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Antipyretic and Antianemic Activities of Three Anti-malaria Recipes from South Benin on Wistar Rats

Seindé Espérance MEDOATINSA[1,2], Cokou Pascal AGBANGNAN DOSSA[1,*], Sossa Pascal ATCHADE[4], Gbèdossou Sophie Reine BOGNINOU[1], Kossivi DOSSEH[3], Tchazou KPATCHA[3], Amegnona AGBONON[3], Hyacinthe AHISSOU[2], Dominique SOHOUNHLOUE[1]

[1]Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), Ecole Polytechnique d'Abomey-Calavi (EPAC), Université d'Abomey-Calavi (UAC), 01 BP 2009 Cotonou, Bénin
[2]Laboratoire d'Enzymologie et de Biochimie des Protéines (LEBP), Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC), Bénin
[3]Laboratoire de Physiologie et de Pharmacologie (LPP), Faculté des Sciences (FDS), Université de Lomé (UL), Togo
[4]Département de Génie de Biologie Humaine (GBH), Ecole Polytechnique d'Abomey-Calavi (EPAC), Université d'Abomey-Calavi (UAC), 01 BP 2009 Cotonou, Bénin
*Corresponding author: cokou2005@yahoo.fr

Abstract The present study aims to evaluate "*in vivo*" the acute toxicity, antipyretic and antianemic activities of three plant recipes used in the treatment of malaria in southern Benin lake cities on Wistar rats. We note the presence of saponosides, phenolic compounds, sterols and terpenes in the recipes studied. The toxicity evaluation of the extracts revealed that they are practically non-toxic (LD_{50}> 5g/kg body weight) according to the Hodge and Sterner classification. All the extracts investigated contain antipyretic molecules but only two extracts (aqueous extract obtain by decoction of the child's recipe: 36.07 ± 0.48°C, hydroethanolic macerated of the adult's recipe: 36.07 ± 0.33°C) showed significant antipyretic activity, similar to that of aspirin (36.03 ± 0.25°C) used as reference molecule in the present study. After evaluating the antianemic activity, we note that the extracts are not hemolytic.

Keywords: *toxicity, antipyretic, antianemic, metabolites, plants, malaria*

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1. Introduction

Malaria is a parasitosis due to the presence and multiplication in the human organism of a protozoan of the genus Plasmodium transmitted by the bite of a female mosquito, the Anopheles, causing intermittent fevers which determines an erythrocytopathy with haemolysis. Among the clinical signs of this disease, we have fever and anemia. Anemia is due to the mass destruction of parasitized erythrocytes while the release of hemozoin into the blood circulation is the basis of the feverish state of the malaria [1,2]. The World Health Organization (WHO) estimates the incidence of malaria at 198 million cases. The mortality practically always due to *Plasmodium falciparum* is 584000 per year, the majority of cases is recorded in the countries of sub-saharan Africa where every minute a child dies [3]. In Benin, malaria is the leading cause of hospitalization and death as well as the general population, pregnant women and children under five years of age [4]. Malaria represents approximately 20% of cases of diseases treated in traditional medicine [5]. According to the WHO, in some developing countries in Asia, Africa and Latin America, 80% of the population depends on traditional medicine, especially in rural areas, because of the proximity and accessibility of this type of care at affordable cost and mainly because of lack of access to modern medicine these populations [6]. Therefore, traditional medicine can be considered as an integral part of primary health care, to improve access to care. Unfortunately, these drugs do not benefit from scientific control and are a constant danger with respect to therapeutic doses and lethal doses, and hence the safety margin problem. Thus, it is necessary to evaluate clinical efficacy, ensure the safety of medicinal plants, strengthen the knowledge of traditional herbalists and ensure adequate follow-up of patients. The aim of this study is to evaluate "*in vivo*" the acute toxicity; antipyretic and antianemic activities of three recipes of plants used in the treatment of malaria in the lake cities of South Benin.

2. Material and Methodology

2.1. Material

2.1.1. Plant Material

It is composed of 3 plants recipes, obtained from an ethnobotanical survey conducted throughout the South Benin lakeside population (Ganvie, Aguegue-Daho). These recipes are used in the treatment of malaria in pregnant women, adults and children. The composition of recipes is presented below. After collection, the plant material has been identified in the National Herbarium of the Department of Botany, Abomey-Calavi University. The samples were dried over laboratory temperature (25-30 °C) until their stabilization and then reduced in powder with an electric grinder (Brand RETSCH, Type SM 100).

Child	
Species	**Family**
Pteleopsis suberosa	Combretaceae
Waltheria indica	Sterculiaceae
Lippia multiflora	Verbenaceae
Combretum indicum	Combretaceae
Ehretia cymosa	Boraginaceae
Hyptis suaveolens	Lamiaceae

Adult	
Species	**Family**
Morinda lucida	Rubiaceae
Cassia siamea	Caesalpinoides
Cymbopogon citratus	Poaceae
Rytigymia umbellulata	Rubiaceae
Flacourtia indica	Flacourtiaceae
Pavetta corymbosa	Rubiaceae
Croton zambesicus	Euphorbiaceae
Cocos nucifera	Arecaceae

Pregnant woman	
Species	**Family**
Hibiscus surrattensis	Malvaceae
Pavetta crassipes	Rubiaceae
Cajanus cajan	Papilinoideae
Dissotis rotundifolia	Melastomataceae
Cymbopogon citratus	Poaceae
Pleiocarpa pycnantha	Apocynaceae
Spathodea campanulata	Bignoniaceae
Cymbopogon giganteus	Poaceae
Phymatodes scolopandria	Polypodiaceae
Dichapetalum madagascariensis	Dichapetalaceae
Newbouldia laevis	Bignoniaceae

The number of plants per recipe varies from 6 (child) to 11 (pregnant woman). Rubiaceae is the most represented family followed by that of Poaceae. *Cymbopogon citratus* was found in 2 recipes (adult, pregnant woman) on the three objects of the present study.

2.1.2. Animal

Male and female wistar rats were produced by the Department of Physiology/Pharmacology of University of Lome (Togo). Animals were kept under ambient temperature, with a 12 hours light/dark cycle and had free access to food and water.

2.1.3. Laboratory Material

Spectrophotometer, electronic thermometer equipped with probe, centrifuge, capillary tubes, needles.

2.1.4. Reagents

Yeast of beer, NaCl, Dinitrophenylhydrazine, ether, ethanol.

2.2. Methodology

2.2.1. Phytochemical Screening

The determination of metabolites was done by differential coloring reaction and/or precipitation of the major families of chemical compounds contained in plants. So, sterols and terpenes have been identified by the Liebermann test [7]. The characterization of the phenolics compounds was made by the reaction with ferric chloride [8]. Flavonoids identification was carried out by the test of cyanidine [9]. The compounds belonging to the group of tannins have been highlighted by the reaction of Stiasny [10]. The free or combined quinone compounds have been disclosed by the reaction of Borntraeger [11]. The saponosides research is based on foam test; degree of aqueous decoction dilution giving a persistent foam after shaking [12]. Alkaloids were identified by Mayer test and confirmed by Bouchardat test.

2.2.2. Acute toxicity

In order to evaluate the acute toxicity of the extracts, the rats were fasted 12 hours with free access to water. The extracts were administered by gavage at the dose of 5 g/kg of body weight of the animal. They were divided into seven lots, one control group receiving distilled water. After the gavage of the extracts and the distilled water, the rats were observed immediately and then every 30 minutes, for three hours on the first day and once a day for 14 days. During this period, symptomatic disorders (diarrhea, shivering, salivation, convulsion, agitation, lack of appetite, motor difficulties and dyspnea) were sought (Bakoma *et al*., 2013) [13].

2.2.3. Antipyretic Test

This test was carried out according to the methodology used by Dosseh *et al*., in 2014 [14] followed by some modifications. The extracts were tested at the dose of 500 mg/kg of body weight of the rats. Hyperthermia was induced by injection of 20% beer yeast intraperitoneally at a dose of 5 mL/Kg body weight of the rat. Before the injection of the beer yeast, the rats were fasted for 12 hours and the initial rectal temperature was taken. 13 hours after the injection of the brewer's yeast, the rectal temperature was taken again. Only rats that showed an increase in temperature of at least 0.5 °C were used for experiments. Distilled water and extracts were administered orally and the temperature was measured at 1, 2, 3, 4, and 5 hours after drug administration. In sum, 8 lots of rats were formed as follows:

Lot1 distilled water (negative control),

Lot2 aspirin (positive control),

Lot3 Aqueous extract obtain by decoction of the child's recipe

Lot4 Macerated hydroethanolic of the child's recipe

Lot5 Aqueous extract obtain by decoction of the pregnant woman's recipe

Lot6 Macerated hydroethanolic of the pregnant woman's recipe

Lot7 Aqueous extract obtain by decoction of the adult's recipe

Lot8 Macerated hydroethanolic of the adult's recipe

2.2.4. Antianemic Test

It was conducted following the technique used by Diallo in 2008 [15] with some modifications. Blood samples were taken before, during and at the end of the test in order to evaluate the haematological parameters of the rats on day 0, day 7 and day 14. The injection of phenylhydrazine to day 1 and day 2 has allowed us to induce hemolytic anemia in rats. They were gavaged with extracts at the dose of 500 mg/kg of body weight of the rats from day2 to day13.

The rats were divided into five lots:

Lot1 Anaemia rats + distilled water (negative control)

Lot2 Normal rat + distilled water (positive control)

Lot3 Anaemia rats + aqueous extract obtain by decoction of the children's recipe

Lot4 Anaemia rats + aqueous extract obtain by decoction of the pregnant woman's recipe

Lot5 Anaemia rats + aqueous extract obtain by decoction of the adult's recipe

The osmotic resistance of red blood cells of rats in the study was determined J7. The method is to induce haemolysis. This test will assess the degree of fragility of red blood cells. A range of saline (NaCl) from 0% to 0.9% should be prepared according to the Redondo protocol (1995) [16]. At each of these different concentrations, 50 µL of the blood is added. The mixture is homogenized (shaken slightly) and then incubated for 60 minutes. After incubation, the mixture is centrifuged at 1085 rpm for 10 minutes. The supernatant is collected and the absorbance read at 540 nanometers with the spectrophotometer (4049LKB, Biochrom, Cambridge, CB4 4FJ Angland). The percentage of haemolysis is determined by the ratio of the absorbance test. The curve giving the percentage of haemolysis is produced as a function of the concentration of sodium chloride (NaCl). This curve is compared with that of a normal control.

$$\%\ \text{Haemolysis} = \frac{Absorbance Test}{Absorbance(Blood + \text{distilled water})} x100.$$

The results were statistically analyzed by One-way ANOVA followed by Dunnett's multiple comparison. A difference is considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Phytochemical Screening

Various metabolites have been found in the recipes studied by a series of coloring and/or precipitation reactions specific to each class of active ingredients as shown in Table 1.

Table 1. Metabolites identified

		Child	Pregnant woman	Adult
Saponosides		+	+	+
Reducing compound		+	+	+
Phenolics compound		+	+	+
Flavonoids		+	+	+
Anthocyanins		+	+	-
Condensed tannins		+	+	+
Gallic tannins		+	+	+
Leucoanthocyanes		+	-	+
Alkaloids		+	+	-
Anthraquinone	Anthraquinones free	-	-	-
	O-Heterosides	+	-	-
	O-Heterosides in reduced genins	-	-	-
	C- Heterosides	-	-	-
Quinones		-	-	-
Mucilage		-	-	-
Sterols and terpenes		+	+	+

+: Presence; -: Absence.

All investigated recipes are rich in saponins, phenolic compounds, sterols and terpenes. By against mucilages, quinones and anthraquinones were absent. Two of the three recipes contain alkaloids. The presence of alkaloids, flavonoids, sterols and terpenes recognized for their antiplasmodial property [17] in the recipes would justify their use in the treatment of malaria in Benin.

3.2. Acute Toxicity

3.2.1. LD_{50}

After evaluating the acute toxicity of extracts, we have observed no symptomatic disorder (diarrhea, shivering, salivation, convulsions, agitation, lack of appetite, motor difficulties and dyspnea) nor recorded any deaths in the ranks of the rats subjected to this study. The results show that the investigated extracts are practically non-toxic with a lethal dose 50 (LD_{50}) greater than 5 g/Kg [18].

3.2.2. Body weight Variation

The results of the 3 weekly weighings carried out in order to follow the weight evolution of the treated and control animals during the experimental study (14) are reported in the table below. During the first week, we note a weight gain in rats fed with distilled water, aqueous extract recipe of the child, the pregnant woman, adult, while in the second week, a weight loss was observed in rats that took the aqueous extract of the child's recipe and the recipe of the pregnant woman. Nevertheless, the ANOVA test showed that this difference is not significant at the 5% threshold for the different groups. Thus, our extracts administered orally in a single dose (5 g/kg) don't affect the evolution of the body weight of rats. The body weight variation is an indicator of adverse effects of chemical compounds [19], we can conclude that our extracts do not have adverse effects in a single dose orally at a dose of 5g/Kg.

Table 2. Percentage variation in body weight (g)

	Day 0	Day 7	Day 14
Control	192.00±14.01	199.70±16.60	204.70±19.88
Aqueous extract of child	215.30±08.37	219.70±11.05	215.70±11.78
Hydroethanolic extract of child	254.70±01.76	253.00±04.73	256.70±04.67
Aqueous extract of pregnant woman	148.00±02.00	148.30±01.76	143.70±01,20
Hydroethanolic extract of pregnant woman	162.70±04.67	163.70±04.70	163.30±05.84
Aqueous extract of adult	183.70±01.76	196.30±04.05	197.00±05.77
Hydroethanolic extract of adult	210.70±01.33	194.00±01.15	212.00±02.31

Table 3. Antipyretic activity

Treatment	Temperatures					
	T0	T1	T2	T3	T4	T5
Negative control	37.50 ±0.10	37.87±0.27	37.87±0.30	37.40±0.35	37.23±0.35	37.23±0.35
Positive control	36.93±0.32	37.57±0,14	37.17±0.17	37.00±0.23	**36.67±0.35**	**36.03±0.25***
Hydroethanolic extract of child	37.53±0.07	37.87±0.39	38.03±0.52	37.67±0.35	37.23±0.18	37.20±0.20
Aqueous extract of child	37.17±0.03	37.13±0.18	36.77±0.48	**36.47±0.61**	**36.23±0.64**	**36.07±0.48***
Hydroethanolic extract of pregnant woman	37.20±0.25	37.53±0.09	37.40±0.15	37.30±0.12	37.17±0.09	36.87±0.18
Aqueous extract of pregnant woman	37.27±0.13	37.37±0.12	37.27±0.03	37.03±0.09	36.90±0.06	36.80±0.00
Hydroethanolic extract of adult	37.47±0.33	37.20±0.29	37.07±0.22	**36.83±0.27**	**36.67±0.18**	**36.07±0.33***
Aqueous extract of adult	37.40±0.12	37.40±0.17	36.80±0.12	**36.67±0.07**	**36.67±0.07**	36.63±0.03

3.3. Antipyretic Test

Table 3 shows the results obtained following the evaluation of the antipyretic activity of the aqueous extract obtain by decoction and the hydroethanolic macerated of the three recipes. It results that the extracts investigated involve in a linear fall in the rectal temperatures of the Wistar rats. Compared to the negative control, we note that all extracts contain antipyretic molecules. The aqueous extract obtains by decoction of the child's recipe and the hydroethanolic macerated of the adult's recipe showed a significant antipyretic activity comparable to that of aspirin. The antipyretic potential of no steroidal anti-inflammatory drugs whose acetylsalicylic acid is due to their ability to inhibit prostaglandin synthase at the level of the hypothalamus [20]. Our extracts would act by the same mechanism. This antipyretic effect of the extracts will contribute to their anti-inflammatory effects. Malaria is a febrile illness; these extracts are indicated in cases of malaria but also during other febrile diseases.

3.4. Antianemic Test

The antianemic capacity of the extracts was investigated through the impact of their oral intake on the haematological parameters and on the osmotic resistance of red blood cells of rats.

3.4.1. Haematological Parameters

After the evaluation of the anti-anemic activity of our extracts, red blood cell and hemoglobin are shown in the tables below.

Table 4. a) rate of erythrocytes; b) Hemoglobin level

a) Red cell rate (10^6)			
	Day 0	**Day 7**	**Day 14**
Positive control	8.79±0.16	8.34±0.24***	7.88±0.41
Negative control	8.09±0.44	4.55±0.23***	7.10±0.12
Aqueous extract of pregnant woman	8.00±0.15	4.82±0.16***	7.30±0.17
Aqueous extract of adult	8.67±0.30	4.98±0.10***	7.73±0.37
Aqueous extract of child	8.05±0.20	4.72±0.05***	7.14±0.15
	Day 0	Day 7	Day 14
b) Hemoglobin level (g/dL)			
	Day 0	**Day 7**	**Day 14**
Positive control	15.03±0.14	14.17±0.26***	14.97±0.07
Negative control	14.57±0.49	11.30±0.30***	15.40±0.32
Aqueous extract of pregnant Aqueous extract of woman	14.87±0.35	11.73±0.41***	15.40±0.26
Aqueous extract of adult	15.37±0.13	12.13±0.20***	15.43±0.47
Aqueous extract of child	15.37±0.35	11.53±0.14***	15.07±0.24

*** Significant difference at P <0.05.

On day 7, compared to normal rat, we observed a significant decrease in haematological parameters (number of red blood cells, hemoglobin) in the rats to which we administered phenylhydrazine. It shows that phenylhydrazine has a haemolytic power as observed by the team of Agbor (2005) [21] and that of Turaskar (2013) [22]. We observed a slight increase in haematological parameters in the anemia rats treated with our extracts compared to untreated anemia rats. Our extracts therefore antagonize the effect of phenylhydrazine. We observed no significant differences in haematological parameters at Day 0 (anemia was not yet induced) and Day 14. The normalization of the haematological parameters at day 14 is explained by the fact that the organism generates new red blood cells to replace those destroyed by phenylhydrazine [23].

3.4.2. Osmotic Resistance

On day 7 the osmotic resistance of erythrocytes was assessed. The figures below show the percentage of haemolysis.

The percentage of haemolysis of the red blood cells of the anaemia rats treated with our extracts is less considerable than that of the anaemia rats gavaged with distilled water. It is thus deduced from these results that the extracts studied increase the osmotic resistance of the erythrocytes of the rats treated after induction of anemia by phenylhydrazine. It has been reported that phenylhydrazine causes oxidative damage to the erythrocytes leading to an increase in reactive oxygen species [24]. The presence of phenolic compounds recognized as antioxidants in these extracts reverses the oxidative effect of phenylhydrazine. Anemia, decreased blood hemoglobin level, is a very common symptom which may be subtended by a very large number of diseases. Among these diseases, we can mention malaria. In view of the results obtained, we note that our extracts are not haemolytic. Their use in the treatment of malaria responsible for haemolytic anemia is beneficial.

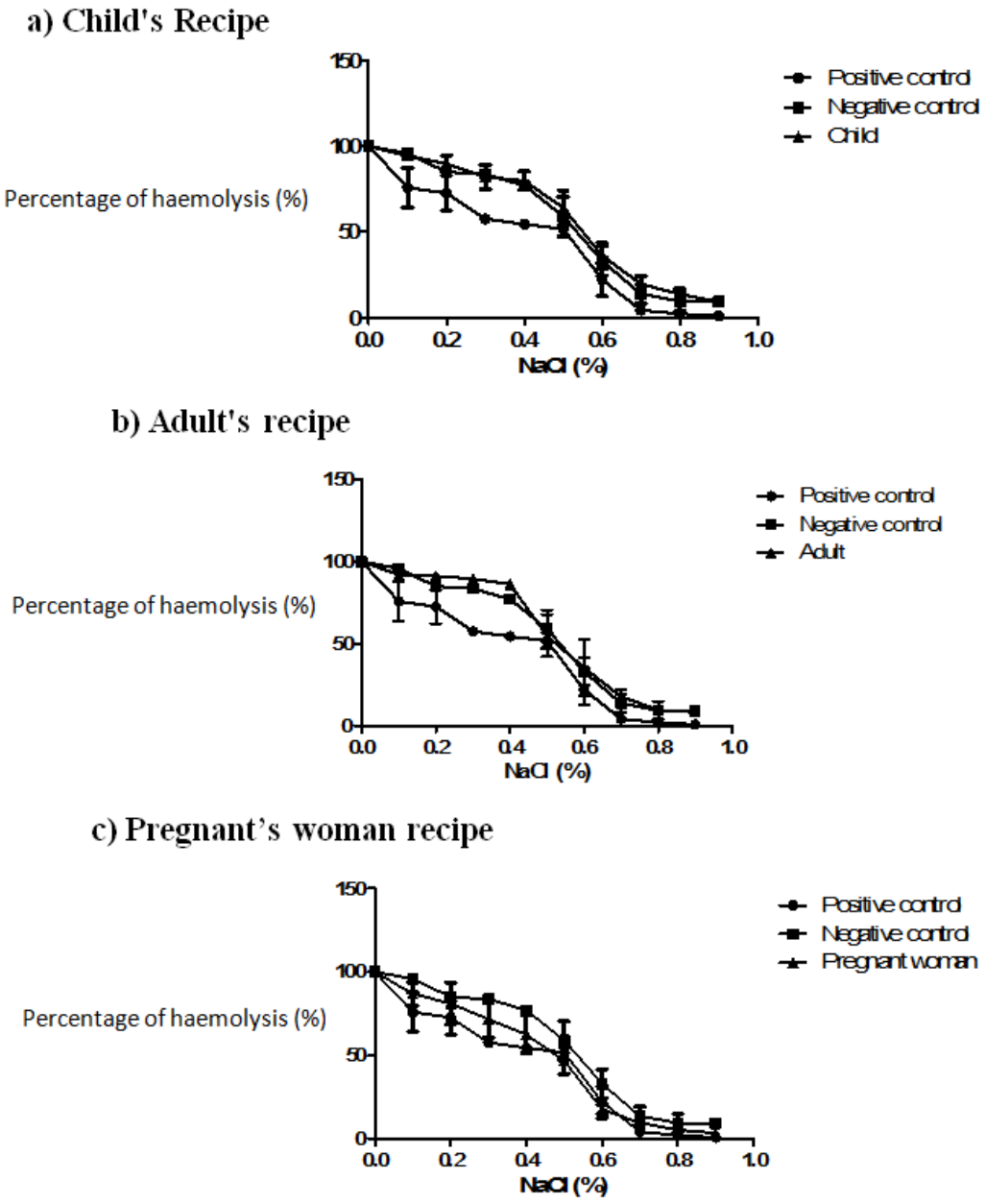

Figure 1. Percentage of haemolysis: (a) Child; (b) Adult; c) Pregnant woman

4. Conclusion

At the end of this study, it appears that the investigated extracts are rich in secondary metabolites and not toxic with LD_{50} greater than 5 g/Kg. Two of six extracts evaluated possess significant antipyretic activity. Significantly, no antianemic activity was observed at the dose of 500 mg/Kg, nevertheless the investigated extracts are not haemolytic in view of the results obtained following the evaluation of the osmotic resistance.

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