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Research Article
Evaluation of the Antiplasmodial Activity and Lethality of the Leaf Extract of *Cassia alata* L. (Fabaceae)

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Abstract

Objective: *Cassia alata* L. (Fabaceae), one of the three plants contained in Saye, a polyherbal antimalarial remedy was assessed for its antimalarial potential and safety in mice. **Methodology:** Organic extracts were prepared from the leaves and tested on the D_{10} chloroquine-sensitive strain of *Plasmodium falciparum* using the parasite lactate dehydrogenase assay. The 4 days suppressive test using *Plasmodium berghei* in mice was used to evaluate the *in vivo* antiplasmodial activity of the extracts. Animals were treated by oral route, once a day with 50, 100, 250 and 400 mg kg^{-1} b.wt., of the extracts. The acute toxicity of the extracts was assessed in mice according to Thompson and Weil method. The lethal effects of the extracts on animal's body weight, tissues, biochemical and haematological parameters were determined at 823.5, 1235.5, 1853 and 2779.5 mg kg^{-1} b.wt., respectively. **Results:** The dichloromethane/methane (1:1, v/v) extract of *Cassia alata* was the most active against *Plasmodium falciparum*. The mean percent suppression of parasitemia in mice was equal to 22.5, 41.8 and 45.2% at 50, 250 and 400 mg kg^{-1} b.wt., respectively. No death and no clinically significant changes were recorded in mice. The maximum non-lethal dose was more than 16875 mg kg^{-1} in animals. No significant changes were observed in body weight, tissues morphology, biochemical and hematological parameters at doses above or equal to 2779.5 mg kg^{-1} b.wt. **Conclusion:** The dichloromethane/methanol leaf extract of *Cassia alata* had a good to moderate *in vitro* and *in vivo* antiplasmodial activity and was found to have low toxicity at high doses in tested animals.

Key words: *Cassia alata*, malaria, lethality, *P. falciparum*, *P. berghei*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Africa and particularly in Burkina Faso plants are still widely used for the treatment of ailments such as malaria. *Cassia alata* L. is a shrub belonging to the family Fabaceae, growing up to 12 m high in grasslands around towns and villages throughout West Africa[1,2]. Leaf extracts of *Cassia alata* have been reported to possess several pharmacological properties including anti-inflammatory[3,4], analgesic[5], larvicidal[6], anti-bacterial[7,10], fungicidal[10-13], hypoglycemic[14-16], antiseptic in skin disease[17,18], anti-tumor[19], laxative[20-22], emetic, astringent, anti-pyretic and anti-oxidant activities[4]. In previous studies, the prophylactic and antimalarial effects of say e, a polyherbal remedy, which contains leaves of *Cassia alata* L., rhizomes of *Cochlospermum planchoni* and the whole plant of *Phyllantus amarus* were demonstrated[23-25]. The water extracts of saye and its individual plants (*Cassia alata*, *Cochlospermum planchonii* and *Phyllanthus amarus* were found to have a weak antiplasmodial activity *in vitro*[23,26]. The current study was undertaken to evaluate the antiplasmodial activity and toxicity of the organic extracts of *Cassia alata.*

MATERIALS AND METHODS

Plant material: The leaf powder of *Cassia alata* Linn. was supplied by Phytofla laboratories (Banfora, Burkina Faso) who has a licence (N°2015/255/MS/DGPML/DMTP) for selling the phytomedicine.

Sequential extraction: Sequential extraction using four organic solvents (hexane, dichloromethane (DCM), dichloromethane/methanol (1:1) and methanol (MeOH) was performed. The volume (mL) of solvent used for each extraction was estimated by multiplying by 10 the mass in grams of the powdered plant material used. Evaporation of the solvent after extraction and filtration was performed using the rotavapor R-200 (Buchi Labortechnik AG, United Kingdom) at a constant temperature (60°C) and pressure below 1 bar.

In vitro antiplasmodial activity testing

Continuous parasite culture: *Plasmodium falciparum* parasites were cultivated as described by Trager and Jensen[27] with minor modifications. The CQ-sensitive strain D10, derived from FCQ-27 from Papua New Guinea[28] was used. The parasites were maintained in RPMI 1640 culture medium supplemented with phenol red, albumax II (bovine serum albumin) (25 g L^{-1}), HEPES (N-[2-hydroxyethyl]-piperazine-N'-[2-ethansulphonic acid]) (6 g L^{-1}), 4.25% of sodium bicarbonate and gentamycin (50 mg L^{-1}). Washed O^+ human RBCs and serum were added to the culture medium. The RBCs were washed twice with medium before use. The parasites were cultivated in sealed flat bottom flasks and maintained at 37°C in an atmosphere of 93% N_2, 4% CO_2 and 3% O_2. The haematocrit and parasitaemia were kept between 2-4% by the addition of RBC. Parasites were synchronized at the ring stage by treatment with 5% D-sorbitol. The parasitaemia was determined microscopically using Giemsa stained thick/thin blood smears on slides.

Antiplasmodial activity testing: The experiments were carried out under sterile conditions in duplicate in 96-well microtiter plates. Aliquots of 100 µL of complete medium were dispensed in all the wells except in rows 3 and 8. In rows 3 and 8, aliquots of 200 µL of drug stock solutions (200 µg mL^{-1}) previously prepared (2 mg mL^{-1} of crude extract in 10% of $DMSO_4$ and complete medium) were added in duplicate and two fold serial dilutions were carried across the plate to achieve 5 dilutions (100, 50, 25, 12.5 and 6.25 µg mL^{-1}). The parasitaemia of Red Blood Cells (RBC) was adjusted to 2% haematocrit in complete medium; 100 µL of this suspension was added to rows 2-12. The first row served as a blank and was filled with 100 µL of complete medium and 100 µL of non-parasitized RBCs at 2% haematocrit. Row 2 without drug served as positive control. The plates were covered with a sterile lid, put in a sterile chamber, gassed with 4% CO_2, 3% O_2 and 93% N_2 and then incubated at 37°C for 48 h Un control avec la CQ a t-il ete fait?

Measuring pLDH activity: The parasite lactate dehydrogenase (pLDH) assay was used to measure parasite viability as described by Makler *et al.*[29]. The pLDH activity was measured using MalstatTM reagent (1 mL L^{-1}), APAD (0.33 g L^{-1}) and 0.24 mM phenazine ethosulphate (PES)/1.96 mM nitro blue tetrazolium NBT (Sigma). After 48 h of incubation, the parasites were re-suspended and 15 µL of the suspension were transferred in wells of a new plate where, 100 µL of Malstat and 25 µL of NBT/PES solution were added. The plate is placed in the dark for 5 min for the development of the colors. Absorbance was recorded at 620 nm using a multi-purpose plate reader (PHERA star FS, BMG LABTECH, software V3.10 R6).

The IC_{50} of the extract was estimated from a dose response curve by non-linear regression analysis using the IC Estimator, version 1.2 described by Kaddouri *et al.*[30] and Le Nagard *et al.*[31].

***In vivo* antiplasmodial activity testing:** The experiment was performed in NMRI female and male mice (8 weeks old and

weighing 25.7±3 g) according to the 4 days suppressive test described by Peters[32]. At day 0, mice were inoculated intraperitoneally with 10^7 red blood cells parasitized with *Plasmodium berghei* (ANKA strain). Two hours post infection the treatment was carried out by using six mice per treatment group. Mice were treated orally, once daily, from 0-3 day with 200 µL of 50, 100, 250 and 400 mg kg^{-1} b.wt., of the dichloromethane/methanol (1/1) extract of the leaves of *Cassia alata*. The control group received instead of the extract, 200 µL of distilled water used to dissolve the extract. On day 4 post-infection, blood smears were obtained from the tail of the mice were fixed in methanol, stained with Giemsa 10% and read with a microscope, under a 100x oil immersion objective.

The parasitemia (Mean±95% confidence interval) for each group of mice is recorded and then, the percentage suppression of parasitemia was calculated with respect to control group.

Lethality studies

Experimental animals: Male and female NMRI mice, 8-10 weeks old and weighing 28.6±1.3 g were used for the experiment. The animals were maintained in healthy environmental conditions (temperature of 24±3°C) and 12 h photoperiod) given water *ad libitum*. All the experiments were performed in compliance with animal welfare guidelines for foreign institutions (OLAW A5926-01).

Determination of the lethal dose 50% (LD_{50}%): Lethal dose 50% (LD_{50}) was assessed according to the modified method of Litchfield and Wilcoxon[33] in NMRI mice of 9 weeks old. The dose rates were spaced so that they were in a geometric progression. The dichloromethane/methanol (1:1) extract was dissolved in water and administered to mice in single dose per os. The test was performed at 5000 mg kg^{-1} b.wt., dose. As no death was recorded, the test was repeated with higher doses (7500, 11250 and 16875 mg kg^{-1} b.wt.). Animals were monitored for 14 days to identify all drug dose related changes including body weight, mortality, gross lesions and behavioural and clinical abnormalities.

Haematological, biochemical and histopathology studies: The test was performed according to the modified method of Thompson and Weil[34]. The mice were randomly divided into five groups of six. Group 1 served as control and animals in groups 2-4 were respectively treated with 823.5, 1235.25, 1853 and 2779.5 mg kg^{-1} of the DCM/MeOH (1:1) extract per body weight. Animals were monitored daily for changes in clinical signs and behaviour. Body weight was recorded on day 0 before extract administration, day 7 and 14 post- treatment. On the 14th day, animals were sacrificed under diethylether anaesthesia; all major organs such as heart, lungs, spleen, liver and kidneys were collected, weighed on a microbalance (sensitivity 0.01 g, Sartorius, Germany) and inspected for any morphological changes. Samples of major vital organs were then fixed in 10% formalin solution.

Blood sample collection: Whole blood (2.0-2.5 mL) was collected with a sterile syringe from anaesthetized mice; 1 mL was transferred into a tube with ethylenediaminetetracetic acid (K_2EDTA) and 1 mL in a second tube without any anticoagulant.

Determination of biochemical parameters: Blood samples in the tubes without anticoagulant were allowed to stand for 2 h at room temperature and then centrifuged (Rotina®380R, Hettich lab technology, Germany) at 4000 rpm for 10 min. The serum aspirated into clean tube was used to measure the following biochemical parameters: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total Cholesterol (TC), triglycerides (Trig), Total Protein (TP), glucose (Glu), urea and creatinine (Crea) using an automated analyzer (ARCHITECT ci4100, Abbott diagnostics, France) at the Souro Sanou University Hospital, Bobo-Dioulasso.

Haematological measurements: Haematological measurements which included White Blood Cells (WBC), Lymphocytes (LY), Monocytes (MO), Granulocytes (GR), Red Blood Cells (RBC), hematocrit (Hct), hemoglobin (Hgb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), platelets (Plt), red cell distribution width (RDW) and Mean Platelet Volume (MPV) were analysed on whole blood collected in tube containing EDTA using a COULTER A^C•T diff, Beckman coulter, California.

Histopathological study: The tissues stored in formalin were routinely processed, embedded in paraffin, sectioned at 3 µm with a rotary microtome; the slides obtained, were then stained with Hematoxylin, Eosin and Safran (HES) and microscopically examined. The microscopic features of organs of treated mice were compared to those of untreated mice.

Statistical analysis: The parasitaemia from each group of mice was recorded and the percentage suppression of parasitaemia per dose was calculated. All values (parasitaemia,

haematological and biochemical parameters) are presented as means±standard deviation. Statistical significance was determined at p = 0.05 level and performed by one-way analysis of variance (ANOVA) followed by Dunnett's post-test.

RESULTS

Antimalarial activity: The extracts displayed various parasite inhibition rates on the D10 CQ-sensitive *P. falciparum* strain using the pLDH assay. The most active extract after the *in vitro* testing was the dichloromethane/methanol (1/1) extract with an IC_{50} = 7.02 μg mL^{-1} (95% CI: 5.96-8.08 μg mL^{-1}) (Table 1), which was thus chosen for the *in vivo* experiments. *In vivo*, 22.5% suppression of parasitaemia was obtained at 50 mg kg^{-1} b.wt., 39.3% at 100 mg kg^{-1} b.wt., 41.8% at 250 mg kg^{-1} b.wt. and 45.2% at 400 mg kg^{-1} b.wt.) with the dichloromethane/methanol extract (Table 2).

Lethality results: The maximum non-lethal dose was equal to 16875 mg kg^{-1} in mice. There were no toxicity symptoms observed in the animals. A significant increase in body weight was observed in mice treated at 1235.5 and 1853 mg kg^{-1} b.wt. (p = 0.042). This was followed by a significant body weight lost in mice treated with doses of 2779.5 and 5000 mg kg^{-1} b.wt. (p = 0.002) (Table 3). The loss of weight was more significant (p = 0.0005) in female mice than in male mice. No dose-response effect and no significant differences in hematological parameters were observed between the control and tested groups of mice (Table 4). An incease in lymphocyte counts in mice at 823.5 mg kg^{-1} b.wt., was observed (Table 4) while an increase in total protein was observed in mice treated at 1853 mg kg^{-1} b.wt., but this increase in total protein was not dose dependent (Table 5).

Table 1: *In vitro* antiplasmodial activity of *Cassia alata* organic leaf extracts

Plants	Organic solvent	Activity	IC_{50} (mg mL^{-1})
Cassia alata	Hexane	Active	-
	DCM	Active	-
	DCM/MeOH	Highly active	7.02
	MeOH	Not active	
Cochlospernum planchonii	DCM/MeOH	Not active	-
Phyllanthus amarus	DCM/MeOH	Not active	-
Saye	DCM/MeOH	Not active	-

Table 2: Reduction of parasitaemia according to the doses of dichloromethane/methanol extract of *Cassia alata* administered to mice

Dose (mg kg^{-1})	Parasitemia (%)	Reduction of parasites (%)	IC_{95} (%)
Control	21.5±7.9	0	50
50	16.7±7.9	22.5	4.5
100	13.0±4.4	39.3	3.0
250	12.5±7.5	41.8	4.2
400	11.8±3.3	45.2	1.7

Table 3: Mean weight gain in mice treated with high dose of the dichloromethane/methanol (1:1) extract of *Cassia alata* leaves

			Treatment group per dose (mg kg^{-1})			
Mean weight gain (g)	Sex	Control group	5000	7500	11250	16875
Day 7	Male	4.5±1.1	2.8±0.5	0.9±1.7	1.6±0.2	-1.8±0.9
	Female	2.7±1.3	1.6±1.7	-0.1±1.1	-0.8±0.5	0.8±1.8
Day 14	Male	4.6±1.9	3.7±1.5	2.0±1.8	0.6±1.6	1.1±0.4
	Female	2.8±0.7	2.8±0.9	1.0±1.5	0.1±0.2	1.5±1.9

Table 4: Haematological parameters between treated and control group mice after treatment with the dichloromethane/methanol extract of *Cassia alata*

		Treatment group per dose (mg kg^{-1})				
Haematological parameters	Control group	823.5	1235.25	1853	2779.5	*p-value
WBC (×10^3 μL^{-1})	3.0±0.6	5.3±1.4	3.5±1.1	2.4±1.2	3.3±2.0	0.023
LY (%)	92.9±1.1	93.6±1.9	94.7±1.4	93.7±1.2	92.6±2.0	0.263
MO (%)	5.2±1.0	5.1±1.7	4.2±1.1	5.1±0.9	5.3±1.1	0.621
GR (%)	1.9±1.8	1.3±1.4	1.1±0.7	1.2±0.5	2.2±1.1	0.556
LY# (×10^3 μL^{-1})	2.8±0.5	5.0±1.3	3.3±1.0	2.8±0.3	3.0±1.9	0.035
MO# (×10^3 μL^{-1})	0.2±0.1	0.3±0.1	0.1±0.1	0.2±0.1	0.2±0.1	0.070
GR# (×10^3 μL^{-1})	0.1±0.0	0.2±0.1	0.1±0.0	-	0.1±0.0	0.479
RBC (×10^6 μL^{-1})	7.4±0.3	7.2±0.4	6.3±1.1	6.9±1.3	6.4±1.8	0.392
Hgb (g dL^{-1})	13.8±0.7	12.3±0.8	11.1±1.8	12.2±1.9	11.3±2.9	0.112
Hct (%)	38.5±2.2	38.3±2.9	34.3±5.6	36.5±6.2	33.6±10.1	0.505
MCV (fL)	52.0±1.0	53.5±1.8	52.6±1.3	53.5±1.7	52.0±1.1	0.241
MCH (pg)	18.6±0.5	17.2±0.5	17.1±0.4	18.4±2.4	17.0±0.3	0.085
MCHC (g dL^{-1})	35.8±1.0	32.2±0.5	32.4±0.4	34.5±4.4	32.5±0.8	0.032
RDW (%)	20.2±1.2	20.4±0.6	21.5±1.3	21.0±1.7	20.0±1.3	0.293
Plt (×10^3 μL^{-1})	650.0±168.4	776.7±80.8	539.8±405.9	635.0±263.8	566.0±293.7	0.607
MPV (fL)	5.5±0.1	5.2±0.26	5.9±0.2	5.8±0.3	5.3±0.3	0.024

*p-value: After comparison of means between groups by one way analysis of variance, WBC: White blood cells, LY#: Lymphocytes, MO#: Monocytes, GR#: Granulocytes, RBC: Red blood cells, Hgb: Hemoglobin, Hct: Hemato crit, MCV: Mean car puscular volume, MCH: Mean corporation hemoglobin, MCHC: Mean corpucular hemoglobin concentration, Plt: Platelets, RDW: Red cell distribution width, MPV: Mean platelet volume

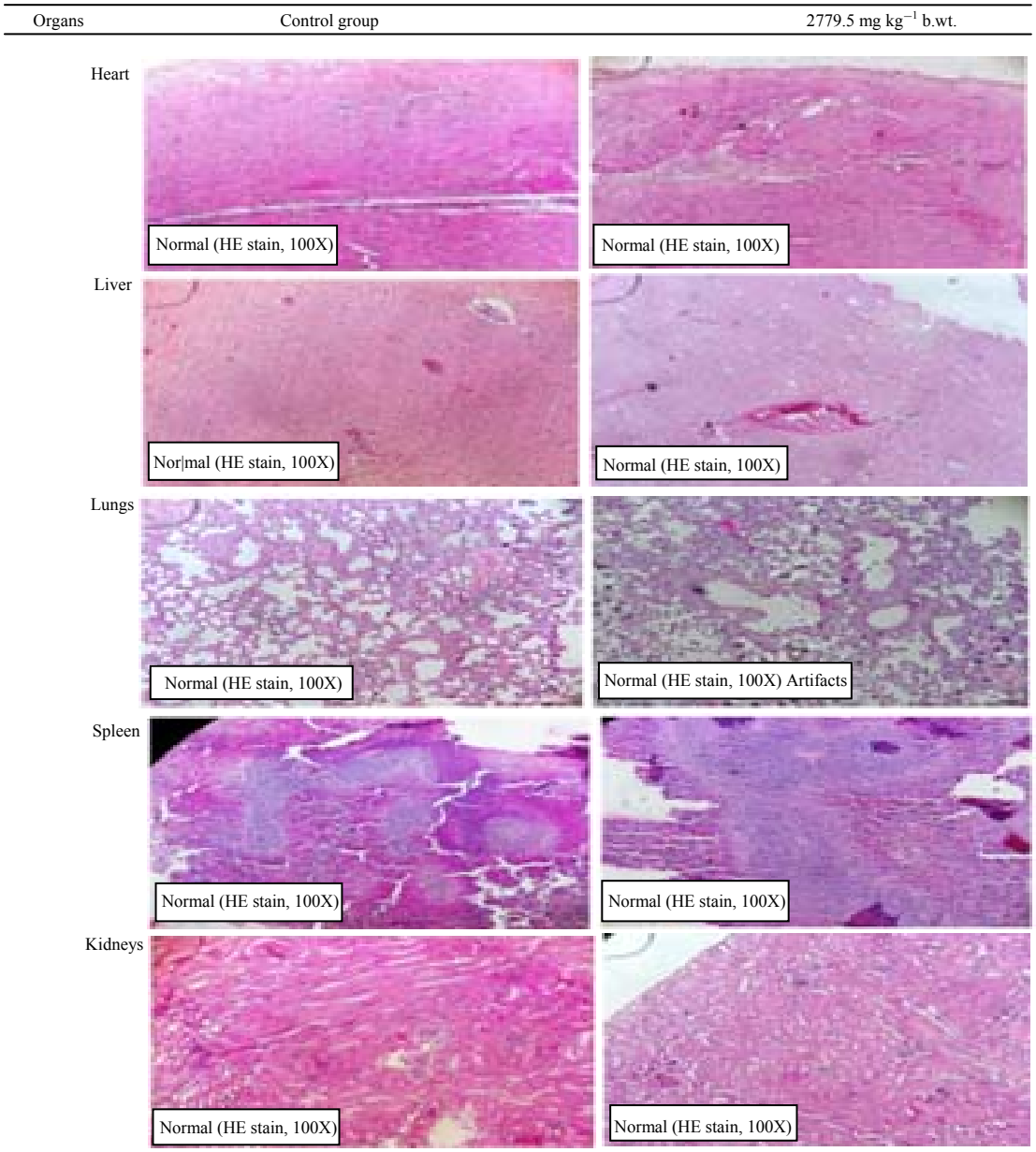

Fig. 1: Photomicrograph sections of organs from control and experimental animals after administration of the dichloromethane leaf extract of *Cassia alata* at the highest dose of 2779.5 mg kg^{-1} (normal architecture for all the organs)

A mild increase in total cholesterol (2.9±0.3 and 3.7±0.3) was observed in extract-treated groups mice. There was no difference in triglyceride concentration between the two groups of animals (Table 5). The extracts at 823.5, 1235.25 and 1853 mg kg^{-1} b.wt., significantly reduced the concentration of glucose in the blood of the mice (Table 5). Urea increased in mice treated at 1235.25 mg kg^{-1} b.wt., while creatinine significantly showed an increase in all treated groups (Table 5). There was no difference in AST concentration between treated and control groups while ALT significantly increased in treated groups (Table 5).

Histopathological study: There were no significant differences observed in organ weights between treated mice and control mice (Table 6). Micrographs of sections from liver, lung, kidney, spleen and heart are presented in Fig. 1. The histopathological examination of the organs of both control and treated groups did not display any morphological changes after treatment with the dichloromethane extract at all doses.

Table 5: Biochemical parameters between treated and control group mice after treatment with the dichloromethane/methanol extract of *Cassia alata*

		Treatment group per dose (mg kg^{-1})				
Biochemical parameters	Control group	823.5	1235.25	1853	2779.5	*p-value
ALT (U L^{-1})	20.7±4.8	36.8±8.80	35.3±3.6	35.4±7.8	36.3±5.9	0.002
AST (U L^{-1})	86.7±29.5	109.6±38.2	96.0±29.2	151.8±73.3	115.5±67.9	0.316
Creatinine (µmol L^{-1})	30.2±1.0	37.5±3.41	35.6±0.2	34.5±0.9	34.6±2.3	0.000
Urea (mmol L^{-1})	4.8±0.2	5.3±1.01	5.7±0.2	6.5±0.7	5.3±0.3	0.001
Glycemie (mmol L^{-1})	12.3±1.6	9.8±2.08	9.6±1.1	9.2±1.0	11.2±2.2	0.018
Cholesterol (mmol L^{-1})	2.9±0.3	3.5±0.34	3.4±0.2	3.7±0.3	3.4±0.7	0.017
Triglyceride (mmol L^{-1})	1.0±0.1	1.2±0.29	1.2±0.7	0.8±0.2	0.9±0.2	0.329
Total protein (g L^{-1})	49.2±2.2	49.0±4.20	54.0±2.8	55.2±1.3	53.2±3.3	0.005

*p-value: after comparison of means between groups by one way analysis of variance, ALT: Alanine transminase, AST: Aspartate aminotransferase

Table 6: Body and organ weight between treated and control group mice after treatment with the dichloromethane/methanol extract of *Cassia alata*

		Treatment group per dose (mg kg^{-1})			
Weight (g)	Control group	823.5	1235.25	1853	2779.5
Body weight					
Day 1	27.4±7.7	28.3±0.9	27.1±0.8	26.9±0.8	30.20±0.8
Day 7	28.4±7.0	28.3±1.2	26.7±0.7	28.6±0.8	29.40±1.2
Day 14	29.8±6.5	30.9±0.8	29.3±1.1	30.9±1.7	31.40±0.8
Body weight gained in 14 days	2.5±1.3	2.6±1.2	2.2±1.2	4.0±1.5	1.10±0.9
Liver	1.5±0.2	1.5±0.1	1.5±0.1	1.4±0.1	1.56±0.1
Kidneys	0.4±0.1	0.4±0.0	0.4±0.0	0.4±0.0	0.40±0.1
Heart	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.17±0.0
Lung	0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.0	0.29±0.0
Spleen	0.2±0.1	0.2±0.0	0.2±0.0	0.2±0.0	0.18±0.0

DISCUSSION

According to the threshold established by Rasoanaivo *et al.* (2004), the activity displayed on *P. falciparum* by the dichloromethane/methanol (1/1) extract of *Cassia alata* L. was good to moderate (Table 2), the efficacy displayed *in vivo* on *P. berghei* was moderate Rasoanaivo *et al.*[35]. A similar activity (44.9% inhibition at 100 mg kg^{-1} b.wt.) was previously displayed by the macerated aqueous extract of *Cassia alata* while the efficacy with the decocted extract (19.2% at 100 mg kg^{-1} b.wt.) was less[26]. These results, together with those from previous works, appear very valuable in view of the wide use of *Cassia alata* and Saye.

The results of acute toxicity revealed that the extract was safe in mice when given by the oral route. The body weight changes in mice at 2779.5 ($p = 0.002$) and 5000 ($p = 0.037$) mg kg^{-1} b.wt., could be indicators of adverse effects of the extract or could be that the animals lost appetite for food. However, no changes in any of the parameters measured and no deaths were observed. The decrease in body weight is possibly due to appetite suppression more in males than in females. This suggested that the dichloromethane/methanol (1:1) extract of *C. alata* leaves does not present toxicological risk at doses below 2779.5 mg kg^{-1} b.wt. The significant increase in lymphocyte count in mice at 823.5 mg kg^{-1} b.wt., could explain an immunostimulatory effect of the extract as previously seen with the ethanolic extract of *Cassia alata*[8]. The results are in agreement with those obtained by Pillai *et al.*[36]. The increase in cholesterol concentration at all doses was not clinically significant in the animals and the histopathological study did not show anomalies in the tissues (Fig. 1). High level of total cholesterol is known to induce hyperlipidemia and to be a risk factor of atherosclerosis, diabetes and hypertension[4]. The reduction of glucose concentration in treated mice is not consistent with the results previously obtained by Palanichamy *et al.*[37]. Indeed, a methanolic extract of the leaves of *Cassia alata,* which was found to reduce the blood sugar in streptozotocin-induced hyperglycaemic rats, had no effect on glucose levels in normoglycemic rats. Elsewhere, the hypoglycemic activity of *Cassia alata* was first demonstrated by Adjanahoun *et al.*[14], Olagunju *et al.*[15] and Varghese *et al.*[16]. The slight increase of liver enzyme (ALT), urea and creatinine were still within the normal range and suggest that the extract did not affect the liver function. The histopathological analysis supports the toxicity results in the sense that the few differences observed in biochemical and hematological parameters between control and tested animals were not significant to leave sequelae in animals.

CONCLUSION

The investigations carried out to demonstrate the pharmacological and safety properties of *Cassia alata*, one of the three plants contained in the herbal remedy "Saye", have led to very valuable data. The dichloromethane/methanol (1/1) extract of the leaves of the plant has exhibited a good to moderate inhibitory effect on malaria parasites *in vitro* as well as *in vivo* at doses of 50 mg kg^{-1} and above and was safe to mice at doses below 2779.5 mg kg^{-1} b.wt., under study conditions. As "Saye" is already used in humans, these findings should be very useful for future clinical studies.

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REFERENCES

1. El-Mahmood, A.M. and J.H. Doughari, 2008. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. Afr. J. Pharm. Pharmacol., 2: 124-129.
2. Hennebelle, T., B. Weniger, H. Joseph, S. Sahpaz and F. Bailleul, 2009. *Senna alata*. Fitoterapia, 80: 385-393.
3. Villasenor, I.M., A.P. Canlas, M.P.I. Pascua, M.N. Sabando and L.A.P. Soliven, 2002. Bioactivity studies on *Cassia alata* Linn. leaf extracts. Phytother. Res., 16: 93-96.
4. Moriyama, H., T. Iizuka, M. Nagai, H. Miyataka and T. Satoh, 2003. Antiinflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoid glycoside. Yakugaku Zasshi, 123: 607-611.
5. Palanichamy, S. and S. Nagarajan, 1990. Antifungal activity of *Cassia alata* leaf extract. J. Ethnopharmacol., 29: 337-340.
6. Kumarasinghe, S.P.W., N.D. Karunaweera, R.L. Ihalamulla, L.S. Arambewela and R.D. Dissanayake, 2002. Larvicidal effects of mineral turpentine, low aromatic white spirits, aqueous extracts of *Cassia alata* and aqueous extracts, ethanolic extracts and essential oil of betel leaf (*Piper betle*) on *Chrysomya megacephala*. Int. J. Dermatol., 41: 877-880.
7. Somchit, M.N., I. Reezal, E.I. Nur and A.R. Mutalib, 2003. *In vitro* antimicrobial activity of ethanol and water extracts of *Cassia alata*. J. Ethnopharmacol., 84: 1-4.
8. Awal, M.A., A. Nahar, M.S. Hossain, M.A. Bari, M. Rahman and M.E. Haque, 2004. Brine shrimp toxicity of leaf and seed extracts of *Cassia alata* Linn. and their antibacterial potency. J. Med. Sci., 4: 188-193.
9. Makinde, A.A., J.O. Igoli, L. Ta'ama, S.J. Shaibu and A. Garba, 2007. Antimicrobial activity of *Cassia alata*. Afr. J. Biotechnol., 6: 1509-1510.
10. Chatterjee, S., S. Chatterjee and S. Dutta, 2010. A survey on VAM association in three different species of *Cassia* and determination of antimicrobial property of these phytoextracts. J. Med. Plants Res., 4: 286-292.
11. Ibrahim, D. and H. Osman, 1995. Antimicrobial activity of *Cassia alata* from Malaysia. J. Ethnopharmacol., 45: 151-156.
12. Khan, M.R., M. Kihara and A.D. Omoloso, 2001. Antimicrobial activity of *Cassia alata*. Fitoterapia, 72: 561-564.
13. Ajose, F.O.A., 2007. Some Nigerian plants of dermatologic importance. Int. J. Dermatol., 46: 48-55.
14. Adjanahoun, E., R.A. Ahyi, L. Ake-Assi, J.A. Elewude and S.D. Fadoju *et al*., 1991. Traditional Medicine and Pharmacopoeia: Contribution to Ethnobotanical Floristic Studies in Western Nigeria. OAU/STRC, Nigeria, Pages: 420.
15. Olagunju, J.A., A. Loremikan and Z.O. Gbile, 1998. Hypoglycaemic and lipolytic activities of isosaline extract of the leaves of *Anthocleista djalonensis A. Chev.* in alloxanized diabetic rats. Proceedings of the 1st International Workshop on Herbal Medicinal Products, November 22-24, 1998, University of Ibadan, Ibadan.
16. Varghese, G.K., L.V. Bose and S. Habtemariam, 2013. Antidiabetic components of *Cassia alata* leaves: Identification through α-glucosidase inhibition studies. Pharmaceut. Biol., 51: 345-349.
17. Benjamin, T.V. and A. Lamikanra, 1981. Investigation of *Cassia alata*, a plant used in Nigeria in the treatment of skin diseases. Quart. J. Crude Drug Res., 19: 93-96.
18. Igoli, J.O., O.G. Ogaji, T.A. Tor-Anyiin and N.P. Igoli, 2005. Traditional medicine practice amongst the Igede people of Nigeria. Part II. Afr. J. Tradit. Compliment. Altern. Med., 2: 134-152.
19. Veerachari, U. and D.A.K. Bopaiah, 2012. Phytochemical investigation of the ethanol, methanol and ethyl acetate leaf extracts of six *Cassia* species. Int. J. Pharma Bio Sci., 3: 260-270.
20. Elujoba, A.A., O.O. Ajulo and G.O. Iweibo, 1989. Chemical and biological analyses of Nigerian *Cassia* species for laxative activity. J. Pharmaceut. Biomed. Anal., 7: 1453-1457.
21. Thamlikitkul, V., N. Bunyapraphatsara, T. Dechatiwongse, S. Theerapong and C. Chantrakul *et al*., 1990. Randomized controlled trial of *Cassia alata* Linn. for constipation. J. Med. Assoc. Thailand, 73: 217-222.
22. Ogunti, E.O. and A.A. Elujobi, 1993. Laxative activity of *Cassia alata*. Fitoterapia, 64: 437-439.
23. Traore, M., A. Diallo, J.B. Nikiema, H. Tinto and Z.P. Dakuyo *et al*., 2008. *In vitro* and *in vivo* antiplasmodial activity of Saye, an herbal remedy used in Burkina Faso traditional medicine. Phytother. Res., 22: 550-551.
24. Yerbanga, R.S., L. Lucantoni, G. Lupidi, G.U. Dori and N.R. Tepongning *et al*., 2012. Antimalarial plant remedies from Burkina Faso: Their potential for prophylactic use. J. Ethnopharmacol., 140: 255-260.
25. Dakuyo, Z., A.L. Meda, D. Ollo, M. Kiendrebeogo and M. Traore-Coulibaly *et al*., 2015. *SAYE*: The story of an antimalarial phytomedicine from burkina faso. J. Altern. Complement. Med., 21: 187-195.
26. Da, O., M. Traore/Coulibaly, R.S. Yerbanga, B. Koama and N. Ouedraogo *et al*., 2014. Antiplasmodial and antioxidant activities of saye: A traditional herbal remedy for malaria. Am. J. Biochem. Mol. Biol., 4: 155-166.

27. Trager, W. and J. Jensen, 1976. Human malaria parasites in continuous culture. Sciences, 193: 673-675.
28. Ekong, R.M., K.J.H. Robson, D.A. Baker and D.C. Warhurst, 1993. Transcripts of the multidrug resistance genes in chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Parasitology, 106: 107-115.
29. Makler, M.T., J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins and D.J. Hinrichs, 1993. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg., 48: 739-741.
30. Kaddouri, H., S. Nakache, S. Houze, F. Mentre and J. Le Bras, 2006. Assessment of the drug susceptibility of *Plasmodium falciparum* clinical isolates from Africa by using a *Plasmodium* lactate dehydrogenase immunodetection assay and an inhibitory maximum effect model for precise measurement of the 50-percent inhibitory concentration. Antimicrob. Agents Chemother., 50: 3343-3349.
31. Le Nagard, H., C. Vincent, F. Mentre and J. Le Bras, 2011. Online analysis of *in vitro* resistance to antimalarial drugs through nonlinear regression. Comput. Methods Programs Biomed., 104: 10-18.
32. Peters, W., 1975. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of *P. berghei* in screening for blood schizontocidal activity. Ann. Trop. Med. Parasitol., 69: 155-171.
33. Litchfield, Jr. J.T. and F. Wilcoxon, 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.
34. Thompson, W.R. and C.S. Weil, 1952. On the construction of tables for moving-average interpolation. Biometric, 8: 51-54.
35. Rasoanaivo, P., E. Deharo, S. Ratsimamanga-Urverg and F. Frappier, 2004. Guidelines for the Nonclinical Evaluation of the Efficacy of Traditional Antimalarials. In: Traditional Medicinal Plants and Malaria. Willcox, M., G. Bodeker, P. Rasoanaivo and J. Addae-Kyereme, (Eds.), CRC Press, Boca Raton, ISBN: 978-0-415-30112-1, pp. 255-270.
36. Pillai, P.G., P. Suresh, G. Mishra and M. Annapurna, 2011. Evaluation of the acute and sub acute toxicity of the methanolic leaf extract of *Plectranthus amboinicus* (Lour) Spreng in Balb C mice. Eur. J. Exp. Biol., 1: 236-245.
37. Palanichamy, S., S. Nagarajan and M. Devasagayam, 1988. Effect of *Cassia alata* leaf extract on hyperglycemic rats. J. Ethnopharmacol., 22: 81-90.