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PHARMACOKINETICS AND TISSUE DISTRIBUTION STUDY OF SICKLE CELL HOME REMEDY MIXTURE

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ABSTRACTS

Aim: Sickle cell home remedy mixture for the relief of symptoms associated with sickle cell disease has been toxicologically investigated and ascertained in the rat model in the previous study. However, this study was designed to ascertain the level of absorption, distribution, bioavailability, metabolism and excretion of the drug which is medically refers to as pharmacokinetic (PK) to ensure its effectiveness and safety.

Methods: The structural profiles, metabolites and the *in vivo* concentration of the mixture in blood plasma and different tissues homogenate (liver, kidney, liver, blood, heart and lung) of rats model collected at different time points after oral and intravenous (IV) administration at a single dose of 200mg/kg body weight were done using high performance liquid chromatography (HPLC) and LTQ-Orbitrap XL mass spectrometer (Thermo Electron, Begin China).

Results: A 55.3% bioavailability was recorded in both the male and female rats after 30 minutes of oral administration. There was no significant difference ($P<0.05$) increase in the level of plasma concentration between male and female rats at the same time points of administration. Three distinct metabolites (alkoxide, acetaldehyde and sodium triglycerides) were identified in the rat's feces and urine 24 h after administration of which the metabolic pathway were mainly hydrolysis and oxidation using cytochrome P450. Regarding the study data, One-way ANOVA was done employing Statistical Package for Social Science (SPSS) software version 21. Significant differences between male and female experimental rat groups were judged by Waller Duncan test. All data were articulated as mean ± and standard deviation (SD).

Conclusion: With the results of this Pharmacokinetic study, sickle cell home remedy mixture dose intervals can be determined and adjusted more accurately for effectiveness and safety of the end users. Moreover, the clinical trial will be used to fully understand *in vivo* disposition of this mixture and evaluate the mechanism of its biological response.

KEYWORDS: Pharmacokinetic, tissue distribution, sickle cell, home remedy, mixture.

INTRODUCTION

Sickle cell home remedy mixture comprises four substances; palm oil, ethanol, salt and water thermally homogenized and filtered. The crude palm oil, known also as red palm oil, extracted either by wet or dry processes, contains both healthy beneficial compounds, such as triacylglycerol (TAGs), vitamin E, carotenoids, phytosterols, as well as impurities, such as phospholipids, free fatty acids (FFAs), gums, and lipid oxidation products; the latter can be removed by means of refining processes (Derry *et al*., 2000). According to Sambanthamurthi *et al*, (2000), crude palm oil represents the richest natural source of carotenoids (500–700 ppm), tocopherols and tocotrienols (600–1200 ppm), all these contribute to its nutritional and medicinal properties (Mba *et al.*, 2015). The antioxidant component of palm oil exerts its medicinal importance against reactive oxygen species, which plays a significant role in aging, in cardio vascular diseases and in cancer prevention. Furthermore, tocotrienols have been reported to be natural inhibitors of cholesterol synthesis (Edem *et al*., 2002; Ong *et al*., 2002 and Sen *et al*., 2007). Concerning the ethanol component of this sickle cell home remedy mixture, although several reports of studies have been published on the side effect of alcohol or ethanol on human health the bottom line is that the majority of risk associated with the consumption of ethanol depend on the concentration intake by the individuals. According to the report of Food and Nutrition Board, Diet and Health (1989), and Farid (2008), the health effect of alcohol

vary between individuals and depend on the amount and type of alcohol consumed. In his report, ethanol is the psychoactive component in any alcoholic beverages, it is the substance that makes one drunk and has powerful effects on human's mood and metal state. Moderate intake of alcohol has been reported to reduce the risk of heart disease, raise High Density Cholesterol (HDL) in the blood stream, decrease blood pressure, lower the blood concentration of fibrinogen, reduces the risk of diabetes, and reduces stress and anxiety (Turner *et al.*, 1981; Hales and Hales, 1986; Klatsky et al., 1986).

Salt that is sodium chloride ($NaCl_2$) which was the third components of sickle cell home remedy mixture has been reported to have a lot of benefits and side effects on individual health depending on the concentration used by individual and his health status (Jennifer Le, 2019). According to the U.S. Dietary Reference Intake (2018), 500mg of sodium is required for human's body proper functions. Too much salt intake can damage the heart, aorta and kidney, causes heart failure, high blood pressure, stiffen of blood vessels and heart attack. On the other hand, the deficiency of salt in human's diet has been reported to be associated with some sever health conditions among which are: hypotremia that is abnormal low amount of sodium chloride in the blood which leads to the symptoms such as nausea, vomiting, headaches, altered mental state/confusion, lethargy, seizure and coma (WHO, 2016). In view of the above merits and demerits of sickle cell home remedy mixture's components, the concentration of each item in the mixture has been toxicologically evaluated and adjusted to a very safety level in comparison to the local and unscientific methods of formulation of the drug This is consistent with the study of Heintze and Funchs (2015) and Ademokoya *et al*., (2019) who examines the safe use of home remedy components to solve health problems. This approach will go a long way to in some health management systems.

MATERIALS AND METHOD

Drug preparation (sickle cell home remedy mixture)

Sickle cell home remedy mixture was prepared from locally made red palm oil, table salt ($NaCl_2$), 95% ethanol and potable water. The composition and concentration of these items are stated in Table 1. The salt, ethanol and water were purchased from licensed agrarian shop at Akungba metropolis, however, the palm oil was processed locally from palm fruits. The component were mixed together according to the proportion already evaluated in the toxicological study as stated in Table 1. The mixture was boiled at 100^{o}C for 15 minutes, cooled and filtered using membrane filtration assembly. The preparation was stored in refrigerator for further studies.

Experimental animals

Males and females Wister albino rats 28g body weight each, used for this experiment were purchased from Centre of Basic Science and Animal Study, University of Ibadan, Nigeria. They were brought to Microbiology Laboratory animal's cage, Adekunle Ajasin University Akungba Akoko where they had access to rat feed and water all through the experimental period. The rats were given 4 h post-dose fasting period in other to avoid food effects on the bioavailability of the drug. They were handled according to standard regulation for the use of laboratory animals. The assessment was carried out according to the ethical quid line of the Committee for Control and Supervision of Experimental Animal, January, 2000.

Table 1: Composition and concentration of items used for the preparation of sickle cell home remedy mixture used for pharmacokinetic study.

Component	Composition	Concentration
1	Salt	2g
2	Ethanol	10ml
3	Palm oil	5ml
4	Water	1000ml

Pharmacokinetic, tissue distribution and metabolism of sickle cell home remedy formulation (SCHRF)

This study gained the approval of Adekunle Ajasin University Akungba Akoko Academic and Research Grants Committee. The experiment was conducted in the Central Research Laboratory of the institution.

Experimental strategy

Oral administration study for pharmacokinetic of sickle cell home remedy formulation was conducted according to the methods of Carrillo et al, (2000), Shayeganpour et al, (2005, 2008) and Thomas et al, (2019) with a single dose of 200mg/kg BW of the drug. It was administered orally to 36 male and female Wister Albino rats grouped in 6 time points of administration. The above arrangement also was duplicated for intravenous administration study for pharmacokinetic evaluation, and a control group was setup. After 30 minutes of administration, serial blood samples were collected through cannula attached to the lateral tail vein of each rat in six different points (1/2, 1, 1:1/2, 2, 2:1/2, 3) h from the rat groups into heparinized tubes. Tissues and organs (heart, liver, kidney, and lung) were also harvested after they were killed. The blood samples were centrifuged at 4000 rpm for 10 minutes according to the method of Marcio *et al* (2014) to separate the plasma and was kept in fridge at -20^{o}C. The organs and tissues for study were rinsed with sterile distilled water, weighed before analysis.

Analysis of sickle cell home remedy mixture

Sickle cell home remedy mixture plasma and tissue concentration and its metabolites were determined using HPLC and LTQ-Orbitrap mass spectrometry (Thermo Fisher Scientific, Begin China) according to the methods of Rodriques *et al.*, (2012); Wang *et al.*, (2015) and Derry *et al.*, (2015) with slight modifications. Aliquot of the rats' plasma sample was mixed with 0.1 M sodium phosphate buffer (pH 5). The mixture was added to 500µL n-hexane and vortexed for 30 sec. The setup was centrifuged at 17000 rpm for 2 min at 4^{o}C. The supernatant was transferred to a clean glass tube and re-extracted 2 times more with the solvent. The organic extract was evaporated to dryness under a nitrogen gas at 60^{o}C and the residue was reconstituted in organic solvent – methanol. The reconstituted residue (20µL) was injected into the HPLC system for analysis. Moreover, the rat's tissue homogenate (heart, liver, lung and kidney) was added to 400µL of acetonitrile – a protein precipitating agent. The setup was vortexed for 1 min and centrifuged at 17000 rpm for 10 min at 4^{o}C to precipitate the protein content. The upper – layer was decanted to a new tube and 1 ml of n-hexane was added. The mixture was vortexed just like in the case of plasma above. The detection was conducted at 254 nm. Calibrations curves were linear ($r^2 \geq 0.995$) in the range of 25-200µg/ml.

Statistics

The study data were analyzed using One-way ANOVA via Statistical Package for Social Science (SPSS) software version 21. Significant differences between male and female experimental rat groups were judged by Waller Duncan test. All data were articulated as mean ± and standard deviation (SD).

RESULTS

The results of pharmacokinetic parameters assessed by non-compartmental analysis of the tissues and plasma concentration-time profiles of sickle cell home remedy mixture obtained after one dose of 200mg/ml oral administration in both males and females rat is shown in Table 2. The plasma drug concentration was highest in female rats (116.0 mg/ml) after 30 min of administration followed by 115.0 mg/ml in the same time-point of administration. However, the lowest plasma drug concentration was found in female rats 84.3mg/ml after 3 h of drug administration. On the other hand, the male rats had its lowest concentration 84.5mg/ml with no significant difference (P>0.05) between the male and female rat in the same time-point of administration.

Concerning the tissue distribution study, the highest drug concentration 16.8mg/ml recorded in the heart was found in the male rats 30 min after the administration of the drug, while the lowest concentration recorded 15.0mg/ml was found 2 and 3 hours after drug administration in the same gender. Moreover, in the lung, the highest drug concentration 21.2mg/ml was found in both gender but at different time-point of administration. While that of male was found at 2 hours after the administration, the female was found at 2 h: 30 min. The lowest concentration 15.8 mg/ml in the lung was found in male gender 1 hour after the administration. However, in the kidney, both the highest concentration 20.8 mg/ml and the lowest concentration 21.2 mg/ml were found in the male gender but at different time-point of administration 1 h: 30 min and 3 hours after the drug administration respectively.

Table 3 shows the bioavailability of sickle cell home remedy mixture calculated from every time point of administration from a single dose of 200 mg/ml in rat model. The highest value 119.1% was found in the blood plasma of the experimental rats after 3 hours of administration the drug, while the lowest value 55.3% was found after 30 min of drug administration.

Moreover, Table 4 is the result of the analysis of Pharmacokinetic parameters estimated by non-compartmental evaluation of the plasma concentration-time profile of sickle cell anemia home remedy mixture administered in a single 200mg/kg.bw dose. The mean maximum plasma peak concentration (C_{max}) was 116% while the time taking to reach this peak (t_{max}) was 30 min after administration. The drug bioavailability measured by the area under plasma concentration time curve (AUC) in this study was119.1mg/ml, while the elimination half-life ($Ct_{1/2}$ was 90 min.

Furthermore, the Plasma concentration time curve of intravenous and oral administration of 200 mg/ml sickle cell home remedy mixture in rat model is shown in Figure 1. The highest plasma concentration 120 mg/ml reached in oral administration was attained at 90 min after dosing the rats. While the lowest was 50 mg/ml attained at 180 min after the administration. On the other hand, the highest plasma concentration recorded in intravenous root of administration 152 mg/ml was attained after the drug administration, while the lowest value 42 mg/ml was recorded180 min after administration.

Table 2: Pharmacokinetic parameters assessed by non-compartmental analysis of the tissues and plasma concentration-time profiles of sickle cell home remedy mixture obtained after one dose of 200mg/ml oral administration in male and female Wister Albino rats.

Tissue evaluated	Sex	PK at each time-points of administration						
		0	30	1	1:30	2	2:30	3
Heart	Male	200	16.8	15.5	15.5	15.0	15.8	15.0
Female	200	16.6	15.3	15.5	15.2	15.9	15.1	
Liver	Male	200	16.2	21.9	16.2	15.8	15.3	16.7
Female	200	16.1	21.8	16.1	15.4	15.2	16.8	
Lung	Male	200	20.8	15.8	20.3	21.2	21.1	20.0
Female	200	20.6	15.9	20.1	20,1	20.2	20.1	
Kidney	Male	200	20.6	20.5	20.8	20.7	20.3	21.2
Female	200	20.7	20.4	20.6	20.5	20.3	21.3	
Blood plasma concentration	Male	200	115.8	108.2	102.3	115.5	115.3	84

Table 3: Bioavailability of sickle cell anemia home remedy mixture calculated from every time point of administration from single dose of 200mg/kg bw.

Parameters	Time- points of administration the drug					
	30 min	1 h	1:30	2	2:30	3
(AUC) Intravenous administration	152	126	101	87	74	42
AUC Oral administration	84	100	120	80	60	50
Bioavailability	55.3	79.4	118.8	92.0	81.1	119,1

Table 4: Pharmacokinetic parameters estimated by non-compartmental evaluation of the plasma concentration-time profile of sickle cell anemia home remedy mixture administered in a single 200mg/kg.bw dose.

Parameter	Sickle cell anemia home remedy mixture
Cmax	116.0%
$Ct^{1/2}$	90 minutes
t_{max}	30 minutes
AUC	119.1µg.h/mL

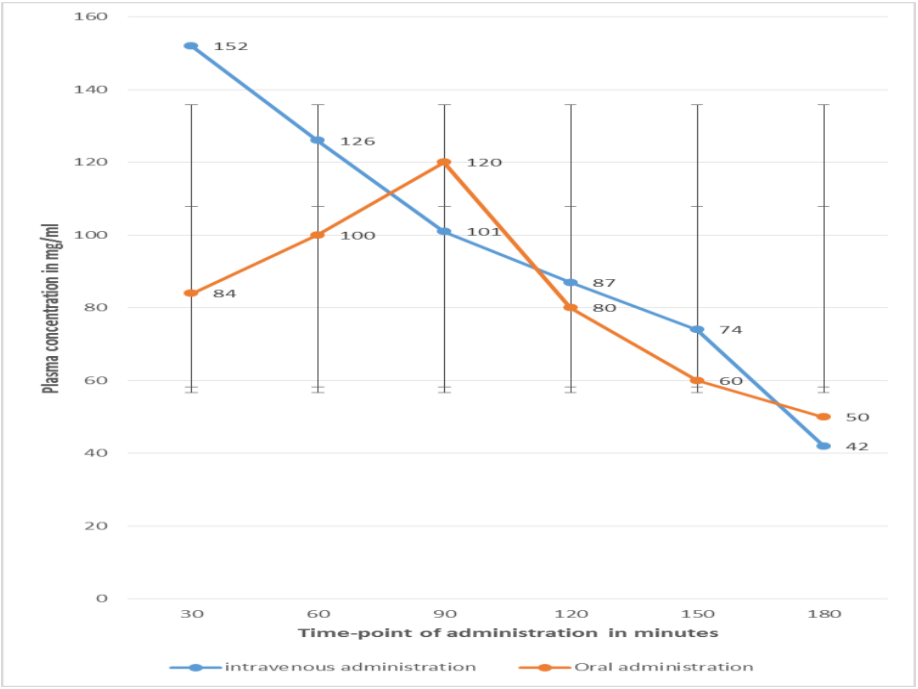

Figure 1: Plasma concentration time curve of intravenous and oral administration of 200 mg/ml sickle cell home remedy mixture in rat model.

DISCUSSION

The study of the time course of absorption, distribution, metabolism and excretion called pharmacokinetic according to Jennifer Le (2019); Moore *et al.,* (2015) and Heintze and Funchs (2015) of sickle cell anemia home remedy mixture was carried out in this study. One of the ethics of pharmaceutical industries has been to carry out the pharmacokinetic (PK) for a new drug isolate in order to achieve the goal of drug's efficacy and decreasing toxicity of patient's drug therapy (Rodriques *et al*., 2012; Wang *et al*., 2015). Therefore, It was found convenient to use Swiss Albino rats to determine the pharmacokinetic properties of this drug isolate since the Food and Drug Administration (FDA) has a guidance for industry on the conversion of animal dose to human equivalent dose based on body surface area (U.S. Department of Health and Human Services (1987). After, one hour of administration of this drug, the maximum plasma concentration (C_{max}) recorded 116.0%. Unlike the work of Thomas *et al* (2019). In their report, there was delay in attaining the maximum plasma concentration (t_{max}) of 60-70% amiodarone drug tested. This discrepancy could be due to the nature of the drugs. In this study, the drug – sickle cell anemia home remedy mixture was in liquid form which does not require dissolution before absorption especially when administered orogastically, but in the second authors' work, the drug – amiodarone tested was a solid caplets which requires dissolution before absorption. In addition to this, the delay to reach t_{max} for amiodarone could also be due to the co-administration of this drug with *carica papaya* in the rats tested. It is a well-known fact that the rate and extent of absorption of drug depends on the route of administration, the formulation and chemical properties of the drug and physiologic factors that can impact the site of absorption (Greenblatt *et al*., 1980). It was documented by these authors that amiodarone had low solubility (Thomas *et al.,* 2019). In this study, the highest plasma concentration obtained – 116.0% could be due to a time allowance allowed between the feeding of the experimental rats and the administration of the drug which actually could have prevented the interference in the absorption of the drug by the food. In this study, out of 200 mg/kg bw drug administered to the rats model after 30 minutes, a 55.3% bioavailability was recorded in both the blood plasma, tissues and organs tested.

Concerning the drug distribution, appreciable concentration was found in the tissue or organs tested. For instance, in the liver the highest concentration (21.9%) was found 1 hour after the drug administration. This value when compared with Thomas *et al.,* (2014), a wide range of difference would be noticed. In their report, after 24 hours post-dose of their drug to the rats tested, 2.5µg was found in the rat's liver. This discrepancy could be due to the differences in binding capacity of individual drug to the blood plasma which these authors also reiterated in their report. Many drugs such as digoxin, amiodarone and so on have great affinity to bind blood plasma protein like albumin (Derry *et al*., 2015; Jennifer Le, 2019), therefore, little concentration would be found in the tissues and organs of animals they were administered. Moreover, in this study, it must also be highlighted that the volume of distribution (V_d) of this drug varied from organ to organ. This finding is not anomaly because various factors affect drug distribution in animal's body. Apart from the proportion of blood flow rate in the different tissues, the age of the subjects, body composition, cardiac function and many others are involved (Jennifer le, 2019).

Concerning the metabolism of this drug, the enzyme cytochrome P450 system made the drug more polar through oxidation-reduction process. The drug was catalyzed by biotransformation into sodium ethoxide commonly called alkoxide (white precipitate). Other noticeable metabolites of this drug were sodium triglycerides and sodium ethanoate which were excreted via liver and kidney and found in faces and urine of rats' model.

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