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Basic Science

Ethanolic Extract of *Emilia sonchifolia* Leaves Possess Erythropoietic and Hepatoprotective Effect in Mice Infected with *Plasmodium Berghei Berghei*

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Abstract

Aim: This study was designed to investigate the effect of ethanolic extracts of the leaves of *Emilia sonchifolia* on the haematological parameters and histomorphology of the liver of male Swiss albino mice infected with *Plasmodium berghei berghei (Pbb).*

Material and Methods: 35 mice were divided into; Group 1 (control) given normal saline 0.3 ml, Group 2 passaged with *Pbb* only, Group 3 passaged with *Pbb,* and then treated with Coartem®, Group 4 treated with *E. sonchifolia* 325 mg/kg only, Group 5 treated with *E. sonchifolia* 650 mg/kg only, Group 6 passaged with *Pbb* then treated with *E. sonchifolia* 325 mg/kg, while Group 7 was passaged with *Pbb* then treated with *E. sonchifolia* 650 mg/kg. *Pbb* was passaged intraperitoneally, while the test drug and extracts was given via orogavage once daily.

Results: The result showed significantly (P<0.001) reduced RBC parameters at in Group 5 treated with 650 mg/kg similar with Group 2 compared to Group 1, while there was significant (P<0.01) increased WBC and differentials in Parasitized groups compared with Group 1. The micrographs showed slightly inflamed nuclei in Group 4, with few nuclei shrinkage Group 5, whereas in the parasitized groups treated with the extract there appeared to be hepatoprotection compared to Group 2.

Conclusion: In conclusion, the extract promotes erythropoiesis at 325 mg/kg, but was haemolytic at 650 mg/kg, and exerts its effect possibly through an agonistic and a synergistic activity of its rich bioactive ingredients. It showed mild toxic effect in the histomorphology of the non-parasitized mice at 325 mg/kg and 650 mg/kg, and also appeared to offer hepatoprotection in parasitized mice compared to the parasitized group that had no treatment.

Introduction

Emilia sonchifolia (Lin.) is a bushy annual herb distributed mainly in Asian countries [1]. It has been traditionally used as important medicinal plant in most tropical and subtropical countries, including in the South-South region of Akwa Ibom State, Nigeria. Globally, an estimated 3.3 billion people were at risk of malaria in 2011, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria [2]. *Emilia sonchifolia* has also been reported to possess anti-fever activities [3, 4] antimicrobial activity [5] analgesic and anti-inflammatory activities [6-8]; anticancer activities [9-11]; antioxidant activities [12-15]; anti-diabetic [16]; anti-cataract activities [17-20]; anticonvulsant activity [21]; antinociceptive effect [22].

Compounds like simiral, beta-sitosterol, stigmasterol, palmitic acid and honey acid were obtained from the whole plant of *E. sonchifolia* [23]. Few pyrrolizidine alkaloids, senkirkine and doronine were isolated from the aerial parts of *E. sonchifolia* [24].

There is a growing disillusionment with modern medicine and also the misconception that herbal remedy, being natural may be devoid of adverse and toxic effects often associated with allopathic medicines [25]. The dangers associated with the potential toxicity of herbal therapies demand that herbal practitioners be kept abreast of the report on renal and hepatic toxicity resulting from ingestion of medicinal herbs [26]. Researchers with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines [27].

There has been no report on the effect of *Emilia sonchifolia* on haematological indices as well as histomorpholgy of the liver in a parasitized mice model; hence this study was designed to contribute this information.

Materials and Methods

Experimental animals

Thirty five male Swiss albino mice where obtained from the animal holding facility of Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria and acclimatized for two weeks before the start of the experiment. They were allowed access to water and feed *ad libitum*. All procedures involving animals in this study conformed to the guiding principles in the care and use of animals [28] and the institution's code of ethics for the use of laboratory animals.

Plant collection

Fresh leaves of *Emilia sonchifolia* was obtained at the medicinal farm of the Department of Pharmacology and Toxicology, University of Uyo during the October period of 2012. They were identified and authenticated by the Curator at the Herbarium with voucher numbers UUH/10(e) for *Emilia sonchifolia* deposited.

Plant extraction

The extraction was done with 700 g of fresh leaves *of Emilia sonchifolia,* macerated in 96% ethanol (Sigma Aldrich, Germany) in a flat bottom flask and were kept for 72 hours at room temperature. The macerated leaves were then filtered and the filtrate concentrated in water-bath at 45^{o}C to dryness with a yield of 15.71 g.

Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.3 ml of infected blood containing about 1×10^{7} *Plasmodium berghei berghei* parasitized erythrocytes. The inoculums consisted of 5×10^{7} Plasmodium berghei berghei erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations [29]. The origin of the parasites was from 3 donor mice of a Chloroquine sensitive strain of *Plasmodium berghei berghei* (ANKA) obtained from the National Institute of Medical Research (NIMER) Yaba, Lagos, and was maintained via cycles of subpassage at the Department of Pharmacology and Toxicology, University of Uyo, where they were monitored until the scheduled time when experimental mice were infected.

Dosage

All extracts dosage was determined after toxicity test (LD_{50}) Median lethal dose, which used the modified Lorke's method [30]. The 10% and 20% of the LD_{50} of the extracts was administered as low and medium dose. Eighteen mice divided into six groups of three mice each were administered with the ethanolic extract (1000 mg/kg, 3000 mg/kg, 3500 mg/kg, 4000 mg/kg, 4500 mg/kg and 5000 mg/kg respectively), and the mice were fasted for 24 hours prior to the administration of the extract and 3 hours before testing, drinking water was removed. The manifestation of physical signs of toxicity such as; writhing, restlessness, decreased motor activity, aggressiveness, weakness, gasping for air and possible death was recorded within 24 hours.

Drug

Coartem® Dispersible tablets, an anti-malarial agent (Artemether 20 mg/Lumefantrine 120 mg) manufactured by Novartis Pharmaceutical Corporation Suffern, New York, USA for Norvatis Pharma AG Basle, Switzerland under licence from the PRC with NAFDAC REG. NO: A4-1680 was purchased from a Pharmacy in Uyo metropolis, and single tablets on each successive days was dissolved in normal saline, then administered in the test group based on body weight.

Phytochemical screening

The preliminary phytochemical constituents of the leaves was determined [31]

Experimental design

Group 1 served as control and the other 6 groups served as experimental groups. Group 1-which served as the Control and was given (0.3ml) normal saline (11 days), Group 2-were passaged with *Plasmodium berghei berghei* (11 days), Group 3-were passaged with *Plasmodium berghei berghei* (6 days) and then treated with Coartem® (5 days), Group 4-were administered with *E. sonchifolia* 325 mg/kg (11 days), Group 5-were administered with *E. sonchifolia* 650 mg/kg (11 days), Group 6-were passaged with *Plasmodium berghei berghei* (6 days) then treated with *E. sonchifolia* 325 mg/kg (5 days), Group 7-were passaged with *Plasmodium berghei berghei* (6 days), then treated with *E. sonchifolia* 650 mg/kg (5 days). Parasitized animals were passaged once intraperitoneally lasting for duration of 6 days before treatment with extract commenced once daily for 5 days, except in group 2 where there was no treatment.

Determination of haematological parameters

Blood was collected from the left ventricle of each animal in a vial containing 0.5 M EDTA. Haematological indices were determined after day 11

of treatment using an Automated Mindray BC-5300 Haematolog Analyzer Made in China at the University of Uyo Teaching Hospital.

Tissue collection

Each mouse was humanely sacrificed by chloroform inhalation and the liver was dissected, immediately weighed and rinsed with normal saline and fixed in 10% neutral buffered formaldehyde for light microscopy investigation [31].

Statistical analysis

One way analysis of variance (ANOVA) was applied to compare the relationship of the groups, and Dunnett post-hoc test was used to compare the experimental groups and the control. All values were presented as mean ± standard error of mean (SEM), and values were considered significant at $p<0.05$.

Results

Phytochemical constituents of Emilia sonchifolia

The results of the preliminary phytochemical screening showed that *E. sonchifolia* was positive for the presence of alkaloids, flavonoids, saponins, tannins, terpenes, and cardiac glycosides at varying degrees of lowly, moderately and highly present (Table 1).

Table 1: Phytochemical Constituents of *Emilia sonchifolia*.

Phytochemical	*Emilia sonchifolia*
Alkaloids	+++
Flavonoids	++
Phlobatannins	-
Saponin (Frothings' test)	+
Saponins (Fehlings' solution + NA_2CO_3)	++
Saponins (Fehlings' solution)	++
Tannins	++
Terpenes	+
Anthraquinones	-
Deoxy-sugar	-
Cardiac glycosides	++

Method: Trease and Evans, 1989 [32]; Key: + = lowly present, ++ = moderately present, +++ = highly Present, - = absent

Effect on haematological indices

The result from Table 3 shows that the RBC in Group 2 passaged with *Plasmodium berghei berghei* was significantly ($P<0.001$) reduced compared to the Group 1 (control), and a similar value was obtained for other RBC parameters like HGB, PCV, MCH and MCHC. Group 5 treated with *E. sonchifolia* at 650mg/kg was also significantly ($P<0.001$) reduced compared to the Group 1 (control). However, the WBC in Group 2 passaged with *Plasmodium berghei berghei* showed a significantly ($P<0.01$) increased value compared to Group 1 (control), and this was similar in Group 5 treated with *E. sonchifolia* at 650 mg/kg, while Group 3 passaged with *Plasmodium berghei berghei,* then treated with Coartem® had a WBC significantly increased at ($P<0.05$).

Table 2: Acute Toxicity Test (LD_{50}) for *Emilia sonchifolia*.

Groups (n= 3)	Dosage (mg/kg)	Mortality	% Mortality
Group 1	1000	0/3	0
Group 2	3000	0/3	0
Group 3	3500	3/3	100
Group 4	4000	3/3	100
Group 5	4500	3/3	100
Group 6	5000	3/3	100

$LD_{50} = \sqrt{ab}$; a = 3000; b = 3500; $LD_{50} = \sqrt{3000 \times 3500}$ (mg/kg) = 3250mg/kg. 10% of 3250.00 mg/kg = 325.00 mg/kg = low dose; 20% of 3250.00 mg/kg = 650.00 mg/kg = Medium dose.

Neutrophils was only significantly ($P<0.05$) increased in Group 2 passaged with *Plasmodium berghei berghei* compared to Group 1 (control), whereas Lymphocytes was only significantly ($P<0.05$) increased in Group 4 treated with *E. sonchifolia* at 325 mg/kg compared to Group 1 (control). The result of Platelet indicated that Group 2 passaged with *Plasmodium berghei berghei* was significantly ($P<0.001$) reduced compared to Group 1 (control); while Group 6 passaged with *Plasmodium berghei berghei,* and then treated with E. sonchifolia 325 mg/kg was significantly ($P<0.01$) reduced compared to Group 1 (control), whereas Groups 4 and 5 were both significantly reduced compared to Group 1 (control) at ($P<0.05$)

Effects on the histology of the liver

Group 1 showed normal histological architecture of the liver with the Central vein (Cv), the plates of hepatic cells (Hc), Portal vein (Pv), Endothelial cells (Ec), Sinusoids (S) all appearing unaffected (Figure 1A). Group 2 passaged with *Plasmodium berghei berghei* only showed; Hypertrophy, Inflammation, Numerous Karyorrhectic hepatic cells (Kc) and Necrosis (N), and was strongly affected. Central vein (Cv), the Portal vein (Pv), plates of hepatic cells (Hc), sinusoids (S) (Figure 1B). Group 3 passaged with *Plasmodium berghei berghei, and then treated with* + Coartem® showed the Portal vein (Pv), Central vein (Cv), plates of hepatic cells (Hc), Sinusoids (S), sparse karyorrhectic cells (Kc) and Necrosis (N), few inflamed areas (If) and is strongly affected (Figure 1C), compared to the control. Group 4 treated with *Emilia sonchifolia* 325mg/kg showed slightly Inflamed nuclei , the Central vein (Cv), portal vein (Pv), Bile duct (Bd), Hepatic artery (Ha), Binucleate cells (Figure 1D), mildly affected compared to the control. Group 5 treated with *Emilia sonchifolia* 650mg/kg showed the Central Vein (Cv), Portal Vein (Pv), Hepatic artery (Ha), Bile duct (Bd), Lymphatic vessels (Lv), Sinusoid (S), plate of hepatic cells (Hc).

Table 3: Effect of the ethanolic extract of *Emilia sonchifolia* on haematological parameters in male mice infected with *Plasmodium berghei berghei.*

Groups	RBC	HGB	PCV	MCV	MCH	MCHC	WBC	NEU	LYM	PLT
Group 1 Normal Saline 0.3 ml	7.21±0.35	111.00±2.49	40.10±1.20	55.28±0.51	15.40±0.12	277.00±2.24	7.27±0.58	33.24±3.98	61.32±4.52	1086.28±30.70
Group 2 PBB only	2.39±0.73***	41.00±8.79***	17.26±4.11***	73.50±2.43***	18.04±0.99**	243.40±5.06**	36.37±11.32**	48.06±6.07*	50.56±6.66	441.00±106.07***
Group 3 PBB+Coartem®	6.72±0.86	98.20±10.15	35.12±2.23	54.14±5.03	14.36±0.65	275.40±14.15	28.16±13.48*	38.64±8.78	60.00±9.36	955.00±93.24
Group 4 ES 325 g/kg	7.60±0.18	116.20±1.74	40.04±0.81	52.36±1.04	15.20±0.38	290.20±1.32	16.22±3.19	18.22±3.63	80.12±4.36*	807.40±31.58*
Group 5 ES650 mg/kg	2.31±0.22***	39.40±1.89***	17.40±0.97***	83.62±4.17***	18.70±0.92***	226.40±2.89***	42.29±4.69**	30.44±2.42	69.20±2.42	732.00±95.08*
Group 6 PBB+ ES 325 g/kg	5.52±1.06	85.40±14.08	30.24±4.94	55.54±1.75	15.56±0.51	281.40±1.44	21.24±6.89	32.40±3.52	65.58±4.26	757.00±148.10**
Group 7 PBB+ ES 650 mg/kg	7.36±0.58	113.40±10.59	38.66±3.40	52.50±1.09	15.28±0.55	290.20±3.50	13.10±2.27	29.48±2.76	68.18±2.95	944.20±113.67

Values in means + S.E. (Standard error), n=5,*P<0.05, **P<0.01, ***P<0.001, when compared with control.

It showed few nuclei shrinkage and some inflamed nuclei (Figure 1E), compared to the control. Group 6 passaged with *Plasmodium berghei berghei,* and then treated with *E. sonchifolia* 325 mg/kg showed the Portal vein (Pv), Bile duct (Bd), Hepatic artery (Ha), few karyorrhectic cells among healthy hepatic cells (Kc), few inflammation (If) and clumping cells, and was strongly affected (Figure 1F), compared to the control.

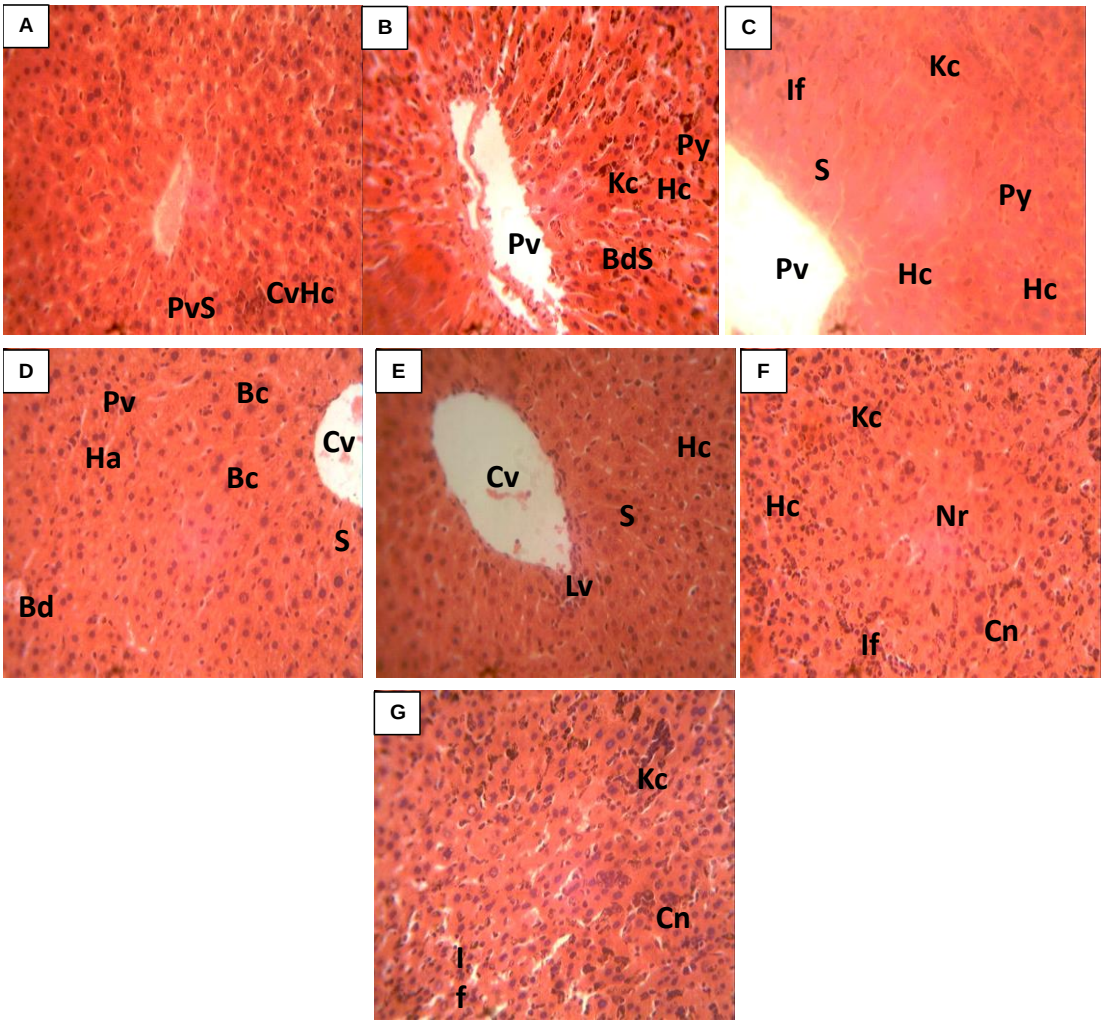

Figure 1: Photomicrographs showing histological sections of the liver of (A) Group 1 served as Control administered (0.3 ml) normal, (B) Group 2 were passaged with Plasmodium berghei berghei only, (C) Group 3 were passaged with Plasmodium berghei berghei and then treated with Coartem®, (D) Group 4 were administered with E. sonchifolia 325 mg/kg only, (E) Group 5 were administered with E. sonchifolia 650 mg/kg only, (F) Group 6 were passaged with Plasmodium berghei berghei then treated with E. sonchifolia 325 mg/kg only, (G) Group 7 were passaged with Plasmodium berghei berghei then treated with E. sonchifolia 650 mg/kg, at magnification of 400 X stained with Haematoxylin and Eosin.

Group 7 Plasmodium berghei berghei and then treated with E. sonchifolia 650 mg/kg showed the portal vein (Pv), Bile duct (Bd), few karyorrhectic cells (Kc), Clumping nuclei (Cn) and Inflammation (If) was strongly affected (Figure 1G), compared to the control.

Discussion

This study was designed to determine the LD_{50} of the plant, establish the phytochemical constituents, and evaluate the effect of *Emilia sonchifolia* on haematological parameters and the histomorphology of the liver of Swiss albino male mice infected with *Plasmodium berghei berghei*. The result from the acute toxicity test indicated that 3250 mg/kg in mice, however 2874.02 mg/kg has been reported [5] obtained using similar method, the reason for this differences may be due to the season and the location in which both plants were cultivated and obtained for the different experiments. The preliminary phytochemical constituent in our study was similar to that reported by [5], although they did not report the degree of the bioavailability of these constituents in the leaves.

The result from the haematological indices in Table 3 indicates that in Group 5 treated with *E. sonchifolia* 650 mg/kg, showed RBC parameters that were significantly ($P<0.001$) reduced indicating haemolytic activity compared with the Group 1 (control). This may be due to the rich presence of saponins in the extract, see Table 1. Saponins promote hemolysis of RBC by increasing the water transport by the water channel aquaporin rather than by acting on the lipid phase [33]. It acts through structural changes in the membrane of RBC, by causing a decrease in the level cholesterol which thus affects the susceptibility of RBC membrane [34]. Interestingly, in Group 4 treated with *E. sonchifolia* 325mg/kg, there appeared to be erythropoietic effect compared to Group 1 (control), and similarly in Group 7 treated with (*E. sonchifolia* 650 mg/kg), which was parasitized before treatment. Reasoning that, perhaps the extract only promotes erythropoiesis at a low dose or in the presence of the berghei parasite, by a mechanism not well understood. However, flavonoid has been reported in vitro studies to have antidiarrheal and antioxidant activity [35], anti-allergic, antiflammatory [36], antimicrobial (antibacterial) [37-39], anti-cancer [40], antiviral [37, 39, 41], antifungal [37, 39]. Hence there appears to be an active interplay of both agonistic and synergistic effect from the extract. Alkaloid is also richly present in the extract and most plants contain several alkaloids. Their mixture is extracted first and then individual alkaloids are separated [41]. Many alkaloids are still used in medicine, usually in the form of salts, including the following: Quinine as antipyretics, antimalarial; Morphine as analgesic; Reserpine as antihypertensive; Codeine as cough medicine, analgesic; Ergot alkaloids as sympathomimetic, vasodilator, antihypertensive; Caffeine as Stimulant, diuretic, Adenosine receptor antagonist just to mention but a few, with most of the known functions of alkaloids related to protection [42, 43].

The WBC shown in Table 3, indicates that Groups 2, 3, and 5 had significantly increased values ($P<0.01$) compared to Group 1 (control). Therefore it may imply that, at the dose 650 mg/kg, *E. sonchifolia* triggered sufficient lysing of RBC to activate the elevated levels of WBC present in circulation. WBCs are the mobile units of the body's protective system, and acting together, these cells provide the body with powerful defenses against tumors and viral, bacterial, and parasitic infections [44]. The changes in the Platelet values in the treatment groups compared with the control may reflect the animals' response at the start of bleeding from needle prick, to obtain thin blood smear for parasitemia (data not reported).

Ethanolic extract of *Emilia sonchifolia* has been reported to cause splenotoxicity in a dose-dependent manner in mice [45]. The photomicrographs shows that the liver of the treated groups showed varying degree of adaptive responses which consisted of inflammation, hyperplasia, hypertrophy of the hepatocytes with reduced sinusoidal sizes, nuclei shrinkage and pyknotic nuclei especially in the parasitized groups. Group 2 with Figure 1B had poor staining intensity compared to the Group 1 with Figure1A; this perhaps resulted from the severity of the parasite trauma to the parenchyma of the liver. This is closely observed in Group 3 parasitized and treated with Coartem®. In the non-parasitized Groups; 4 and 5 (Figures1D and E) there was presence of few inflamed nuclei, but in the parasitized Groups 6 and 7 (Figures1F and G), the parenchyma was severely affected. These changes may be indicative of an underlying cellular trauma and morphological change in the tissue cytoarchitecture a normal reaction of the liver tissue to insults [46].

In concluson, oral administration of *Emilia sonchifolia* has a Median lethal dose of about 3250 mg/kg in mice. The extract is very rich in the presence of alkaloids as well as flavonoids and saponins, and promotes erythropoiesis at 325 mg/kg, but is haemolytic at 650 mg/kg, hence possess a dose-dependent negative effect, possibly through an agonistic and a synergistic activity of its rich bioactive ingredients. It showed mild toxic effect to the histomorphology of the non-parasitized mice at the low and medium doses, and also appeared to offer hepatoprotection in the parasitized mice compared to the group that was parasitized but without treatment.

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