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Research Article
Hepatoprotective Effects of *Plasmodium berghei* Infected Swiss Mice Treated with some Plant Extracts

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Abstract

Hepatoprotective effects of ethanol leaf extract of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* against *Plasmodium berghei* induced malaria in Swiss mice were investigated. Eighty four swiss mice of both sexes were used. All the mice were infected intraperitoneally with 0.2 mL parasitized blood suspension and parasitemia assessed by Giemsa stain thin blood films after three days (72 h). The mice were divided into 6 groups namely; A, B, C, D, E and F. Groups B, C, D and E were subdivided into three: B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, D_3, E_1, E_2 and E_3. Both groups and subgroups contained six mice each. The subgroups were treated with the extracts of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* each for five consecutive days with 200, 400 and 800 mg kg^{-1} b.wt., via oral intubation once daily. The results indicated a general significant ($p<0.05$) decrease in the average body weight of the parasitized untreated mice and a dose dependent significant reductions ($p<0.05$) in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in parasitized treated mice. Dose dependent significant elevations ($p<0.05$) were observed in total protein and albumin concentrations of parasitized treated mice. The results suggest that these plants possess hepatoprotective potential.

Key words: Hepatoprotection, hepatotoxicity, *Plasmodium berghei*, herbal drugs

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

INTRODUCTION

Hepatic disease is a term used to describe illness which affects cells, tissues, structures, or functions of the liver and it is a worldwide problem[1]. Liver is one of the most vital organs of human body that has a wide range of functions such as a centre of metabolism of nutrients and excretion of waste metabolites, handles metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating, protein synthesis and production of biochemical substances necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital functions[2]. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual. Liver cell injury is caused by various toxicants such as certain chemotherapeutic agents, chronic alcohol consumption, microbes among others.

The use of natural remedies for the treatment of diseases including liver disease has a long history and medicinal plants and their derivatives are still used all over the world in one form or the other for this purpose. The more widely used of plants than allopathic drugs as hepatoprotective agents is because they are inexpensive, better cultural acceptability, better compatibility with the human body and minimal or no side effects[3]. Scientific evaluation of plants has shown that active principles/ingredients in plants are responsible for therapeutic success[4] and some of the chemical constituents are; phenols, Coumarins, Lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids, xanthenes etc.[5].

Malaria is a devastating global health problem and It is known as the world's most important tropical parasitic infectious disease to human[6]. Malaria is not a single disease but rather a complex one because of other diseases associated with it and it is its relationship with other diseases that made malaria a global health problem. Malaria ranked third as one of the most six killer diseases in the world today and in Africa, it ranked first as the most killer disease[7]. Reports have shown that malaria kills one child in every 30 sec[8] and about 300-500 millions of cases with a mean death of 2 million are reported every year[9].

Globally, the control of malaria has witnessed a serious deterioration. Severe malaria is almost exclusively caused by *Plasmodium falciparum* in humans, but other forms of *Plasmodium* include *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. When the infected anopheline mosquito takes a blood meal, sporozoites are inoculated into the blood stream. Within an hour, sporozoites enter hepatocytes and begin to divide into exoerythrocytic merozoites (tissue schizogony). During the hepatic stage of the parasite, it caused damage to hepatic cells/architecture via surplus generation of reactive oxygen species.

Large numbers of chemical formulations that have hepatoprotective activity are in global market today. However, these formulations are some times inadequate and can have some serious adverse effects on individual(s) taking them. Based on these toxic effects, it has become very necessary to look for an alternative measures to replace currently used drugs with uncertain safety[1].

Liver damage caused by malaria infection may be prevented or reduced by treatment with plants that forms part of our diet, hence, this study aim to examine the hepatoprotective effects of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* in mice infected with *P. berghei.* This research is motivated by death of information on changes in liver function test of mice infected with *Plasmodium berghei* treated with some plant extracts.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* were collected from Ogboji-Agoutu Ezzagu in Inyaba Development Centre of Ebonyi State, Nigeria. Dr. (Mrs) K. C. Nnamani of the Department of Applied Biology of Ebonyi State University, Abakaliki graciously authenticated and identified the plants. Apparently healthy leaves of the plants were removed from plant stalk, rinsed with clean water and shade dried to a constant weight. The dried plant samples were ground to fine powder with grinding machine, packaged in glass jars and stored at 4°C until analysis.

Extraction of plant materials: Exactly 150 g of powder samples of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* were soaked at 35°C in 500 mL of absolute ethanol (analytical grade, 98%) each for 24 h. They were filtered using whatman paper into a graduated beaker and exposed to mild heat at 40°C in water bath to remove the ethanol until solid crude extracts were obtained in variable amounts depending on the plant. The obtained crude extracts were dissolved in normal saline and administered to experimental animals at different concentrations based on body weights.

Experimental animals: Eighty four Swiss mice aged 2 months weighing 17-34 g of both sexes were obtained from Chris King

Animal Farm of Nnamdi Azikiwe University Awka, Anambra State and transferred to Animal House of Department of Biochemistry, Ebonyi State University, Abakalki. The animals were housed in metal cages under controlled conditions and acclimatized for 7 days under standard environment conditions and fed *ad libitum* on their normal diets.

Rodent parasite (Plasmodium berghei NK65): The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice after every 5 days. The re-infected mice were moved to the Animal House of Department of Biochemistry, Faculty of Biological Science, Ebonyi State University Abakaliki where the study was carried out. Prior to the start of the study, one of the infected mice was kept and observed to reproduce signs of disease similar to human malarial infection.

Preparation of *Plasmodium berghei* and inoculation of animals: The swiss mice were all inoculated by intraperitoneal (IP) injection with standard inoculums of *Plasmodium berghei* NK65 with 1×10^7 infected erythrocytes. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mice to mice every 5 days. Ten animals at a time were used as infected donors and as parasite reservoir. The donor mice were monitored for signs of infection which included lethargy, anorexia, ruffled appearance, shivering and heat-seeking behavior. Blood was taken from the third day (72 h) via the cut tip of the tail to confirm level of parasitemia in the donor mice, using the White Blood Cell (WBC) count method[7]. Blood collected from the tail of the infected donor mice was diluted with phosphate buffer saline pH 7.4 to produce standard inoculums of 0.2 mL containing 1×10^7 *P. berghei* infected erythrocytes. Each mouse was inoculated intraperitoneally on day 0 with 0.2 mL of infected blood containing 1×10^7 *P. berghei*. Parasitemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Giemsa stain[10].

Experimental design: The mice were injected intraperitoneally with standard inoculums of 1×10^7 *P. berghei* NK 65 infected erythrocytes on the first day. Seventy two hours later, the mice were divided into 6 groups namely; A, B, C, D, E and F of 6 mice each. Groups B, C, D and E were subdivided into three: B_1, B_2, B_3, C_1, C_2, C_3, D_1, D_2, D_3, E_1, E_2 and E_3. The subgroups were treated with the extracts of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* each for five consecutive days with 200, 400 and 800 mg kg^{-1} b.wt., via oral intubation daily, respectively. Two control groups, A and F were used. The negative control (A) was treated daily with 5 mL kg^{-1} normal saline while positive control group (F) was treated with 5 mg kg^{-1} b.wt., of chloroquine. All groups were given water and fed *ad libitum*. On the sixth day, mice were starved overnight, sacrificed and livers were collected for various biochemical estimations.

Preparation of serum: Fasting blood was collected from each mice into a sterile, plain tube and then it was centrifuged at 1,200× g for 5 min at room temperature to obtain the serum sample, which was stored frozen at -20°C until analyzed.

Determination of liver function test parameters: Determination of aspartate aminotransferase and alanine aminotransferase were done according to the method described by Reitman and Frankel[11] while alkaline phosphatase (ALP) was done according to Deutsche[10]. Determination of total protein and albumin were by method of Tietz[12].

Statistical analysis: The results obtained were expressed as Mean±S.D of 6 rats in each group. All the average body weights were subjected to statistical analysis using ANOVA. Differences between means were regarded significant at $p<0.05$.

RESULTS

Results of the effects of leaf extracts on the body weight of animals: The result of body weight of mice treated with leaf extract of *Spilanthes uliginosa* (Sw) are showed in Fig. 1a. The body weight of the parasitized untreated animals at 0 mg kg^{-1} showed a significant weight loss ($p<0.05$) on the fifth day and the extract at only 800 mg kg^{-1} caused significant weight gain ($p<0.05$) while 5 mg kg^{-1} of chloroquine did not cause any significant weight gain ($p>0.05$).

The result of body weight of mice treated with leaf extract of *Ocimum basilicum* are showed in Fig. 1b. The body weight of the parasitized untreated at 0 mg kg^{-1} showed a significant weight loss ($p<0.05$) on the fifth day and the extract at only 400 mg kg^{-1} caused a significant ($p<0.05$) weight gain but at 800 mg kg^{-1} caused significant ($p<0.05$) weight loss while 5 mg kg^{-1} of chloroquine did not cause any significant weight gain ($p>0.05$).

The result of body weight of mice treated with leaf extract of *Hyptis spicigera* are showed in Fig. 1c. The body weight of

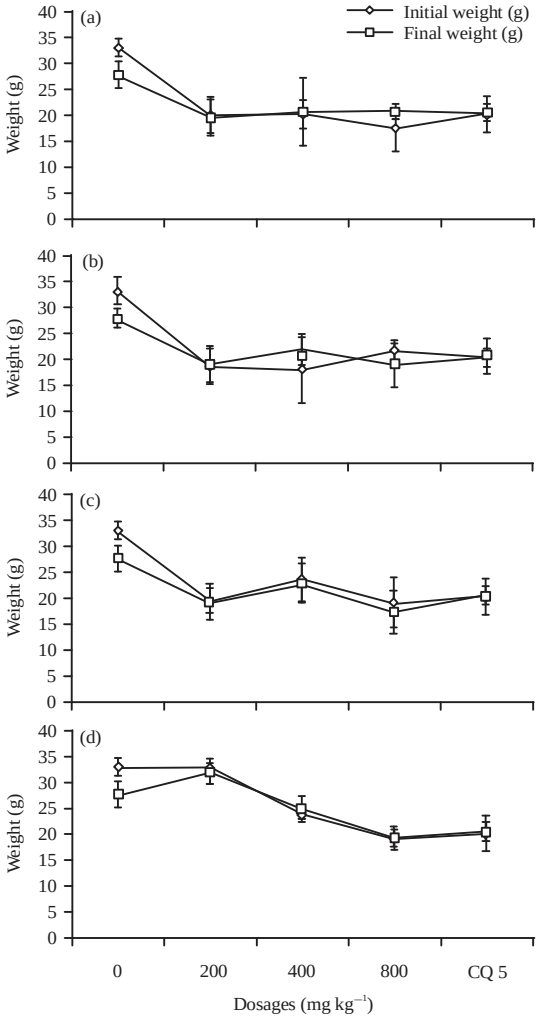

Fig. 1(a-d): Result of the body weight (g) of mice treated with leaf extract of (a) *Spilanthes uliginosa* (Sw), (b) *Ocimum basilicum*, (c) *Hyptis spicigera* and (d) *Cymbopogon citratus*

the parasitized untreated at 0 mg kg^{-1} showed a significant weight loss ($p<0.05$) on the fifth day and the extract at only 800 mg kg^{-1} had a significant weight loss at ($p<0.05$) while 5 mg kg^{-1} of chloroquine did not cause any significant weight gain ($p>0.05$).

The result of body weight of mice treated with leaf extract of *Cymbopogon citratus* are showed in Fig. 1d. The body weight of the parasitized untreated at 0 mg kg^{-1} showed a significant weight loss on the fifth day and the extract at all doses used as well as 5 mg kg^{-1} of chloroquine did not cause any significant weight gain ($p>0.05$).

Results of the effects of ethanolic leaf extract on serum liver enzymes activities of treated and untreated mice: The results of AST activities are shown in Fig. 2a. The results

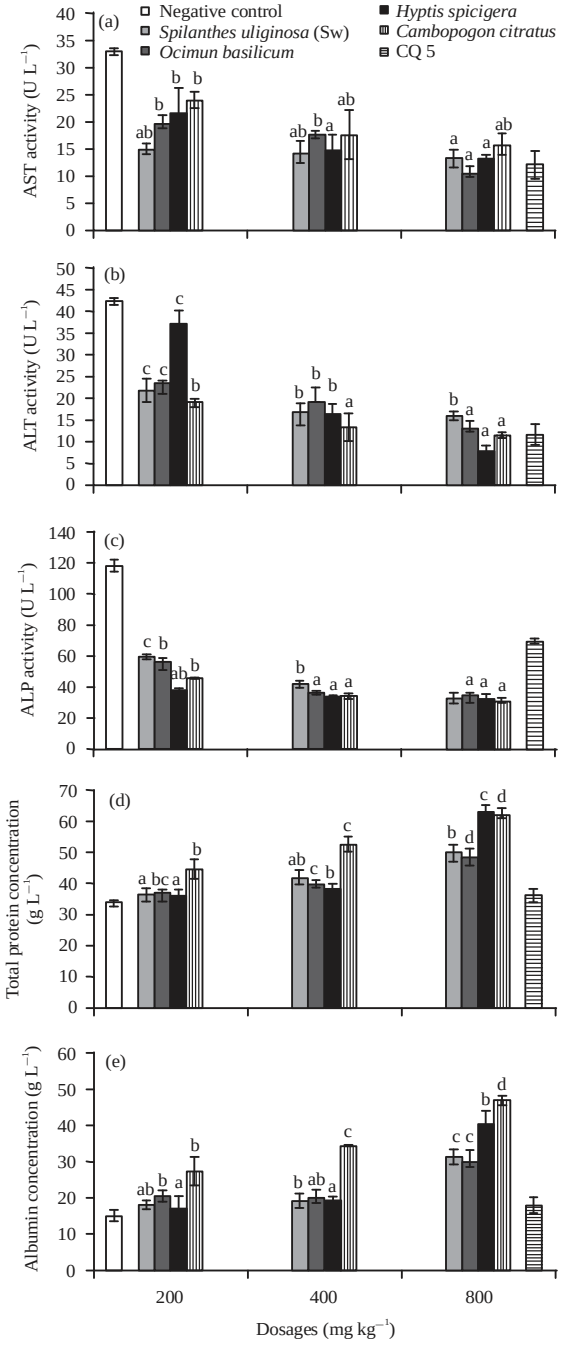

Fig. 2(a-e): Results of (a) AST activities (U L^{-1}), (b) ALT activities (U L^{-1}), (c) ALP activities (U L^{-1}), (d) Total protein concentration (g L^{-1}) and (e) Albumin concentrations (g L^{-1}) of the treated and untreated mice. Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other ($p<0.05$)

showed that there were significant reduction ($p<0.05$) in the activity of AST for all the extracts at all varying doses when

compared to negative control. There was no significant difference ($p>0.05$) between the effects of the standard drug and the extracts of *S. uliginosa* (Sw) at only 800 mg kg^{-1} and that of *H. spicigera* at 400 and 800 mg kg^{-1}. However, there were marked significant difference ($p<0.05$) between the effects of the standard drug and the extracts of *O. basilicum* and *C. citratus* at varying doses used. The extracts *S. uliginosa* (Sw) and *O. basilicum* at 200 and 400 mg kg^{-1} and that of *H. spicigera* and *C. citratus* at 400 and 800 mg kg^{-1} had no significant differences ($p>0.05$) when compared to other doses.

The results of ALT activities are shown in Fig. 2b. The results showed that there were significant reduction ($p<0.05$) in the activity of ALT for all the extracts at all varying doses except *H. spicigera* at 200 mg kg^{-1} when compared to negative control. There was no significant difference ($p>0.05$) between the effects of the standard drug and the extracts of *O. basilicum* at only 800 mg kg^{-1} and that of *C. citratus* at 400 and 800 mg kg^{-1}. However, there were marked significant difference ($p<0.05$) between the effects of the standard drug and the extracts of *S. uliginosa* (Sw) at all given doses used, *O. basilicum* and that of *H. spicigera* at 200 and 400 mg kg^{-1}. The extracts of *S. uliginosa* (Sw) and *C. citratus* at 400 and 800 mg kg^{-1} had no significant differences ($p>0.05$) when compared to other doses while *O. basilicum* and *H. spicigera* at all doses showed a significant difference ($p<0.05$).

The results of ALP activities are shown in Fig. 2c-e. The results showed that there were significant reduction ($p<0.05$) in the activity of ALP for all the extracts at all varying doses when compared to negative control. There were marked significant difference ($p<0.05$) between the effects of the standard drug and that of all the extracts at varying doses used. The extracts *O. basilicum*, *H. spicigera* and *C. citratus* at 400 and 800 mg kg^{-1} doses had no significant differences ($p>0.05$) when compared to other doses while *S. uliginosa* (Sw) at all doses showed a significant difference ($p<0.05$).

DISCUSSION

In living systems, liver is an organ responsible for maintaining and regulating homeostasis. It played important roles in some biochemical pathways which are necessary for growth and fight against diseases. It also supplies nutrients and energy[2]. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual.

There was a general increase in physical activities of the mice treated with the extracts when compared with the parasitized untreated group. The results showed that the physical activities of the extracts-treated mice were better (more active) and this could be due to ameliorating effect of the plant extracts in malaria infection. The observed effect of the extracts may be attributed to some chemical components of the extracts such as alkaloids and saponins which have bactericidal and antispasmodic effects as well as antioxidant compounds which help to protect the animals against the damaging effects of reactive oxygen species imposed by malaria parasites.

There was a general decrease in average body weight of the parasitized untreated mice on the final day relative to those treated (Fig. 1-2) and this observation could be attributed to malaria infection. Infection of animals with malaria parasite leads to loss of weight due to loss of appetite or blood quality of the animals[13] as well as generation of Reactive Oxygen Species (ROS) by the parasites inside host erythrocytes[14]. The gain in weight of animals treated with leaf extract may be attributed to the presence of some metabolites found in the plant that reduce the level of malaria parasite and thereby increase appetite of the animals. These results contrasted with those reported by Haruna *et al.*[13]. These observations could be due to ameliorating effect of the plant extracts on acute fluid loss, proteolysis and lipolysis which is usually associated with weight loss in malaria infection[15].

The extracts at all doses reduced AST, ALT and ALP activities (Fig. 1-2). The values obtained for these enzymes corroborate with the results from oxidative stress parameters which indicated that *Plasmodium berghei*-induced oxidative stress in the mice. An increase in the activities of ALT, AST and ALP in the blood serum of parasitized untreated mice may be as a result of liver injury and altered hepatocyte integrity caused by the *Plasmodium* infection and the consequent release of the enzymes into the blood stream. This result is in agreement with the findings of Patrick-Iwuanyanwu *et al.*[16]. Also, this result is in consistent with other studies which reported that majority of malaria patients shown elevation in serum activities (AST, ALT and ALP) indicating liver damage[17]. Activities of ALT rises in diseases associated with death of hepatocytes like viral hepatitis[18]. AST on the other hands is not found to be specific for liver damages but has been found to be a cardiac marker as it is found in cardiac and skeletal muscles[19]. The serum ALP is related to the function of hepatic cell and increase in serum level of ALP may be due to increased synthesis of the enzymes in presence of increasing biliary pressure[20]. Generally, an increase in this enzyme indicates injury or toxicity to the organ. Several African medicinal plants have been shown to have hepatoprotective effects[21]. This hepatoprotection is possibly due to flavonoids and other chemical composition which exert membrane-stabilizing action that protects the liver cells from injury[20].

The general increased level of total protein and albumin levels in the treated animals showed the protection of the liver integrity (Fig. 1-2). Decreased serum protein concentrations in the parasitized untreated group may have resulted from increased protein utilization by the parasite for the building of new protoplasm during multiplication and the host cells for the synthesis of immunoglobulins and acute phase proteins in response to the invading malaria parasites[22] or may indicate hepatic malfunction since liver is the major source of most serum protein and the synthesis of these proteins are useful indicator of normal hepatic function[23]. However, the synthesis of these proteins is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure[23]. Elevation of these parameters in the treated mice may implicate these plant extracts as sources of protein. Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of disease[24].

Decreased serum total protein concentration observed in this study may be due to reduced concentration of albumin i.e., hypoalbuminemia. Serum proteins are synthesized in the liver, which incidentally is one the major sites infected by the malaria parasite and any illness such as malaria which cause infection of the liver may lead to fall in plasma albumin concentration due to decreased synthesis of albumin. Also illnesses such as malaria may result in increased catabolism of albumin and therefore leads to nitrogen loss. These observations disagreed with the report of Iyawe and Onigbinde[15] which stated that infection result in hyperproteinaemia in mice. The reason(s) responsible for the differences are not clear but may suggest differences in species variation and locations.

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