Journal of Advances in Medicine and Medical Research

28(4): 1-6, 2018; Article no.JAMMR.44452
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Animal Toxicological Studies of Agunmu Iba (Malaria Herbal Medicine)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/44452
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/27800

Original Research Article

Received 12 September 2018
Accepted 29 November 2018
Published 17 December 2018

ABSTRACT

Malaria is a disease that kills millions of people worldwide every year and demographic studies tell us of the resilient or resistant nature of the malaria parasite to orthodox drugs. Agunmu iba is a herbal concoction made locally to cure or prevent malaria in Africa. Agunmu iba extract was investigated for its toxic effects in Wistar rats using liver and kidney functionality indicators. Thirty six albino rats weighing 200.08 ± 10.21g were assigned into six groups (A–F) of six animals each randomly. Test groups A–E animals were administered orally daily with 1 ml of the extract equivalent to 50 mg/kg body weight of the extract for a 15 duration with the control group receiving 1 ml of distilled water orally. On the 16th day, the animals were sacrificed. The extract decreased alkaline phosphatase (ALP) activities in the liver to about 80% loss. No consistent pattern was recorded in the kidney ALP activity and serum bilirubin level, the serum enzyme compared well ($p>0.05$) with the control value. Acid phosphatase activity of the tissues and serum of the animals showed no significant effect. The extract reduced urea, albumin and creatinine levels in the serum. These observed changes in biochemical parameters by the aqueous extract of Agunmu iba may have consequential effects on the normal functioning of the liver and kidney of the animals.

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Keywords: Agunmu Iba; alkaline phosphatise; toxicity studies; liver function indices; Wistar rats.

1. INTRODUCTION

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes.

Medicinal herbs are gaining wide acclamation with a greater number of people seeking remedies and health approaches free from side effects occasionally caused by synthetic drugs. For so many reasons, many people are more concerned about their health and so tend to seek succour with the use of herbal medicines, not only to prevent diseases but also to treat them. This is an honest fact for a wide variety of illnesses vis-à-vis common cold, scratches, malaria fever and stomach disorders which are to a large extent home treated (Kincheloe, 1997). According to statistics generated by WHO, about 75% of the world's population are currently into trado-medicinal therapy for alleviation of their ailments and diseases [1].

Malaria is a global disease that is predominant in the tropics and caused by blood parasites, *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium vivax.* In Nigeria, malaria is mostly caused by *P. falciparum* and *P. malariae*. The female anopheles mosquito transmits these parasites to humans. According to the World Health Organisation (WHO), malaria has great morbidity and mortality than any other infectious diseases of the world. The survey shows that 90 per cent of the world's cases of malaria occur in sub-Saharan Africa. Nine out of 10 cases of this disease occur in this region and record over one million deaths annually. The high mortality rate is recorded in children and pregnant women. The disease also has a negative impact on the economy of prevalent countries. Medicinal plants has gained wild acclaim worldwide in the treatment and prevention of malaria. Until now, the world has relied on plants for the best malaria drugs: chloroquine from Cinchona tree; and Artemisinin from Chinese salad plant, *Artemisua annua.* The emergence of the ineffectiveness of chloroquine [2] in combating malaria led to additional studies, which produced Artemisin.

However, the malaria parasite is also developing resistance to the World Health Organisation (WHO) celebrated drug, Artemisin Combination Therapies (ACT). This has led to intensified search for the next best malaria drug from the source of choice, plants [3]. Unfortunately, indigenous medicinal plants in Nigeria used in combating malaria are yet to be given a look in, as the foreign plants, in spite of documented evidence of their efficacy against resistant strains of malaria.

With the interesting skyrocketing growth in the use of herbal medicine, thorough scientific investigations of these plants is needed so as to ascertain their safety and toxicity levels [4]. An example of such plant that has been thoroughly researched upon by the Traditional Medicine Association of Nigeria and accepted in the management of several ailments such as malaria and enteric fever, diabetes mellitus, infertility, gonorrhoea and diarrhoea [5] due to its acceptable safety levels and minimal toxicity is *Cochlospermum planchonii*.

The aim of this study is to determine the toxicity effect of herbal concoction (Agunmu Iba) on male Wistar rats.

2. MATERIALS

Plant Material: The raw dried leaves of Agunmu Iba were purchased from Oja Timi market, in March 2016, in Ede, Osun state, Nigeria.

Preparation of the Extract: A portion (800 g) of the powder was extracted in 1.6 litres of distilled water followed by thorough shaking for 24 hr with magnetic stirrer. The mixture was then filtered and freeze-dried (Ilshin Freeze-Dryer, Model no: FD5518, Ilshan Laboratory Company Ltd., Seoul, Korea) to give 22.75g (percentage yield of 2.84). A calculated amount of the residue was weighed and reconstituted in distilled water to give the required dose of 50 mg/kg body weight. The herb powder material was mixed with 7up as normal intake of human being and calculated to meet the requirement of the animal's body weight (Afr. J. Traditional Complementary and Alternative Medicines [6].

Experimental Design: Thirty six male albino rats of Wistar strain were bred for approximately three months to the average weight of 200.08 ± 10.21g at the animal housing unit of Federal Polytechnic, Ede, Osun State, Nigeria were used to carry out the work. The animals were divided into six groups of six rats per group (A-F). Each group of the rats were housed in a separate cubicles in a different metabolic cage with facilities of proper ventilation, supply and collection of left over feed, supply and collection of left over water, and were placed under standard conditions (temperature 23 ±1°C; photoperiod: twelve hours natural light and twelve hours dark; humidity: 45–50%). They were allowed free access to rat feed (Broiler's Finisher from Premier 'Feeds, Ibadan') and contaminant free tap water. The animals were acclimatised for 2 weeks before the commencement of the experiment.

The rats in the control group F were orally administered with 1ml of distilled water, while the animals in other groups (A-E) were administered with the same volume corresponding to 50 mg/kg body weight of the extract for days 1, 3, 5, 10 and 15 respectively, the extract of already prepared plant material samples (Agunmu Iba).

Ethical Approval: Ethical approval was obtained from the Institution and it was in accordance with the guidelines and care for animal usage in accordance with the American Physiological Association (2002).

Assay Kits and Other Reagents: The assay kits for alkaline phosphatase (ALP), acid phosphatase (ACP), urea, creatinine and albumin were products of Randox Laboratory Ltd., County Atrim, U.K. Formaldehyde, chloroform, distilled water, sucrose solution and all other reagents used were of analytical grade and were products of Sigma Aldrich, St. Louis, USA.

Preparation of Serum and Tissue Homogenates: At the end of the experiment, the rats were sacrificed under anaesthesia with chloroform then and blood collected via cardiac puncture and allowed to clot for 30 min centrifuged at 3000 *g* for 15 min using bench centrifuge(Remi Laboratory centrifuge model RM-12C BL). The sera were thereafter aspirated into clean, dry, sample bottles using Pasteur pipette and were kept at -20ºC for the respective assays. After collection of blood the animals were dissected and respective organs collected. The kidney was decapsulated while the liver was cleaned of blood and other extraneous materials with clean tissue paper, weighed and homogenised in 0.25 M sucrose solution (1:5 w/v). The tissue homogenates were then kept at -20ºC for further analysis.

Determination of Biochemical Parameters: The biochemical parameters were determined using standard methods as described for alkaline phosphatase [7,8], Urea [9], Creatinine [10], Albumin [11] and Bilirubin [12].

Statistical Analysis: SPSS v. 20 computer software package (SPSS Inc. Chicago, U.S.A) was used for the computation of results obtained from this study. Data are presented as mean ± standard deviation (SD) and comparing data with respect to significant difference was evaluated using ANOVA for comparison between sample means with level of significance assessed at 5% confidence interval.

3. RESULTS

Significant ($P< 0.05$) decrease of alkaline phosphatase activities was observed in the liver of the animals after extract administration. This was readily seen from day 1 of extract administration as shown on Table 1, by then about 4/5ths of the enzyme's activity had been lost. No regular pattern was observed with kidney ALP activity as the values changed constantly throughout the duration of the experiment. ALP activity in the serum was comparable with control values. While for acid phosphatase, the reverse was the case as there were no significant difference between control values and test subjects as shown on Table 2.

Administration of the extract reduced the serum, urea, albumin and creatinine content in the animals. For urea and albumin, this was obvious on day 1 of dosing while for creatinine this change was seen on day 3 as shown on Table 3. For bilirubin, an initial decrease was observed until after the first three doses and only after the serum concentration increased to thrice its control value. After the duration of the experiment the bilirubin level in the serum of was commensurate with control values as shown on Table 3.

4. DISCUSSION

Malomo [13] and Yakubu et al. [14], observed that levels of marker enzymes in tissues and

Table 1. Aqueous extract effect of agunmu iba on alkaline phosphatase activities of rat liver and kidney

Group	Liver(U/L)	Kidney(U/L)	Serum(U/L)
Control	36.43±0.89	112.66±3.54	8.18±0.47
A	14.19±0.05	103.75±2.62	8.17±0.45
B	23.29±0.64	139.87±2.26	8.24±0.71
C	11.43±0.59	133.88±1.47	8.07±0.44
D	13.53±0.12	69.15±1.89	8.16±0.49
E	13.17±0.18	140.75±2.07	8.28±0.26

Values are mean ± SD of 6 determinations.

Table 2. Aqueous extract effect of agunmu iba on the acid phosphatase activities of rat liver and kidney

Group	Liver(U/L)	Kidney(U/L)	Serum(U/L)
Control	63.52±0.10	126.46±0.26	34.01±1.86
A	63.34±0.09	127.13±0.15	35.21±0.68
B	64.19±0.18	126.20±0.01	36.64±1.45
C	63.63±0.03	127.29±0.65	35.29±1.43
D	63.27±0.04	126.80±0.20	33.37±0.48
E	63.89±0.11	124.53±0.22	36.58±0.34

Values are mean ± SD of 6 determinations.

Table 3. Aqueous extract effect of agunmu iba on some liver and kidney functional indices of albino rat

Group	Urea(µmol/1)	Creatinine	Albumin	Bilirubin
Control	3.67±0.51	3.19±0.09	7.19±0.15	4.05±0.06
A	1.78±0.10	3.35±0.01	5.11±0.08	1.47±0.04
B	1.79±0.20	3.14±0.16	2.13±0.08	1.57±0.03
C	2.42±0.14	4.24±0.05	4.16±0.01	13.40±1.15
D	1.85±0.18	2.18±0.01	5.20±0.15	11.36±1.19
E	1.69±0.04	0.47±0.03	3.22±0.01	4.13±0.07

Values are mean ± SD of 6 determinations.

body fluids may be assessment criteria of extent of assault or organ damage of a chemical compound. Such test batteries may also be used to predict tissue cellular damage caused by a chemical compound before tissue histology is carried out [15]. Akanji et al. [16] reported that alkaline phosphatase is a marker whose enzymatic activity is used often to assess the strength of the plasma membrane. any change in this enzyme's activity found in the serum and tissue lysates will most likely be an indication of insult on the membranes itself [17]. Henceforth, a reduction in the liver ALP activities without concomitant increase in the serum enzyme in this study following the administration of aqueous extract of agunmu iba could be linked to either inhibition of the enzyme activity at the cellular/molecular level [16], no activity of the enzymes itself *in vivo* [18]. This could affect or hinder the normal transport of necessary ions or molecules across the membrane [16]. It could also affect other enzyme linked metabolic processes which includes synthesis of nuclear proteins, nucleic acids, phospholipids and phosphate esters cleavage. The irregularity observed in the activity of ALP in the kidney tissues may be linked an effort in ignoring the effect of the extract. This lack in the serum ALP's activity may indicate that the reduction in the liver enzyme was not due to membrane labilisation, but rather to inactivation or inhibition of the enzyme itself. A marker for damage on lysosomal membranes is acid phosphatase. In this study no effect was observed with the extract, a clear cut indication that the lysosomal was kept intact. This absence could be that the extract showed some form of selectivity as far as the 2 organs were concerned. Blass et al. [10], [11] and Saad et al. [19] reported that albumin, bilirubin, creatinine and urea are among the indices used to evaluate liver and kidney functionality. Agunmu iba showed a reduction in

the levels of urea and creatinine in the serum indicating some form of hepato-bilary protection. A decrease in the serum albumin level in the liver could be linked to its diminished functionality which could be attributed to hepatocellular damage [20]. This was in agreement with the findings of Moudgil and Narang [21] who observed this same decrease at 200mg/kg bw of the extract of *Hippobromus pauciflorus* leaves to male rats. The same authors noted that Biluribin is a core metabolic product of blood with measurable indices. Since no effect of this was observed with the extract, it may indicate it was not relevant toxicologically [22].

In summary, agunmu iba extract changed enzyme activity, albumin, bilirubin, creatinine and urea levels in the serum. These observed changes may have substantial effects on ion transportation in the membrane and also in its effectiveness of the internal organs like kidney and liver. As way forward a dose of 50mg/kg bw of agunmu iba extract may not be exceeded when taking such remedies.

5. CONCLUSION

There is no doubt that herbal therapeutic remedies have come to stay in the present world, but caution should be taken by all and sundry who take delight in its 'unproven efficacies'. In Ghana for example, recently [3] carried out a survey of medicinal remedies used in combating malaria and its efficacy was assessed. The study reported some findings showing the drug had more benefit than ACT (artemisin combination therapy). Scientific trials of the clinical nature are strongly needed to ascertain safety and dose range of these remedies, also medicinal plants reputed to have antimalarial properties should be exploited and used in the production of efficacious drugs [23]. The public also needs to be informed as to the needed precautions in using such products.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Institution and it was in accordance with the guidelines and care for animal usage in accordance with the American Physiological Association (2002).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27800