Influence of chloroquine and sulfadoxine on biochemical and hematological indices in albino rats

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Abstract

The influence of chloroquine base drugs, sulfadoxine and their combination on biochemical and hematological indices was studied for five days in adult *Rattus novergicus* of mean weight 150.5±0.5g using standard procedures. At $p<0.05$, there was significant increase in activity of transaminases in the serum and liver of groups administered chloroquine base drugs (CS, CP) and their combination with sulfadoxine (CS+SP, CP+SP). The activity of serum and liver GOT was significantly unchanged in sulfadoxine administered groups (SP) while activity of serum and liver ALP was significantly decreased when compared to saline given group (control). The GOT/GPT ratios obtained were generally less than 1 indicating intact liver cells integrity. Drugs effects on total bilirubin and albumin were of no statistical significance while sulfadoxine significantly increased creatinine level. The tested drugs induced significant increase in hemoglobin, PCV, WBC, neutrophil and lymphocytes levels in experimental rats. Decreased body weights and crenated red blood cells were observed in CS and CP administered groups. The functional observational battery and locomotor signs observed in drugs administered groups include weakness, shortness of breath, rapid heartbeat, nose bleeds, decreased body weight, swollen gum and anorexia. Single therapy of chloroquine base drugs (CS and CP) particularly CS showed more toxic effects on biochemical and hematological indices in administered rats than other tested drugs, sulfadoxine was also observed to suppress the functional activity of chloroquine base in their combination in this study. The idiosyncratic reactions (eosinophilia and basophillia) resulting from allergic host response and other related conditions such as hemoglobinuria, polycythemia, leukocytosis, neutrophilia and lymphocytosis observed in this study may resolve over short period of time upon discontinuation of offending drugs.

Keywords: Antimalarial drugs, Biochemical, Hematological.

Introduction

In tropical and subtropical countries where malaria is rife, there is often acquired tolerance in a large percentage of the population, but even in these cases there is considerable debilitation and wastage of human effort. Tropical races suffer considerably from the weakening effects of such diseases like malignant quotidian malaria and benign tertian (Vines & Rees, 1978). Nigeria is malaria endemic; chemoprophylaxis remains the primary method for malaria prevention since no vaccine is available currently. usually therapy is with Chloroquine 300mg (base) given once per week, started 1-week prior to travel and continued for 4 to 6 weeks after exposure. The pediatric dose is 5 mg/kg up to 300mg of the base drug (Ajiboso, 2004).

Chloroquine is mostly used as a preventive drug against malaria while Sulfadoxine is an effective drug against chloroquine-resistant plasmodium (Taylor *et al.,* 1998). Adverse drug reaction (ADR) has been associated with these drugs. Wolfe (1990) has reported the association of Stevenson Johnson syndrome, rashes and headaches with Pyrimethamine-Sulfadoxine. It is also unfit for women near term, nursing mothers and infants under 2 months (ACIP, 1990). Headaches, weakness, dizziness, gastrointestinal problems and itching have been associated with chloroquine phosphate (Ajiboso, 2004). These drugs have also been reported to mediate disturbance in

the cellular and humoral responses of the experimental animals (Ajiboso, 2006). Sood (1999) has associated chloroquine base drugs with hemoglobinuria, polycythemia, neutropenia, lymphocytosis and also reported sulfadoxine as a cause of selective neutropenia.

Keeping in view the adverse drug reactions associated with the common antimalaria drugs (sulfadoxine, quinine and schizontocidal drug called chloroquine) used in Nigeria. Earlier research conducted by Ajiboso (2006) revealed abuse of these drugs by consumers on self medication in Nigeria being malaria endemic country. In view of this, this present work is therefore design to study the influence of chloroquine, sulfadoxine and their combination on biochemical and hematological indices in albino rats (*Rattus novergicus)*.

Materials and methods

Collection and distribution of animals

A total of 30 adult albino rats with a mean body weight of 150.0±0.5 g were obtained from Federal University of Technology Minna, Niger state-Nigeria and randomly divided into six groups: A, B, C, D, E and F. Group A administered normal saline solution (5ml) (control). Groups B, C, D, E and F were administered with sulfadoxine (SP), nivaquine (CS), chloroquine (CP), combined nivaquine with sulfadoxine (CS+SP) and combined chloroquine with sulfadoxine (CP+SP) respectively. Groups B to F received daily doses (5ml) of 100 mg/kg of respective drugs for 5 days.

Location and Duration of Study

This study was conducted in the Biochemistry Research laboratory, Science laboratory Technology Department, the Federal Polytechnic Bida, Niger state-Nigeria, West-Africa. Animal acclimatization, drug administration, biochemical and hematological studies, and evaluation of results lasted for two months (March to May 2012). This study was carried out after approval from the Departmental Ethical Committee on the use and care of experimental animals. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123.

Drug Administration

The test drugs (sulfadoxine, nivaquine, chloroquine and their combinations) used for this experiment were prepared in aqueous solution and administered orally at a dose level of 100 mg/kg body wt for five days. The dose of the treatment was chosen based on previous data of Soniran et al (2011).

Preparation of Saline solution: To prepare a saline solution, 0.88g of sodium chloride (NaCl) was weighed using electronic digital balance (Gibertini, Italy) and dissolved in 100ml of distilled water in a 200ml measuring beaker (Simax, Czechoslovakia).

Preparation of serum: The blood sample in a clean, dry centrifuge tube was allowed to clot and centrifuged at 224*g* x 10 minutes (Ajiboso, 2000). The clear supernatant (serum) was separated from the pellet, kept frozen and used within 24 hours.

Preparation of liver homogenate: The extracted liver was suspended in ice-cold 0.25M sucrose solution (Ajiboso, 2000) buffered with Tris pH 7.4, and was cut into tiny pieces and homogenized until a fine homogenate was produced using Teflon homogenizer.

Determination of biochemical and haematological indices

The transaminases and alkaline phosphatase activities were determined in the serum and liver homogenate according to the methods described by Bergmeyer (1974). Total bilirubin was spectrophotometrically determined in the serum using the method described by Stevenson et al., (1964), the method of bromocresol green (BCG) of Webster (1974) was used to determine serum albumin. Creatinine was determined according to Jaffe's reaction method described by Ferry et al., (1996). The methods described by Akpanabiatu et al. 2012 and Uboh et al. 2010 were used to determine the haematological indices.

Determination of body weight

The method described by Ajiboso *et al*., (2007) was used to determine body weight of

experimental rats. Individual rat was monitored for daily gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was obtained from the relationship given below:Daily gain in weight = Final day Weight – Initial day Weight.

Statistical analysis

Data are presented as Means ± SD and analyzed using ANOVA and Duncan post hoc test and significance was determined at $p < 0.05$.

RESULTS

The influence of chloroquine and sulfadoxine on biochemical and haematological indices in albino rats was carried out in this study. The mean values of results obtained are shown in Tables 1 to 2 below. At $p<0.05$, Table 1 shows that activity of liver GOT was unchanged in group B (SP given) and was significantly increased in groups C to F (C= CS; D=CP; E=CS+SP and F= CP+SP). There was a significant increase in serum GOT activity in all the drugs administered groups when compared to control group A (saline given), serum GOT activity in groups B, C, C, E and F were 4.8 times, 5.8 times, 4.1 times, 4 times and 3.4 times respectively of control group A value. There was no significant difference in activity of GPT in liver of group B while liver and serum GPT activity in groups C to F significantly increased. GPT activity in liver and serum of group C were 3.7 times and 3.1 times of control group A values. Alkaline phosphatase activity was significantly decreased in drugs administered groups. Both serum and liver transaminases and alkaline phosphatase were significantly increased and decreased respectively in group C (CS given) than other drugs administered groups. Total bilirubin was significantly decreased in group B and significantly increased in groups C to F. Albumin was also significantly decreased in groups B, D and F while groups E and C showed significant increase in albumin. Creatinine was high in group B while significant decrease in creatinine level was observed in groups C to F.

Table 2 shows the effect of acute toxicity of chloroquine and nivaquine on haematological indices in albino rats. At $p<0.05$, there was increase in haemoglobin, PCV, WBC, neutrophil and lymphocytes levels in drugs administered groups when compared to control group A, the increase in haematological indices was more significant in group C (CS given) than other groups. Monocytes, eosinophil and basophil were not found in groups A, B and E while twice the amount of monocytes in groups D and E was observed in group C (CS given). Eosinophil and basophil were found present in group C (CS given) and absent in other drugs administered groups. The red blood cell morphology examination revealed normal red blood cells shape in groups A, B, E and F while the shape of groups C and D was crenated.

Table 1. Mean results of influence of chloroquine and sulfadoxine on some selected enzymes (U/L) and bimolecules (mg/l) in liver and serum of experimental rats

	Liver				Serum					
Group	GOT	GPT	ALP	Liver GOT/GPT ratios	GOT	GPT	ALP	Total bilirubin	Albumin	Creatinine
A	2.0±0.2^{a}	3.5±0.3^{a}	239.5±0.8^{a}	0.5±0.0^{a}	32.3 ±0.8^{a}	9.7± 0.6^{a}	101.1±0.4^{a}	0.6±0.0^{a}	3.0±0.2^{a}	1.8±0.4^{a}
B	2.0±0.3^{a}	3.7±0.6^{a}	158.6±0.4^{b}	0.5±0.0^{a}	155.5±0.4^{b}	22.5±0.3^{b}	80.5 ±0.8^{b}	0.4±0.1^{a}	2.5±0.0^{a}	5.6±0.4^{b}
C	2.7±0.3^{a}	13.1±0.3^{b}	100.8±0.5^{c}	0.2±0.0^{a}	186.5± 0.6^{c}	30.5±0.4^{c}	21.0 ±0.9^{c}	0.8±0.0^{a}	4.2±0.2^{b}	1.3±0.2^{a}
D	3.0±0.1 ^{b}	9.3±0.3^{c}	225.9±3.0^{d}	0.3±0.0^{a}	132.0±0.9^{d}	28.1 ±0.1^{d}	52.0 ±4.0^{d}	0.9±0.1^{a}	2.2±0.2^{a}	1.3±0.2^{a}
E	3.3±0.6^{b}	12.8±0.4^{b}	117.3±0.4^{e}	0.3±0.0^{a}	131.0± 0.40^{d}	18.4 ±3.8^{e}	29.5 ±0.1^{e}	0.7±0.1^{a}	3.2±0.2^{a}	1.5±0.1^{a}
F	4.0±0.2^{b}	11.2±0.2^{c}	204.7±0.3^{f}	0.4±0.1^{a}	110.7 ±0.4^{e}	19.8±0.1^{f}	89.0 ±0.4^{f}	0.9±0.0^{a}	2.0±0.3^{a}	1.5±0.2^{a}

Mean values of triplicate determinations
Results expressed in Mean±SEM
At $p<0.05$, same letter across the column shows no significant difference
A= Control; B= Pyrimethamine-Sulfadoxine (S); C= Chloroquine Sulfate (CQS); D= Chloroquine Phosphate (CQP); E= CQS+SP; F= CQP+SP

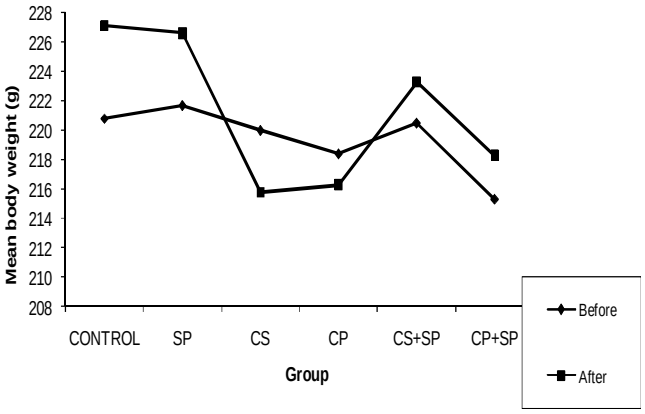
Table 2. Mean results of influence of chloroquine and sulfadoxine on hematological indices of experimental rats

Group	Hb	PCV	WBC	Neut.	Lymph.	Mono	Eos	Baso	RBC Morphology
A	15.2±0.0 [a]	10.7±0.0 [a]	16.3±0.0 [a]	33.3±0.3 [a]	10.1±0.1 [a]	0.0±0.0 [a]	0.0±0.0 [a]	0.0±0.0 [a]	Normal
B	19.3±0.5 [b]	11.2±2.1 [a]	22.3±3.0 [b]	51.0±1.7 [b]	59.3±2.1 [b]	0.0±0.0 [a]	0.0±0.0 [a]	0.0±0.0 [a]	Normal
C	25.3±1.3 [c]	39.1±3.3 [b]	24.1±2.3 [c]	72.1±3.1 [c]	64.7±1.3 [c]	2.0±0.0 [b]	2.0±0.0 [b]	2.0±0.0 [b]	Crenated
D	16.4±1.0 [d]	19.7±3.1 [c]	25.7±1.5 [d]	50.4±3.0 [b]	40.6±2.4 [d]	1.0±0.0 [c]	0.0±0.0 [a]	0.0±0.0 [a]	Crenated
E	21.5±2.1 [e]	26.0±2.4 [d]	21.7±2.7 [b]	74.3±4.0 [d]	60.9±2.6 [e]	1.0±0.0 [c]	0.0±0.0 [a]	0.0±0.0 [a]	Normal
F	18.7±1.7 [b]	17.8±2.0 [e]	18.4±3.0 [e]	51.6±3.8 [b]	42.7±1.5 [f]	0.0±0.0 [a]	0.0±0.0 [a]	0.0±0.0 [a]	Normal

Mean values of triplicate determinations
Results expressed in Mean±SEM
At p<0.05, same letter across the column shows no significant difference
A= Control; B= Pyrimethamine-Sulfadoxine (S); C= Chloroquine Sulfate (CQS); D= Chloroquine Phosphate (CQP); E= CQS+SP; F= CQP+SP

Fig. 1 shows the mean body weights of control and administered groups before and after drugs administration. There was a significant increase in body weights of groups A (control), B (SP given), E (CS+SP) and F (CP+SP) while CS and CP showed decrease in body weights after administration of drugs. Change (gain and loss) in mean body weight between days 1 and 5 is shown in Figure 2, control group A (saline solution) showed highest weight gain of 6.3g while higher weight loss of -4.2g was recorded in group C (CS given).

Fig. 1. Graph of mean body weights of experimental rats between days 1 and 5 before and after drugs administration

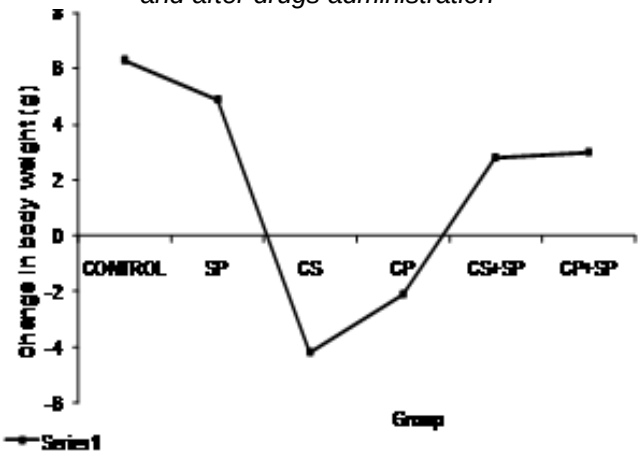


Discussion

In elucidating the mechanism of drugs action, the modification and conjugation processes during biotransformation made the liver the most actively engaged organ because the liver is in possession of enzymes that perform these processes. Drug can exert both medicating and side (toxic) effects; the more active the drug, the faster its toxic properties become manifest (Strove, 1989). During metabolism, the specific activity and toxicity of the drugs used were likely to be susceptible to alterations such as induced activity of an inactive preparation, which may be due to the biologically active groups that have been blocked in the initial preparation become deblocked during metabolism or acquire functional groups that are necessary for eliciting the pharmacokinetic and pharmacodynamic properties of the drugs.

Serum levels of GOT and GPT become elevated whenever disease processes affect liver cell integrity (Strove, 1989). Rise of SGOT level is greater than elevation of SGPT in liver cirrhosis, intrahepatic neoplasm and hemolytic jaundice (Sood, 1999). The offending drugs may cause hepatic complications since SGOT level is greater than SGPT level. However, in toxic hepatitis, GPT is characteristically as high as or higher than GOT (Ellis *et al.,* 1978). The highest liver GOT values obtained in this study

Fig.2. Graph of change in body weights of experimental rats between days 1 and 5 before and after drugs administration



were 6 times higher than control group A values. Studies of the pattern in rise in which GOT and GPT can be as high as 100 times the reference limit are valuable in diagnosing both disease and recovery states of organs where they are located (Ellis *et al.,* 1978). The rise in serum levels of GOT and GPT in this study does not imply that the drugs are offensive to the liver cell integrity from the rise pattern of liver GOT activity which was 6 times as against 100 times reported by Ellis *et al.,* (1978) of control group A, this therefore suggests that the acute toxicity of the drugs has not resulted into toxic hepatitis but elevation of transaminases. To buttress this point, the GOT/GPT ratios less than 1 also suggest normal liver cell integrity. Increased GOT/GPT ratio may implicate the extent of cellular damage (French, 1979). The GOT/GPT increases when liver disease involves the mitochondria, based on the changes in transaminase ratios, the high ratio in the serum may suggest leakage from hepatic cells, and this is an indication of liver dysfunction. Greater activity of GOT (AST) over GPT (ALT) is typical of myocardial infarction. GOT activity is helpful in the early diagnosis of myocardial infarction; the enzyme activity rise in the first few hours after the acute episode (Adolph & Lorenz, 1982). According to Sood (1999) ALP level increases with all forms of jaundice; malnutrition leads to reduced levels of ALP. In this study, the levels of ALP are generally low. The comparative low levels of alkaline phosphatase in this study showed the incapability of the drugs in inducing cholestasis, which is a predominant feature of liver biopsy (Levy, 1993). Increase in serum total bilirubin levels may suggest Gilbert's syndrome (mild jaundice) anemia and hepatitis or gallstones. Jaundice can be caused by excessive breakdown of red cells, impaired liver function or mechanical obstruction of the bile duct, jaundice points to an elevated level of bilirubin in blood (Stroev, 1989). Elevation of bilirubin, alkaline phosphatase and γ- glutamyl transpeptidase levels are characteristics of intrahepatic cholecystitis (Levy, 1993 and Gurlek *et al.,* 1997). Chloroquine sulphate induced increase in albumin level in the blood in this study. High albumin levels usually reflect dehydration (Stroev, 1989). Sulfadoxine, chloroquine phosphate and their combination induced decrease in serum albumin levels. Low-level albumin suggests liver disease, kidney dysfunction, malnutrition and improper utilization of protein in the body (Stroev, 1989; Fujiwara, 1971). Low levels of creatinine are not usually a cause for concern; they can be seen in certain conditions that may result in decreased muscle mass such as observed in CS, CP and CS+SP, CP+SP given groups (Stroev, 1989) while sulfadoxine induced increase in serum creatinine level; this may not represent a real deterioration in renal function (Hottelat *et al.,* 1999).

The drugs administered caused elevation in hematological indices resulting in hemoglobinuria, polycythemia, leukocytosis, neutrophilia and lymphocytosis. Hypoxia leads to polycythemia (increased PCV level), acute stress increases number of WBC. High white blood cell counts may be due to inflammation and immune response (Bagby, 2004). The presence of monocytes in CS, CP and CS+SP and eosinophil and basophil in CS administered groups indicate chronic inflammation and allergic response respectively in administered rats. However, hemoglobinuria and polycythemia have been associated with chronic chemical exposure such as sulfonamides, other substances producing methaemoglobin and sulphaemoglobin (Ajiboso, 2006; Sood, 1999). Crenated blood cells observed in CS and CP administered groups showed destructive property of the drugs on red blood cells. According to Sood (1999) Crenation is the contraction of a cell after exposure to a hypertonic solution due to the loss of water through osmosis . the effects of crenation is visible in red blood cells, or erthrocytes, as they become distorted in shape rather than maintaining the usual disc-like shape with dimple that the blood cell normally has instead.

Growth is regulated by several factors, which include availability of energy, amino acids (White *et al.,* 1973). In an earlier study on growth rate, Dolan *et al.,* (1997) concluded that weight gain is mostly observed in the control group of experimental animals, this is in accordance with the observation made on the control group in this study. Loss in body weight of rats administered CS and CP resulted from anorexia (withdrawal from food), withdrawal from foods was noted as the main factor that affected the body weights. During starvation, glucose reserve is empty mostly in a longer period of starvation; glucose must therefore be formed from non- carbohydrate sources such as glycerol and amino acid for survival (Stryer, 1995). These amino acids can be gotten from protein in the diet, since the animals withdrew from food; the protein in the diet is simply unavailable. Therefore, the only source of these amino acids is the breakdown of proteins in the skeletal muscle; this therefore leads to decrease in total body mass. The functional observational battery and locomotor signs observed in CS and CP administered groups include weakness, shortness of breath, rapid heartbeat, nose bleeds, decreased body weight, swollen gum and anorexia. Single therapy of chloroquine base drugs (CS and CP) particularly CS showed more toxic effects on biochemical and hematological indices in experimental rats than other tested drugs in this study. However, if administration of drugs continues with these drugs, hepatic complications in form of intrahepatic neoplasm and liver cirrhosis may result. O'Grady *et al.,* (1993) had reported that liver dysfunction usually presents within 2-3 weeks of initiation of treatment and can persist for several months despite discontinuation of the offending drugs. Acute toxicity of administered drugs may resolve over a protracted period of time as a result of short period of drugs administration.

Conclusion

Single therapy of the tested drugs (CS and CP) particularly CS (nivaquine) was more toxic than their combination therapy (CS+SP and CP+SP) within the short period of administration. The acute toxicity observed on biochemical and hematological indices was associated with clinical features of blood, hepatocellular and renal necrosis. The idiosyncratic reactions (eosinophilia and basophillia) resulting from allergic host response and other related conditions such as hemoglobinuria, polycythemia, leukocytosis, neutrophilia and lymphocytosis observed in this study may resolve over short period of time upon discontinuation of offending drugs.

Recommendations

The following recommendations are hereby made: 1. Strict compliance to Doctor's prescription,2. Avoidance of self-medication, 3. The need for Nigerian governments to formulate law and appropriate penalty against petty trading of drugs, 4. Thus the need to restrict access to drugs to hospital and pharmaceutical stores, and also, 5. The need to sensitize the public on the danger in drug abuse

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