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Acute toxicity test of some concoctions used for the management of malaria in Ado-Ekiti, Nigeria

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Abstract

Practice of complementary and alternative medicines continue to gain popularity in the recent times. These medicines are products of plants prepared by local herb sellers. Many herb sellers can be sighted on the street of many towns in Ekiti State, Nigeria selling their products to community people. The medicines are either prepared as powder or concoctions. Concoctions that are used to manage malaria were randomly purchased from three herb sellers in Ado-Ekiti metropolis, Ekiti State. These were subjected to acute toxicity test in Wister strain albino rats of either sex in order to determine their biosafety. Water was administered to the test animals in lieu of concoction for the control group. No mortality was recorded 24 hours post-administration of the concoctions to the test animals. The biochemical parameters examined in the test animals were alanine amino transferase, aspartate amino transferase and creatinine, all these parameters showed no significant difference (P>0.05) from the control group. The photomicrographs of the cellular structures of the visceral organs of the test animals also showed no morphological alterations. The acute intake of the concoctions did not cause any noticeable adverse effects on the biochemical and histological composition of the test animals. The concoctions appeared not to cause any potential harm to the animals and could be considered safe pharmacologically.

Keywords: Concoction; Toxicity Test; Biosafety; Herbal Medicine; Biochemical Parameters; Histopathology; Rat; Ekiti

1. Introduction

Malaria is one of the leading causes of morbidity in the world, being also a long-standing epidemic in tropical Africa. Twenty-nine countries accounted for 96% of malaria cases globally, and four countries; Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%) and Mozambique (4%) accounted for almost half of all cases globally [1]. Various interventions have been developed to control vector and parasites in order to curb malaria infection in humans. The use of mosquito repellent sprays, creams and mosquito insecticide treated nets are some strategies commonly used to control the malaria vector. Drugs, either alone or in combination, have also been developed for use as curative or prophylactic interventions. Malaria has long been treated with chloroquine, Artemisinin based combination therapy (ACT) such as artemether-lumefantrine or dihydroartemisinin-piperaquine [2, 3]. However, resistance of the parasites to some of these medicines [4], have presented formidable challenges to local people which have caused many to resort to the use of herbal medicines produced by the local herb sellers [5]. Although herbal medicines are considered to be safe for consumption but they may not be completely free from producing some adverse effects on humans, this makes toxicological evaluation important in order to access their bio-safety limits [6]. Toxicological evaluations are widely used to assess the toxicity in animals which is then used as a guide to choose a safe dose in humans during preclinical and clinical trials. The signs of noxious actions as a result of acute toxicity tests may manifest immediately or after a

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period of latency [7]. The Information obtained from these tests usually provide basis for assessing the safety or hazard of new chemical compounds [8]. These data also serve as a way to compare the potencies of drugs in experimental and clinical settings [9]. With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the bio-safety and quality of medicinal plant materials, finished herbal medicinal concoctions and products have become a major concern for health authorities, pharmaceutical industries and the public [10].

Many herb sellers can be sighted on the street of Ado-Ekiti and other major towns in Ekiti State, Nigeria, hawking their products for the treatment of various health challenges including malaria. These products are usually prepared either as powdery form or concoctions. Even though, there is oftentimes no standard dosage for the concoctions but an adult usually takes between 2.5ml to 5ml/kg body weight on single dose (personal observation). So far, no scientific investigations have been carried out on the bio-safety of these concoctions that are sold to community people particularly in Ekiti State, Nigeria, thus, this study becomes necessary with the objectives to examine the bio-safety of these concoctions in humans, especially the ones used for the management of malaria in Ekiti State. In this research, effects of toxicity of some concoctions used for the management of malaria from some herb sellers (hawkers) in Ado-Ekiti were examined.

2. Methods

2.1. Collection of herbal concoctions

Three herbal concoctions used for the management of malaria in Ado-Ekiti, Ekiti State, were randomly purchased from some three different herb sellers. Ado-Ekiti is the capital city of Ekiti State with population of 234567 [11]. The concoctions were labeled 'A', 'B' and 'C'. These three concoctions were examined separately using experimental animals in acute toxicity test.

2.2. Experimental animals and ethical approval

Wister strain albino rats of either sex (100-110g) were acquired from the animal house, College of Medicine, Ekiti State University, Ado-Ekiti. The animals were brought to the Department of Zoology's animal house and acclimatized to the environment a week before the commencement of the experiment. All animals were kept in cages and fed on standard rodent diet with clean drinking water *ad libitum*. The experimental protocols were authorized by Ethics and Research Committee Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State, Nigeria (approval number EKSUTH/A67/2023/03/006). Animal maintenance and treatment were also performed according to the guidelines set by Animal Research: Reporting of In-Vivo Experiments (ARRIVE).

2.3. Experimental design

Table 1 Experimental design in acute toxicity test of concoctions 'A' 'B' and 'C'

Phase 1 of the Experiment				
	Group 1	**Group 2**	**Group 3**	**Control**
Herbal concoction dose	10ml/kg BW	20ml/kg BW	40ml/kg BW	water
Concoction A	3 Rats	3 Rats	3 Rats	3 Rats
Concoction B	3 Rats	3 Rats	3 Rats	
Concoction C	3 Rats	3 Rats	3 Rats	
Phase 2 of the experiment				
	Group 1	**Group 2**	**Group 3**	
Herbal concoction dose	100ml/kg BW	150ml/kg BW	200ml/kg BW	
Concoction A	1 Rat	1 Rat	1 Rat	
Concoction B	1 Rat	1 Rat	1 Rat	
Concoction C	1 Rat	1 Rat	1 Rat	

The experiment was conducted in two phases as described by Bulus *et al*. [12]. During the phase 1 which was the Day 1 of the experiment, three (3) rats were randomly placed in a group per cage (n = 3). There were three groups for each of

the three concoctions 'A', 'B' and 'C' (i.e. concoction 'A' consisted of three groups that were given different concentrations of 'A' and likewise concoction 'B' and 'C'). During the phase 2 which commenced on Day 2 of the experiment, only one rat was placed in a group (n = 1). Similarly, there were three groups for each of the three concoctions 'A', 'B' and 'C'. However, the control group consisted of 3 animals per cage (Table 1).

2.4. Acute toxicity test of the concoctions

The acute toxicity test was conducted in a method similar to that of Lorke [13] as described by Bulus *et al*. [12]. The administration of the respective concoctions was done orally with a cannula. The rats were deprived of water at least 3 hours (but not food) before the administration of the concoctions. Rats from group 1 were given oral dose of 10 ml/kg body weight (b.w). Group 2 received 20ml/kg b.w. while the animals in group 3 were administered with 40ml/kg b.w. Animals in control group were given water as placebo. The administration of the concoctions in ml/kg body weight against the conventional practice of mg/kg body weight was to mimic the exalt way community people take the concoctions. After the administration of the concoctions, animals were carefully observed for physical signs of toxicity for a period of 24 hours and also to determine the median lethal dose (LD_{50}). During the Day 2 of the test, doses were administered based on the outcome of the first phase. Animals (n = 1) in group 1, 2, and 3 were given 100ml, 150ml and 200ml/kg b.w. respectively. Similarly, animals were carefully observed for physical signs of toxicity for a period of 24 hours and also to determine the median lethal dose (LD_{50}). All animals in the two phases of the experiment were monitored for duration of 14 days after which they were sedated under soft chloroform and then euthanized. Blood samples were collected for biochemical assays and essential organs (livers, kidneys and intestines) were harvested for histology.

2.5. Biochemical assays of the animals

The serum was separated from non-heparinized blood and the serum biochemical parameters examined were aspartate amino transferase (AST), alanine amino transferase (ALT) and creatinine using commercial Spectrum diagnostic kits (AGAPE reagent, AGAPE DIAGNOSTICS SWITZERLAND GmbH, Knonaustrasse 54-6330 Cham Switzerland).

2.6. Assay of Aspartate Aminotransferase and Alanine Aminotransferases' Activities (AST and ALT) respectively

ALT/AST enzymes present in most of the tissues especially in cardiac muscles, skeletal, muscles and kidney are released into the blood as a result of any injury to these tissues. The effect of the concoctions on serum aminotransferase activity of treated rats was assayed using the method of Reitman and Frankel [14]. In order to determine assay of aspartate aminotransferase activity, 0.5 ml of substrate was placed in a clean test tube and warmed at 37°C in a water bath for 2 min. Serum (0.1 ml) was added to the test tube, mixed and incubated for 60 min. After that, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the test tube. The test tube was allowed to stand for 20 min followed by the addition of 5 ml of 0.4N NaOH. The Optical density of the mixture was read at 505 nm against water. The enzyme activity was read off a standard curve produced with oxalo-acetic acid. The serum blank contained 0.5 ml of substrate and 0.5 ml of 2, 4- dinitrophenylhydrazine and 0.1 ml of serum. The test tube was allowed to stand for 20 min at room temperature and 5 ml of 0.4N NaOH added. The Optical density of the mixture was read at 505 nm against water.

2.7. Alanine aminotransferase (ALT) Activity

For assay of alanine aminotransferase activity, 0.5 ml of substrate was placed in a clean test tube and warmed at 37°C in a water bath for 2 min. Serum (0.1 ml) was added to the test tube, mixed and incubated for 30 min. After incubation, 0.5 ml of 2, 4-dinitrophenylhydrazine was added to the test tube. The test tube was allowed to stand for 20 min followed by the addition of 5 ml of 0.4N NaOH. The optical density of the mixture was read at 505 nm against water. The enzyme activity was read off a standard curve produced with Na pyruvate. The serum blank contained 0.5 ml of substrate and 0.5 ml of 2, 4- dinitrophenylhydrazine and 0.1 ml of serum. The test tube was allowed to stand for 20 min at room temperature and 5 ml of 0.4N NaOH added. The optical density of the mixture was read at 505 nm against water.

2.8. Creatinine Estimation (Jaffe method)

Creatinine reacts with picric acid in an alkaline solution to form a reddish coloured complex. The reaction is commonly known as the Jaffe reaction and the red colored product as the Janovski complex. Label 16 x 100 mm test tubes for each control and tested samples. To each control and sample tubes, add 1ml sodium tungstate reagent, 1mL sulfuric acid reagent and 1ml of distilled water. Mix well. Add 1 mL of each control and samples serum to the appropriate tube.

Mix well and centrifuge for 5 min at 1500 Random Per Minute (RPM) Label clean 16 x 100 mm test tubes for a blank, standard, and each control or samples for which a "filtrate" was prepared. Add reagents/samples to each tube according

to the following chart. It is acceptable to add picric acid reagent and NaOH sequentially to all tubes after all other materials have been added. Each test tube contained the same final volume. Mixed well and allowed to stand for 15 min at room temperature. The contents of test tubes were transferred to the appropriate cuvettes and the absorbance at 510 nm was read against the blank solution. The results were recorded on the data sheet. Determine the creatinine values for the control and tested samples by proportional calculation using the concentration of the standard and its absorbance.

2.9. Histopathological examination of some visceral organs of the animals

The histological examination of the harvested organs was done as being described by Slaoui and Fiette [15]. The pieces of the organs were fixed in 10% neutral buffered formalin for 48 hours. Afterwards, the organs were gradually dehydrated in ethanol solution in ascending gradient at different concentrations (50%, 70%, 90% an 100%, 3x per change for 10 min), cleared in xylene for 3 times for 5 min, infiltrated and embedded in paraffin 3 times for 10–15 min. Serial sections, 5µm thick was prepared and stained with hematoxylin-eosin on a microscope slide. All stained sections were examined under a digital microscope (VJ-2005 DN MODEL BIO-MICROSCOPE®) and the photomicrographs of the tissues were also taken with the same microscope to evaluate the histopathological changes.

2.10. Statistical Analysis

Data were analyzed with IBM SPSS version 20 and values were expressed as mean ± SD. Significance was determined using one-way analysis of variance (ANOVA). Probability value (*P*-value) of $P \leq 0.05$ was regarded as significant.

3. Results

Table 2 Effects of concoctions 'A', 'B' and 'C' on biochemical parameters of the rats

Concoction Type	Phase	Treatment	Serum Parameters					
		Dose (ml/kg)	ALT (IU/L)	P-value	AST (IU/L)	P-value	Creatinine (IU/L)	P-value
Control group		0	68.33±5.8		82.00±14.4		70.33±17.6	
ConcoctionA	Phase 1	10	79.33±13.6	0.433	99.00±28.5	0.316	69.33±14.1	
		20	67.67±5.7		66.00±18.3		57.33±18.0	
		40	63.00±20.4		78.67±20.9		81.67±5.6	0.239
	Phase 2	100	77		94		90	
		150	100		43		74	
		200	93		55		40	
Concoction B	Phase 1	10	77.67±5.5	0.783	76.67±24.7	0.982	73.33±22.8	
		20	75.00±20.7		88.33±5.7		63.00±6.9	
		40	81.33±13.6		83.33±20.8		61.00±23.5	0.937
	Phase 2	100	90		74		56	
		150	73		83		79	
		200	69		77		67	
Concoction C	Phase 1	10	74.00±19.5	0.626	91.33±12.7	0.423	44.67±11.7	
		20	48.33±11.5		61.33±4.0		76.67±19.6	
		40	69.67±30.0		88.67±35.8		66.67±11.4	0.174
	Phase 2	100	57		94		40	
		150	75		52		74	
		200	83		94		40	

No death was observed in all animals throughout the 14 days duration of the study. Macroscopic observation of the animals treated with the different concoctions 'A', 'B' and 'C' showed no apparent change in appearance (colour and size) of the systemic organs at different doses when compared with the control group. The levels of hepatic and renal enzymes analyzed were also not significantly (P>0.05) different from that of control group (Table 2). The different doses of concoction 'A' administered to the treated animals do not have significant effects on the levels of both ALT (P=0.433>0.05) and AST (P=0.316>0.05) when compared with the control group (Table 2). Similarly, there existed no significant effect (P=0.239>0.05) on the creatinine level of the treated animals and the control group (Table 2). Concoctions 'B' and 'C' similarly showed no statistically significant difference in the biochemical parameters examined among the treated animals and the control group (Table 2). Likewise, no morphological alterations were observed in the cellular structures of the visceral organs of the treated animals examined even at a very high dose of 200ml/kg body weight. The photomicrographs of the cellular structures of liver, intestine and kidney of the animals administered with 200ml/kg body weight and the control group is shown in Figure 1.

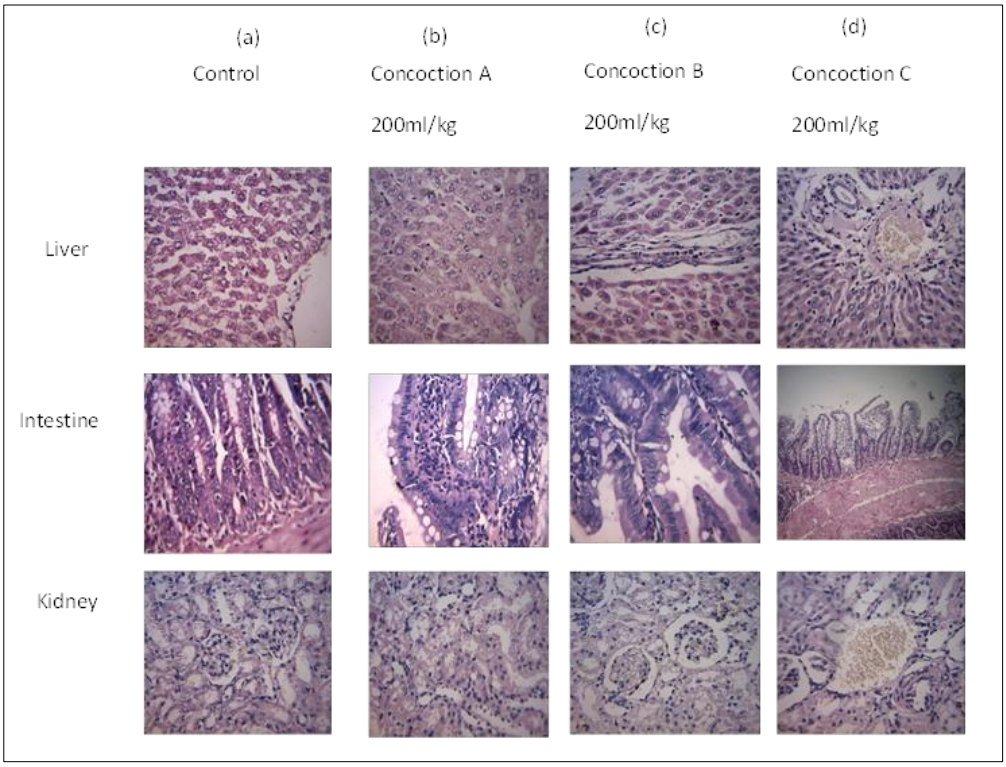

Figure 1 Photomicrographs of the liver, intestine and kidney: (a) Control (b) 200ml/kg of concoction A, (c) 200ml/kg of concoction B and (d) 200ml/kg of concoction C. The cellular architecture of the control and the tested animals after single oral treatment with the 200ml/kg body weight of the respective concoctions appeared normal (H&E x100)

4. Discussion

The index of acute toxicity (LD_{50}) in this study was not calculated as no death was recorded. LD_{50} is the dose expected to kills 50% of test animals 24 hours post-administration of the test drugs. The test animals in this study showed normal clinical signs after the administration of the respective concoctions. Observing clinical signs attributable to exposure to high dose of test substances such as time of onset, remission and recovery from those signs, sequence and timing of events that lead to death of test subjects are important aspect of toxicological studies [8, 16]. Toxicological manifestations of an administered substance are usually detected from behavioural and/or biochemical changes elicited in treated animals. In this study, behavioural pattern of the test animals was similar to the control group. The administration of the three different concoctions neither caused any observable changes in the histology of the liver, kidney and intestine nor in the biochemical parameters of the liver and kidney functions of the treated animals. Analysis of diagnostic serum parameters for liver functions (ALT and AST) and kidney functions (Creatinine) showed that the values were within normal range even at high doses, and whereas toxicities of substances are manifested as liver and kidney irregularities due to the involvement of these organs with metabolism [17, 18, 19]. In this study, no significant difference was observed in the levels of serum biochemical parameters in the treated animals and likewise no changes

were seen on histological examination. This is because the concoctions appeared not to cause any damaging effects that could provoke changes in the levels of these parameters even at high doses. Results similar to these were obtained in the acute toxicity test of aqueous extract of one herbal plant, *Alysicarpus ovalifolius* in mice [20]. This indicates that the acute intake of the concoctions did not cause noticeable adverse effects on the organs examined. The concoctions therefore, appeared not to cause any potential harm to the animals even at a very high dose of 200ml/kg body weight and whereas the community people who regularly consume these concoctions only take between 2.5 - 5ml/kg body weight on single dose. This implies that the concoctions appeared to be safe. However, further studies involving human subjects to confirm their safety on humans could not be overemphasized. This is because most medicinal herbal products which are of natural origin and were believed in the past to be safe for consumption are now with multiple reports of hepatotoxicity [21, 22].

5. Conclusion

The results of biochemical and histological analyses in the acute toxicity tests of these three concoctions in this study showed no significant damages to the system of the treated animals even at high doses. This shows that the concoctions are not harmful and could be considered safe for humans. However, further studies especially tests involving humans are necessary to confirm their safety on humans.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Ethical approval to conduct this work was obtained from Ethics and Research Committee Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State, Nigeria (approval number EKSUTH/A67/2023/03/006).

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