forms and shapes in smears of sperm from epididymis according to the works of [22,23].

2. METHODOLOGY

2.1 Laboratory Animals

Female Swiss albino mice were obtained from the animal breeding unit of the Nigerian Institute of Medical Research, Yaba. The mice which were between 12 and 14 weeks old were acquired and quarantined in a pathogen free well ventilated room in order to enable them to acclimatize to their environment. Only mice that are between 12 and 14 weeks old were tested. Drinking water and pelleted feeds were supplied *ad libitum.*

2.2 Sulfadoxine-Pyrimethamine (SP)

The antimalarial medicine used was Malareich (Sulfadoxine BP 500 mg, Pyrimethamine BP 25 mg) and supplied by Medreich Limited, India. One tablet of SP was crushed and 100 mg weighed using a weighing balance. The SP which dissolves readily in physiological saline was dissolved in 20 mls of distilled water to give a concentration of 0.5 mg/ml.

Doses used in this study were selected according to the therapeutic dose used in humans which was calculated based on the average human weight of 65 kg. The values were obtained from the manufacturer's information manual.

2.3 Mutagenic Studies on Foetus

Mutagenic effect of the SP was studied using pregnant Swiss albino mice [24]. Four groups of female virgin laboratory Swiss albino mice (each group had 5 mice) were used for the study. The mice were weighed in a weighing balance and their weight recorded; their weights ranged between 19.5 g and 22 g with an average of 20.9 g. Two males and 5 female mice were put together in a cage and allowed to mate during eostrus in the female. Gestation was ascertained by detection of the presence of vaginal plug as well as presence of sperm in the vagina. The pregnant mice were divided into four groups. Group one received 0.1 ml of SP giving a concentration of 0.54 mg/kg, which was the equivalent of the normal dose taken by adults. Group two received 0.15 ml, which represents 1½X the normal dose, while group three received 0.2 mls, representing 2X the normal dose.

The SP dose was given twice as recommended during pregnancy to a group of mice in the second and third trimester while another group received thrice during the gestation period, which included the first, second and third trimester. The mice received the first dose on the 5th day of gestation while the second dose was given on the 11th day of gestation and the third dose on day 17th day. These days represented the first and second and third trimesters of pregnancy respectively. Group 4 served as the control and received water.

The pregnant mice were sacrificed on day 19th of gestation and foetal abnormalities investigated. The uterus was examined for foetal resorption [24]. The foetuses were removed and examined morphologically for foetal abnormalities. The foetus was preserved in 10% formalin and sectioned transversely using microtone, stained and examined for foetal abnormalities.

2.4 Histopathological Studies to Determine Mutagenic Effect of Sulfadoxine-Pyrimethamine (SP) on the Foetus of Pregnant Albino Mice

Mutagenic effect of SP was studied, using 12 weeks old virgin Swiss albino mice obtained from the animal house in the College of Medicine, Idi-Araba. The female mice were weighed and divided into four groups of 6 mice. The female mice in each group were put together with two male mice and allowed to mate during eostrus of the female. Gestation was ascertained by detection of the presence of vaginal plug as well as presence of sperm in the vagina.

The pregnant mice received 0.1 ml (0.5 mg) of SP (based on their average weight of 20g) which

represents the human therapeutic dose (HTD) for SP. Group one received 1X HTD of SP; group two 1½ X HTD, group three received 2X the HTD while the control group received only water. The first dose was given at gestational period of 5 days, while the second and third doses were given on the 10^{th} and 15^{th} day of gestation respectively. The days represented the first, second and third trimester of pregnancy respectively. The mice were sacrificed on day 18 by cervical dislocation. At autopsy, each female was examined for total implants and any early foetal deaths. Histopathological examinations of the foetuses were undertaken to assess the impact of SP on the foetus.

2.5 Assessment of Sperm Head Abnormalities Following Sulfadoxine-Pyrimethamine (SP) Administration in Male Albino Mice Using Sperm Assay Test

Induction of sperm-head abnormalities was tested according to the method of Wyrobek et al., [25]. This test was considered necessary because the female ova are fertilized by the spermatozoa of the male. Sperm abnormality would be expected to affect the foetus and ultimately the offspring. The male mice were divided into four groups with five mice in each group. The mice were administered 0.5 mg of SP orally representing the HTD. Three different dose level treatments were considered for the SP corresponding to 1X, 1½X and 2X the HTD and each group was treated for each dose level and for each exposure period. These doses were administered orally to the male albino mice for five consecutive days [26]. Three different exposure periods were considered 5, 7 and 10 weeks from the first administration. One group of mice was treated with water only as a negative control.

Spermatozoa were sampled from the cauda epididymes at 5, 7 and 10 weeks from first administration. Spermatogenesis in mice takes about 5 weeks to complete.

The mice were sacrificed by cervical dislocation. The epididymes were excised and minced with fine scissors in physiological saline. Smears were prepared on clean, grease free slides after staining the cells with a mixture of normal saline and 1% eosin-Y (9: 1) for 45 minutes. The slides were air-dried and coded for subsequent examination under oil immersion. Cytological evaluation for sperm-head abnormalities was carried out using a binocular microscope at 100 X magnification. Six separate slides were prepared for each mouse, which are three slides for each epididymes out of which four were randomly selected for scoring. The slides were read blind to treatment.

The sperms were assessed for morphological abnormalities of sperm head shape according to the criteria of Wyrobek and Bruce, [26]. For each animal, 600 sperms were assessed for morphological damage. Differences between the control and experimental groups were analysed by means of the Student's t-test. The test was considered positive when the frequency of abnormal sperm heads was at least double the negative control level, with $P< 0.05$ as the criterion of significance.

2.6 Analysis of Data

Data collected were recorded into pre-coded case record forms. Thereafter, the data was entered using EPI-INFO 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and analysed. Descriptive statistics such as means and standard deviations were used to summarize quantitative variables, while categorical variables were summarized with proportions. Frequency tables and graphs were presented for relevant variables. The student t-test was used to compare two mean values, while the one way analysis of variance (ANOVA) was used to compare mean values in more than two groups. The Chi-square test was used to investigate associations between two categorical variables and also to compare proportions. For significant associations, the odds ratio (OR) and 95% confidence intervals (CI) were computed. A probability-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Histopathological Evaluation of Sulfadoxine-Pyrimethamine on Foetuses of Female Swiss Albino Mice

Table 1 shows that the number of foetuses from each group of mice and their weight, which ranged between 1.6 g and 2 g with an average weight of 1.8 g. The average weight of the control foetus was 1.8 g. There was no difference between the weight of the control foetuses and test groups, $P>0.05$. The groups that received the 1X HTD and 1½X HTD had no dead foetus,

neither did the control show any physical abnormality. However in the group that received 2X HTD, one mouse had IUGR with one dead foetus, giving a foetal death rate of 2% (Table 1). Histopathological investigation of the foetuses revealed the foetuses of the test animals to be normal (Figs. 1-6).

3.2 Sperm Assay Test Following Sulfadoxine-Pyrimethamine Administration

Spermatoazoa abnormalities were recorded in 5, 7 and 10 weeks following the exposure to the SP. Plates 7-9 illustrate both normal and abnormal sperm cells. In the course of scoring the abnormalities, the various abnormalities found included heads with no hook, amorphous head, pin head bent tail and tail folded over head (Fig. 7-9); the pin head seemed to be more predominant followed by coiled tail. These abnormal cells occurred with different frequencies in both treated and control mice. Table 2 shows the effect of different dose levels of SP on sperm head abnormality after 5, 7 and 10 weeks exposure. The negative controls showed 5.3%, 2% and 2% abnormalities respectively compared with 5%, 7% and 3.3% with the 1X HTD. SP did not induce statistically significant increase in sperm abnormality at the 1X HTD over the control, P = 0.0822), nor at the other consecutive dose levels neither was it reproducible at 7 and 10 week exposure periods, Tables 2 and 3.

4. DISCUSSION

4.1 Histopathological Studies on The Foetus of Pregnant Mice

The histopathological studies showed no adverse effect of SP on the foetus of laboratory bred albino mice. Morphological and histopathological investigations on both the foetus from the test and control mice showed no significant difference in weight of the foetus or abnormality of the cells, P>0.5. Studies carried out with SP on animals have shown the safety of SP [16]. Although folate antagonist use in the first trimester is associated with neural tube defects, large case-control studies have demonstrated that sulfadoxine-pyrimethamine administered as IPTp (exclusively in the second and third trimesters and after organogenesis) does not result in an increased risk of teratogenesis [16].

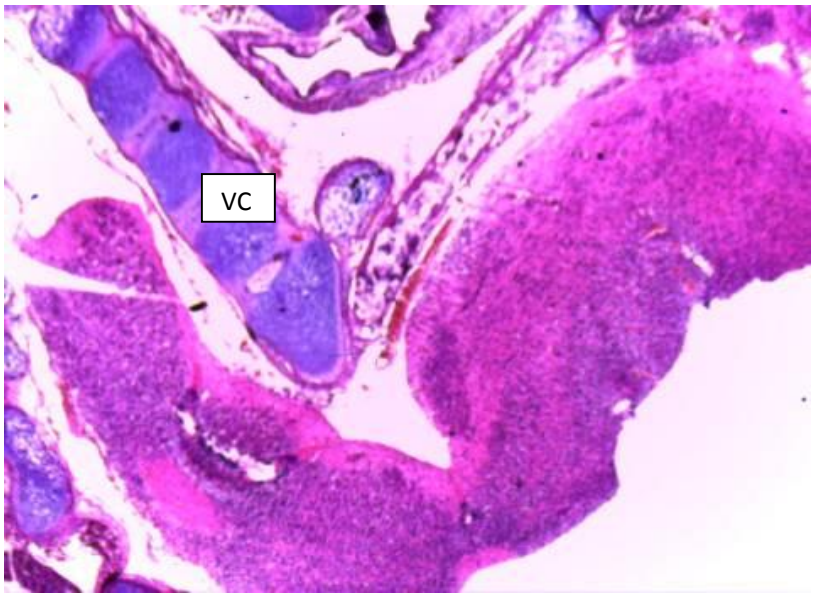

Fig. 1. Transverse section (TS) of foetus of the control female Albino mouse stained with Haemotoxylin and Eosin stains (H and E); shows normal Vertebral Column (VC), (X 100 magnification under oil immersion microscopy)

Table 1. Effect of sulfadoxine-pyrimethamine on foetuses of pregnant albino mice

No. of mice	Doses of SP	Average No. of foetus	Average weight of foetus (g)	No. of dead foetus	Percentage of dead foetus	P-value
5	Control	10	1.8	0	0	
5	HTD	10	1.8	0	0	0.77
5	1½X	10	1.7	0	0	0.44
5	2X	10	1.7	1	2	0.41

There was no statistical significance between the weight of the foetuses of control and test .mice, P>0.05.

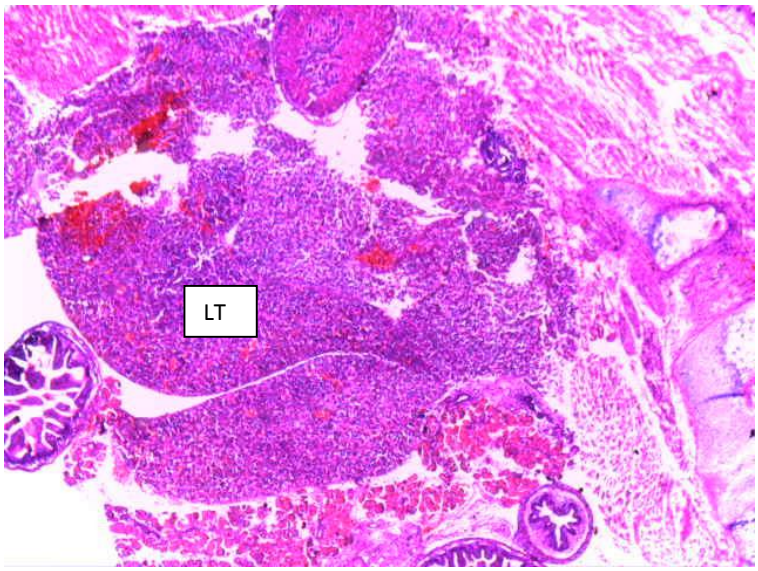

Fig. 2. Transverse section (TS) of the foetus of female Albino mouse administered 1X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Liver Tissue (LT) without any neoplastic features, (X 100 magnification under oil immersion microscopy)

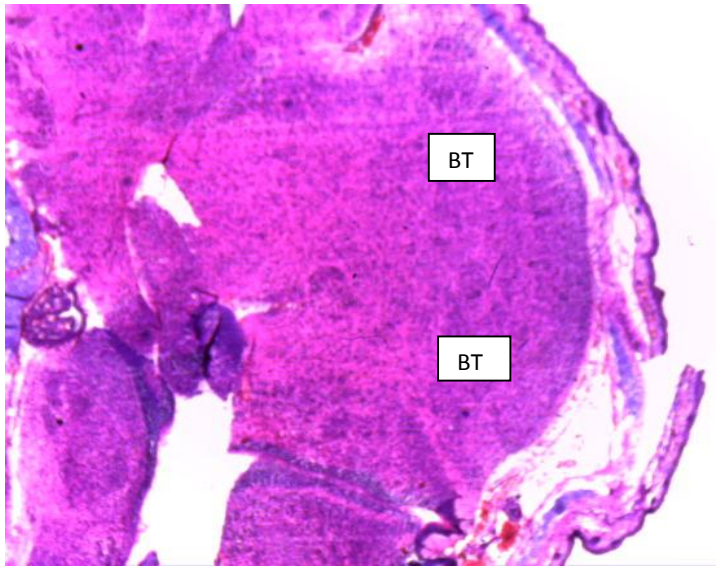

Fig. 3. Transverse section of Brain Tissue of foetus of female Albino mouse administered 1½ HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Brain Tissue (NBT), (X 100 magnification under oil immersion microscopy)

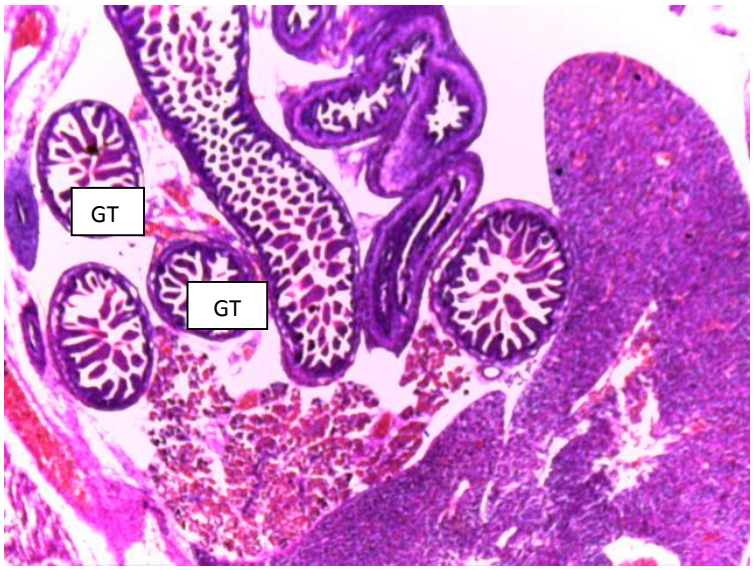

Fig. 4. Transverse section of foetus of female Albino mouse administered 1½ X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Glandular Tissue (GT), (X 100 magnification under oil immersion microscopy)

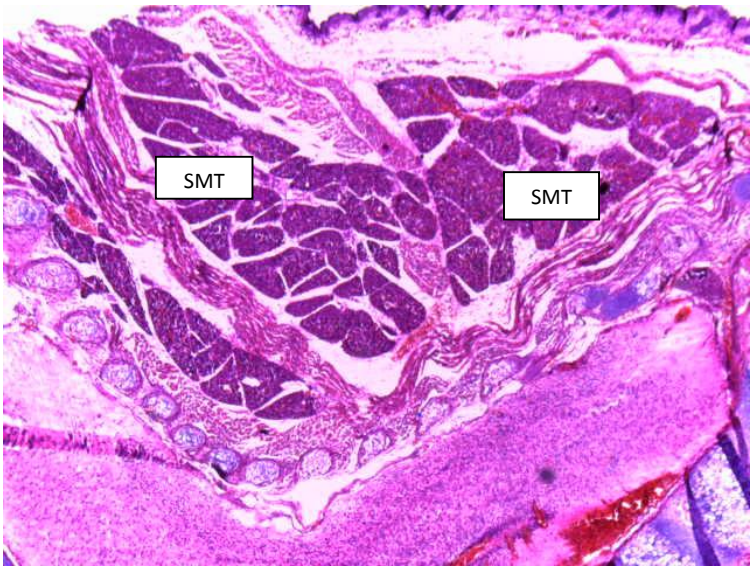

Fig. 5. Transverse section of foetus of female Albino mouse administered 2 X HTD of Sulfadoxine-Pyrimethamine and stained with H and E stains; shows normal Skeletal Muscle Tissue (SMT), (X 100 magnification under oil immersion microscopy)

Pregnancies exposed to quinine or chloroquine and carried to term did not have increased rates of congenital abnormality, stillbirth or low birthweight. These results suggest that for the treatment of uncomplicated malaria in pregnancy [13], there could be challenges with quinine administration. There is the popular belief that quinine can cause abortion in pregnant women; it has been shown that pregnant women therapeutic doses of quinine and chloroquine are safe to use in the first trimester of pregnancy [11]. Despite the fact that quinine is the only recommended drug by Word Health Organization who take toxic dose of quinine will suffer renal failure before experiencing any kind of quinine induced abortion [27]. Quinine can also cause hypoglcaemia in pregnant women, [10]. Quinine overdose may lead to irreversible visual loss,

symptoms of cinchonism which includes convulsion, nausea, vomiting, tinnitus, deafness and vasodilation. The continued use of quinine in the management of uncomplicated malaria is a concern. Clearly, the seven day duration of therapy and thrice daily administration of quinine present a major challenge to completion of therapy, leading to sub-optimal treatment outcomes in these situations [10].

It is known that during spermatogenesis DNA synthesis occurs before the pre-meiotic phase and no further synthesis occurs throughout the duration of spermatogenesis in the cell cycle.

Table 2. Sperm abnormalities in male albino mice after Sulfadoxine-pyrimethamine administration

Sperm abnormalities					
Week 5					
Dosage	**AH**	**CT**	**PH**	**BT**	**Total sperm abnormality**
Control	12	0	20	0	32 (5.3%)
1X	0	0	30	0	30 (5.0%)
1½X	12	0	20	0	32 (5.3%)
2X	12	0	10	0	32 (5.3%)
Week 7					
Control	10	0	0	0	10 (2%)
1X	0	0	20	20	40 (7.0%)
1½X	6	0	8	0	14 (2.3%)
2X	20	0	12	0	32 (5.3%)
Week 10					
Control	12	0	0	0	12 (2%)
1X	0	0	20	10	30 (3.3%)
1½X	0	0	30	0	30 (5.0%)
2X	12	0	0	0	12 (2.0%)

AH=Amorphous head; CT=Coiled tail; Pin head; BT=Bent tail

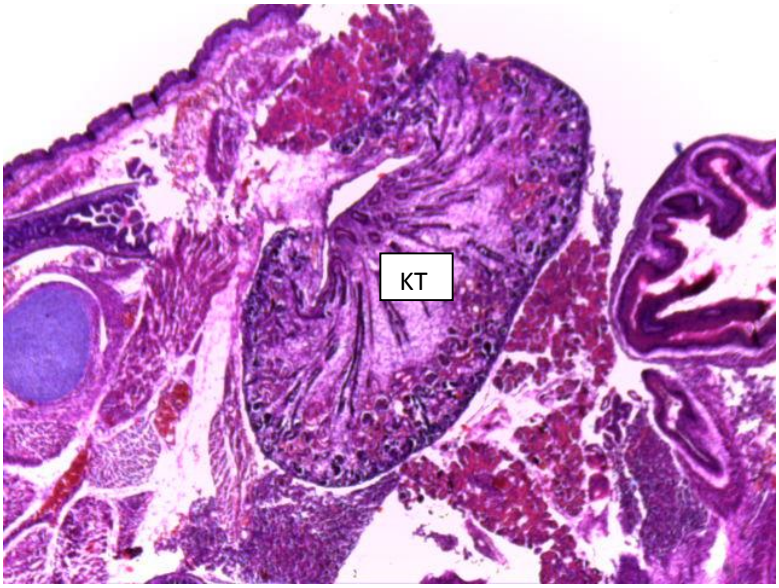

Fig. 6. Transverse section of foetus of female Albino mouse administered 2X HTD of Sulfadoxine-pyrimethamine and stained with H and E stains; shows normal Kidney Tissue (KT), (X 100 magnification under oil immersion microscopy)

Thus, sperm-head morphological abnormalities may be as a consequence of a naturally occurring level of mistakes in the spermatozoon differentiating process and a chemical mutagen might increase the frequency of these mistakes [28]. The abnormalities may be as a result of the mistakes made in packaging the genetic material in the sperm head or perhaps as a result of an abnormal chromosome complement. However, it is probable that sperm with abnormal shapes would contain abnormal genetic material [26].

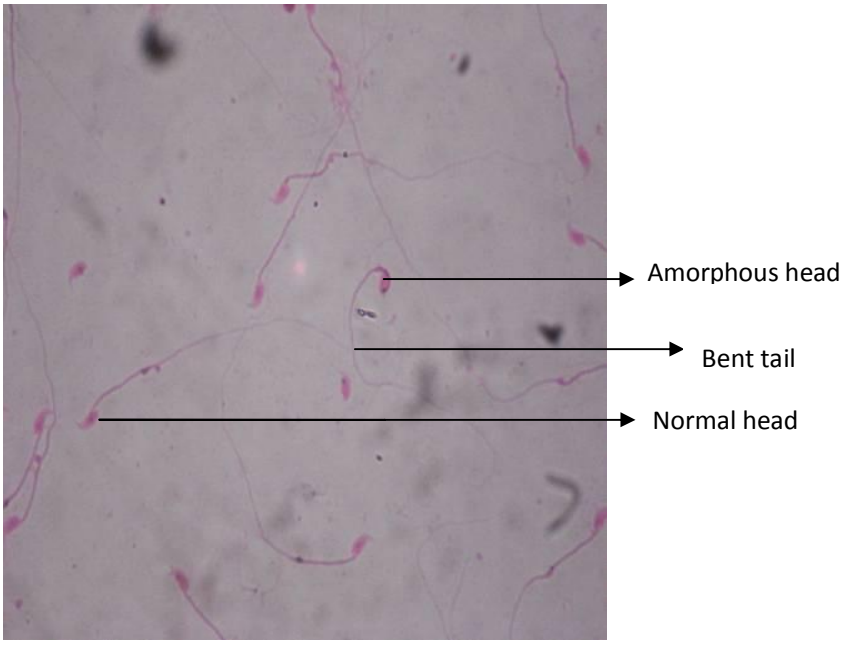

Fig. 7. Control: Sperm of male Albino mouse shows normal sperm heads; bent tail and amorphous head; Stained with Eosin and examined under oil immersion (X 100 magnification)

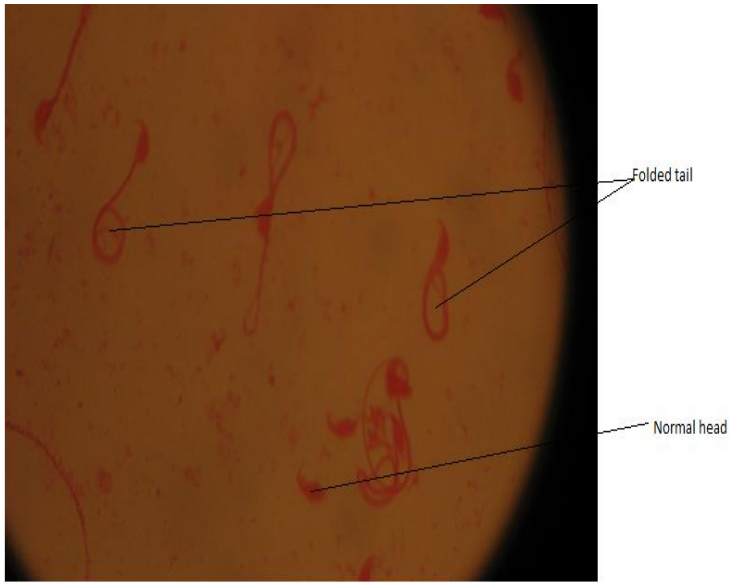

Fig. 8. Male Albino mouse administered 1½X HTD of Sulfadoxine-pyrimethamine; Shows sperm cells with coiled tails and heads; Stained with Eosin and examined under oil immersion (X 100 magnification)

4.2 Assessment of Sperm Abnormality

Sperm abnormalities were observed in both test and control mice that were administered SP. There was no specific type of abnormality that was predominant as they all occurred with different frequencies in both treated and control mice. Adoloju et al*.,* [28], reported the number of pin heads to be highest although a chemical was used in that study instead of medicine. The percentage abnormality in the control for week 5, 7and 10 were 5.3%, 2% and 2% respectively. SP induced statistically significant increase in sperm abnormality at the 1X HTD over the control ($P<0.05$), but not at other consecutive dose levels and this was not reproducible at 7 and 10 week exposure periods.

Thus the abnormality of sperm heads observed for these exposure periods may be due to induced point mutations in the early spermatocytes and spermatogonia at the pre-meiotic stages of spermatogenesis [28]. An *in vivo* evaluation of the induction of abnormal sperm by Sulphamethoxypyridazine-pyrimethamine (Metakelfin) showed that 0.5X the human therapeutic dose gave a statistically significant increase over the negative control value. The study recorded that a higher dose produced fewer abnormalities than the proceeding lower dose level. The study therefore concluded that Metakelfin is probably not mutagenic as induction of sperm head abnormality was not dose dependent [22].

The occurrence of sperm head abnormalities have also been attributed to the chromosomal aberrations that occur during the packaging of genetic material in the sperm head or occurrence of point mutation in testicular DNA [24,28]. Odeigah, reported that exposure to the chemicals could produce pituitary hypothalamic or sex hormonal defects which in turn could affect spermatogenesis or exposure could cause abnormalities in seminal fluid resulting in functional or structural impairment of sperm.

Table 3. Summary of sperm assay

HTD	Total abnormality (%)			Average %	P value
	Exposure period in weeks				
	5	7	10		
Control	32 (5.3%)	10 (2%)	12 (2%)	3.1	
1X	30 (5.3%)	40 (7.0%)	30 (3.3%)	5.2	0.0822
1½X	32 (5.3%)	14 (2.3%)	30 (5.0%)	4.1	0.356744
2X	32 (5.3%)	32 (5.3%)	12 (2.0%)	4.2	0.356744

The percentages are means for groups of six mice for each SP dose

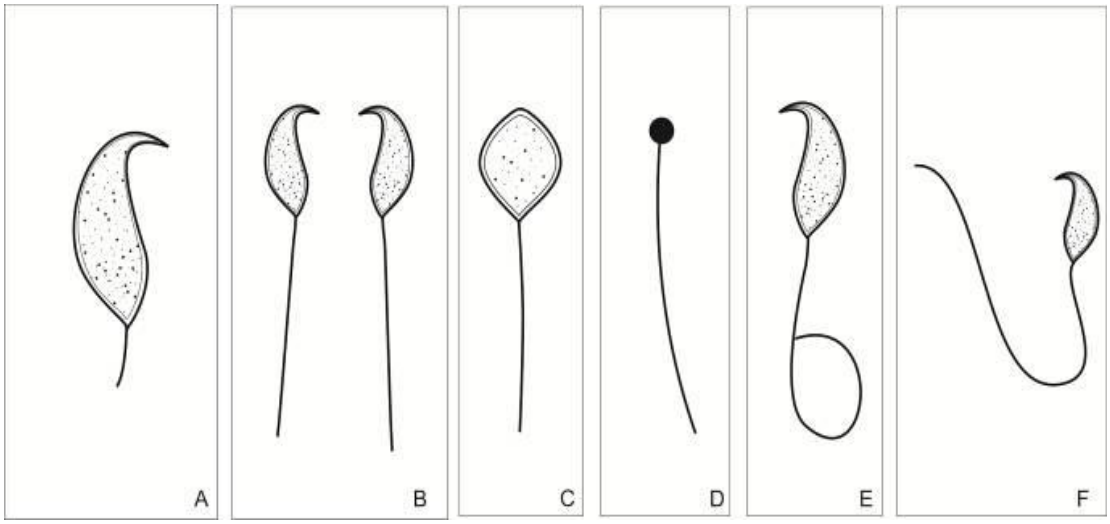

Fig. 9. Sketch of different types of observed spermatozoa abnormalities. A=Normal head without tail; B=Normal heads; C=Amorphous head; D= Pin head; Coiled tail; F= Bent tail

Sperm abnormality may also arise as a consequence of naturally occurring level of mistakes in the spermatozoon differentiating process during spermatogenesis [29]. During spermatogenesis, DNA synthesis occurs before the pre-meiotic phase and no further synthesis occurs throughout the duration of spermatogenesis in the cell cycle.

5. CONCLUSION

It can therefore be concluded that SP is safe for use in pregnancy after quickening.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Malaria chemotherapy. Tech. Reg. Ser. Geneva; 1998.
2. Sachs J, Malaney P. The economic and social burden of malaria. Nature. 2002; 415(6872):680-685.
3. Bruce-Chwatt LJ. ed. Chemotherapy of malaria, 2nd ed. World Health Organization, Geneva; 1981.
4. Markwalder KA, Meyer HE. Possible sulfadoxine-pyrimethamine resistance in Plasmodium falciparum malaria from Kenya (letter). Trans. R Soc. Trop. Med. Hyg. 1982;76(2):281.
5. Djimdé A, Doumbo OK, Cortese JF, Kayentao K, Doumbo S, Diourté Y, Dicko A, Su XZ, Nomura T, Fidock DA, Wellems TE, Plowe CV, Coulibaly D. A molecular marker for chloroquine-resistant falciparum malaria. N. Engl. J. Med. 2001;344(4): 299-302.
6. White NJ. Assessment of the pharmacodynamic properties of anti-malaria drugs *In vivo*. Antimicrobial Agents for Chemotherapy. 1997;41(7):1413-1422.
7. Haynes RK. Artemisinin and derivatives: The future for malaria treatment? Curr Opin Infect Dis. 2001;14(6):719–726.
8. Toure YT, Oduola A. Malaria. Nature. 2004;2(4):276-277. DOI:10.1038/nrmicro 870.
9. Nosten F, McGready R, d'Alessandro U, Bonell A, Verhoeff F, Menendez C, Mutabingwa T, Brabin B. Antimalarial drugs in pregnancy: A review. Curr Drug Saf. 2006;1(1):1-15.
10. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ, D'Alessandro U. Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. Malaria Journal. 2011;10:144. DOI:10.1186/1475-2875-10-144.
Available:http//www.malariajournal.com/content/10/1/144
11. McGready R, Thwai KL, Cho T, Looareesuwan S, White NJ, Nosten F. The effects of quinine and chloroquine antimalarial treatments in the first trimester of pregnancy. Trans R Soc Trop Med Hyg. 2002;96(2):180-4.
12. World Health Organization (2010): Malaria treatment guidelines; 2010.
13. Yeka A, Achan JD, D'Alessandro U, Talisuna AO. Quinine monotherapy for treating uncomplicated malaria in the era of artemisinin-based combination therapy: An appropriate public health policy. Lancet Infect Dis. 2009;9(7):448-452.
14. Gonçalves CA, Silva NL, Mauro MO, David N, Cunha-Laura AL, Auharek SA, Monreal, AC, Vieira MC, Silva DB, Santos FJ, Siqueira JM, Oliveira RJ. Evaluation of mutagenic, teratogenic, and immunomodulatory effects of Annona nutans hydromethanolic fraction on pregnant mice. Genet Mol Res. 2014;11; 13(2):4392-405.
15. World Health Organization (2008). Technical Expert Group Meeting on Intermittent Preventive Treatment in Pregnancy (IPTp). 11–13 July 2007. Geneva; 2008.
16. Peters PJ, Thigpen MC, Parise ME, Newman D. Safety and toxicity of sulfadoxine/pyrimethamine. Implications for malaria prevention in pregnancy using

intermittent preventive treatment. Drug Saf. 2007;30(6):481-501.
17. Morley D, Woodland M, Cuthbertson WFJ. Controlled trial of pyrimethamine in pregnant women in an African Village. British Medical Journal. 1964;1(5384):667-668.
18. Gimnig JE, MacArthur JR, M'bang'ombe M, Kramer MH, Chizani N, Stern RS, Mkandala C, Newman RD, Stekette RW, Campbell CH. Severe cutaneous reactions to sulphadoxine-pyrimethamine and trimethoprim-sulphamethoxazole in Blantyre District, Malawi. American Journal of Tropical Medicine and Hygiene. 2006; 74(5):738-743.
19. Talisuna AO, Staedke SG, D'Alessandro, G. Pharmacovigilance of antimalarial treatment in Africa: Is it possible? Malaria Journal. 2006;5:50.
20. Phillips-Howard PA, Wood D. The safety of antimalarial drug in pregnancy. Drug Saf. 1996;14(3):131-45.
21. Otubanjo OA, Mosuru AA. An *In vivo* evaluation of the induction of abnormal sperm morphology by Sulfamethoxypyridazine-pyrimethamine (Metakelfin). Pakistan Journal of Biological Science. 2007;10(1):156-159.
22. Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proceeding Sciences of United States of America, of the National Academy. 1975; 72(11):4425-4429.
23. Odeigah PGC. Sperm-head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutation Research.* 1997;389(2):141–148.
24. Uche-Nwachi E. Effect of intramuscular SP on pregnant Wistar rats. Ant. Rec. 1998; 250(4):426-9.
25. Wyrobek AJ, Bruce WR. The induction of sperm-shape abnormalities in mice and humans. In: Chemical Mutagens. A. Hollander and F.J. de Serres (Eds.). Plenum Press, New York. 1978;5:257-285.
26. Bruce WR, Heddle JA. The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella* and sperm abnormality assays. Canadian Journal of Genetic Cytology. 1979;21(3):319-334.
27. Danneberg AL, Behel FJ, Johnson J. Use of quinine for self-induced abortion. The Southern Medical Journal. 1983; 76(7):846-849.
28. Aduloju RK, Otubanjo OA, Odeigah PGC. An *In Vivo* Assay of the Mutagenic Potential of Praziquantel (PZQ) Using Sperm Head Abnormality Test. J. Hum. Ecol. 2008;23(1):59-63
29. Bakare AA, Mosuro AA, Osibanjo O. An *In vivo* evaluation of induction of abnormal sperm morphology in mice by landfill leachates. Mutation Research. 2005; 582(1-2):28–34. (DOI:10.1016/j.mrgentox.2004.12.007).582 :28-34.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1176&id=14&aid=9358