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Ethanolic extract of *Clerodendrum violaceum* Gürke leaves enhances kidney function in mouse model of malaria

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Evaluation of the effects of daily oral administration of ethanolic extract of *C. violaceum* leaves (13 mg/kg body weight) for 5 days on some kidney function indices of uninfected and *Plasmodium berghei*-infected mice was done on days 3, 8 and 14 post-infection. The indices studied include serum urea and creatinine concentrations with the specific activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in the kidney. Treatment of *P. berghei*-infected mice with ethanolic extract of *C. violaceum* leaves (13 mg/kg body weight) for 5 days was able to ameliorate significantly the alterations in the various parameters observed in infected untreated mice, comparing favourably with chloroquine treatment in most cases. Administration of extract to uninfected mice had no significant effect on both serum and kidney parameters compared to the uninfected control. The results suggest that the ethanolic extract of *C. violaceum* leaves does not adversely affect kidney function at the dose used in traditional medicine for the treatment of malaria but rather enhances it.

Keywords: Antimalarial, *Clerodendrum violaceum,* Kidney function indices, Leaf extract.

Malaria is one of the most important parasitic infections of humans due to its high morbidity and mortality which greatly affects both economic productivity and livelihood[1]. Approximately 40% of the world's population is at risk of the disease. Each year 300-500 million people suffer from acute malaria and 1.5 - 2.5 million die as a result of the disease annually[2-4], most of which are children under the age of five years. Although medicines for the treatment of malaria are available, many people, especially in developing countries, use herbal preparations of medicinal plants to treat malaria[5]; one of such plants is *Clerodendrum violaceum.*

Clerodendrum violaceum Gürke (Verbenaceae) is commonly called Clerodendrum in English and `Ewe isedun´ in Yoruba (Nigeria). It is a shrub with greenish flowers of up to one inch across. The genus *Clerodendrum* is widely distributed in the tropics and subtropics, with a few species extending into the temperate regions. It is found in countries like Ghana, Zimbabwe, Congo, Cameroon and Nigeria in Africa. In Nigeria, it is found in Lagos, Oyo and Kishi. The fresh leaves are used to resolve enlargement of lymphatic glands. A decoction of the leaves and root is taken for fever. Hot oil-coated leaves are used as dressing for fresh cuts and wounds. Boiled in oil, the roots are used as an embrocation in treating rheumatism. The decoction of the roots is used as antihelmintic[6]. Ethanolic extract of the leaves has been shown to have antimalarial activity[7], authenticating the claim of people in Kishi, Oyo and environs (Nigeria) who use it for the treatment of malaria. Alkaloids and phenolics have been shown to be the predominant phytochemicals in the extract[7].

As the removal of metabolic waste products and maintenance of the chemical composition of body fluids at optimal concentrations is primarily carried out by the kidney and because of renal involvement in malaria, the present study has been aimed at evaluating the effect of administration of ethanolic extract of *Clerodendrum violaceum* leaves on kidney function indices during malaria using animal model.

Materials and Methods

Chemicals and assay kits—Absolute ethanol (Riedel-de Haën) (Sigma-Aldrich Laborchemikalien GmbH, Germany), chloroquine diphosphate salt (Sigma Chemical Company, St. Louis, Mo, USA), methanol (Eagle Scientific Limited, Nottingham),

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Giemsa stain (Anosantec Laboratories, UK.) and immersion oil (Panzonar Laboratory Supplies, Button Road, Canada) were used. Assay kits for alkaline phosphatase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase were obtained from Randox Laboratories Ltd. (Co. Antrim, U.K). The assay kit for γ-glutamyl transferase was obtained from Biolab Reagents, France. All other reagents used were of analytical grade and prepared in all glass-distilled water.

Animals — Adult Swiss albino mice (60) with an average weight of 20 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Ibadan, Oyo state. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum*. The research adhered to the Principles of Laboratory Animal Care (NIH publication #85−23, revised in 1985).

Parasites — A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo state.

Plant materials — Fresh leaves of *Clerodendrum violaceum* were harvested in Oyo town of Oyo State during August, 2006 and were botanically authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria.

Plant extract preparation — Fresh leaves of the plant were dried in the shade at room temperature (25°± 2°C) and pulverized to powder using an electric blender. The powder (200 g) was percolated in 1600 ml of absolute ethanol for 48 h after which it was filtered. The ethanol in the filtrate was allowed to evaporate at room temperature to yield the extract concentrate[8], giving a percent yield of 8.3.

Animal grouping and extract administration — The animals were randomly divided into 5 groups: A, B, C, D, and E of 12 mice each. Animals in groups C, D, and E were inoculated from the same donor mouse. The percentage parasitaemia and the red blood cell count of the donor mouse was first determined using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline were made. Each mouse in the infected groups was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing about 1×10^7 *P. berghei* parasitized red blood cells. Treatment was withheld for 72 h to allow for establishment of infection and was commenced when parasitaemia was established by screening for malaria parasites in tail blood of infected animals after fixing in methanol and staining with Giemsa[9].

Aqueous preparations of the extract (corresponding to 13 mg/kg body weight, a value arrived at from ethno-botanical survey) and chloroquine (corresponding to 4 mg/kg body weight) were made before administering orally to the mice. The administration of the extract and chloroquine which lasted for 5 days is as follows:

Group A (uninfected mice): received an appropriate volume of sterile distilled water.

Group B (uninfected mice): received the aqueous preparation of the extract (13 mg/kg body weight daily).

Group C (infected mice): received an appropriate volume of sterile distilled water.

Group D (infected mice): received the aqueous preparation of the extract (13 mg/kg body weight daily).

Group E (infected mice): received the aqueous solution of chloroquine (4 mg/kg body weight daily).

Sample collection and preparation — Four mice were sacrificed from each of the five groups on days 3, 8 and 14 post-infection by slight ether anesthesia and were then dissected. Blood was collected by cardiac puncture into clean, dry sample tubes containing no anticoagulants. The clotted blood was then centrifuged at 1000 rpm for 15 min and the clear serum supernatant was carefully collected using a Pasteur pipette. The serum samples were stored frozen until needed for analysis. Also, the kidneys of each animal were quickly removed, cleansed of superficial connective tissue and blood. One of the kidneys was then homogenized in ice-cold 0.25 *M* sucrose solution (1:5 w/v) and the other fixed in 10% formalin for histopathological studies. The homogenates were stored frozen overnight to ensure maximum release of enzymes[10].

Biochemical assay and histopathological studies — Activities of alkaline phosphatase (ALP)[11] and aminotransferases [aspartate (AST) and alanine (ALT)][12] were assayed. Protein concentration was determined using the Biuret method[13]. Serum urea concentration was assayed as per Veniamin and Vakirtzi[14] while serum creatinine concentration was assayed by the method of Blass and Thibert[15]. The method of Szasz[16] was used to assay for gamma-glutamyl transferase (GGT) activity.

Histopathological studies were carried out by the procedure of Krause[17].

Statistical analysis — Values are expressed as mean ± SE. The data were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test[18]. Data from the test groups were compared with controls and differences at $P<0.05$ were considered significant.

Results

The administration of extract in uninfected mice caused no significant alteration ($P>0.05$) in all the parameters studied compared to control (untreated and uninfected mice) throughout the experimental period. On days 8 and 14 post-infection, there was significant increase ($P<0.05$) in serum creatinine and urea concentrations in infected untreated mice compared to the uninfected groups (Table 1). Treatment of infected mice with extract was able to ameliorate significantly ($P<0.05$) these alterations, comparing favourably with chloroquine treatment in most cases.

There was significant decrease ($P<0.05$) in the activities of kidney ALP, AST, and ALT in the infected untreated animals on days 8 and 14 post-infection whereas there was a significant increase ($P<0.05$) in serum GGT activity compared to the uninfected groups (Table 1). Treatment of infected animals with extract was also able to significantly ameliorate ($P<0.05$) these alterations, comparing favourably with chloroquine treatment.

Histopathological studies revealed that there was marked congestion of kidney glomeruli with inflammatory cells in the untreated infected group on day 14 (Fig. 1). This congestion was reduced in the treated groups, with the infected chloroquine-treated group showing a better resolution. The uninfected groups showed no aggregation of inflammatory cells.

Discussion

Serum urea and creatinine concentrations are used for the assessment of renal sufficiency[19]. Higher than normal levels of serum urea and creatinine are indications of deficiency in renal function[20,21]. Thus, the increase in serum urea concentration with concomitant increase in serum creatinine concentration in the infected untreated animals suggests that the normal functioning of the kidneys has been compromised. Acute renal failure has been associated with severe falciparum and vivax malaria[22-25]. Two mechanisms have been reported to be involved in the pathogenesis of acute renal failure in

Table 1—Kidney function indices in experimental animals

[Values are means ± SE of 4 determinations]

Groups	Serum			Kidney		
	Creatinine (µmol/L)	Urea (mmol/L)	GGT (IU/mg protein)	ALT (IU/mg protein)	AST (IU/mg protein)	ALP (IU/mg protein)
			Day 3			
Control	34.50 ± 0.53^{a}	3.78 ± 0.19^{a}	2.50 ±0.50^{a}	26.00 ±0.68^{a}	35.00 ± 0.75	102.25 ± 0.69^{a}
Extract - treated	33.00 ± 0.68^{a}	3.70 ± 0.19^{a}	2.50 ±0.90^{a}	27.50 ±0.53^{a}	34.00 ±0.83^{a}	103.50 ± 0.82^{a}
Infected untreated	31.50 ± 0.53^{a}	3.78 ± 0.21^{a}	2.47 ±0.60^{a}	24.30 ±0.61^{a}	33.50 ±0.67^{a}	102.50 ± 0.92^{a}
Infected extract-treated	33.00 ± 0.63^{a}	3.90 ± 0.22^{a}	2.47 ±0.70^{a}	25.30 ±0.61^{a}	34.50 ±0.76^{a}	102.00 ± 0.88^{a}
Infected chloroquine-treated	32.75 ± 0.74^{a}	3.90 ± 0.20^{a}	2.47 ±0.80^{a}	26.80 ±0.61^{a}	33.25 ±0.74^{a}	103.50 ± 1.26^{a}
			Day 8			
Control	34.75 ± 0.86^{a}	3.73 ± 0.19^{a}	2.47 ±0.60^{a}	25.25 ±0.69^{b}	36.25 ±0.61^{c}	103.25 ± 0.85^{c}
Extract - treated	36.00 ± 0.55^{a}	3.75 ± 0.24^{a}	2.47 ±0.80^{a}	26.75 ±0.61^{b}	35.50 ±0.72^{c}	102.50 ± 0.67^{c}
Infected untreated	64.25 ± 0.74^{d}	7.73 ± 0.23^{d}	7.80 ±1.30^{d}	18.75 ±0.61^{a}	19.50 ±0.53^{a}	76.00 ±0.80^{a}
Infected extract-treated	52.25 ± 0.80^{c}	6.00 ± 0.22^{c}	5.07 ±0.90^{c}	20.75 ±0.61^{a}	22.75 ±0.53^{b}	88.50 ±0.53^{b}
Infected chloroquine-treated	45.00 ± 0.84^{b}	4.80 ± 0.22^{b}	4.33 ±0.90^{b}	21.75 ±0.74^{a}	24.50 ±0.61^{b}	91.25 ±0.91^{b}
			Day 14			
Control	36.25 ± 0.90^{a}	3.73 ± 0.23^{a}	2.48 ± 0.50^{a}	24.50 ±0.53^{cd}	35.25 ± 0.61^{c}	101.00 ± 0.55^{d}
Extract- treated	35.25 ± 0.90^{a}	3.80 ± 0.22^{a}	2.49 ± 0.60^{a}	25.00 ±0.63^{d}	34.75 ±0.69^{c}	103.25 ± 0.85^{d}
Infected untreated	81.25 ± 1.00^{c}	10.70 ± 0.27^{c}	15.35 ±1.00^{d}	12.75 ±0.61^{a}	13.50 ±0.67^{a}	63.00 ±0.96^{a}
Infected extract-treated	43.50 ± 0.61^{b}	5.33 ± 0.31^{b}	4.12 ±1.3^{c}	22.00 ± 0.42^{b}	27.50 ±0.53^{b}	75.25 ±1.08^{b}
Infected chloroquine-treated	39.00 ± 0.59^{ab}	4.08 ± 0.41^{a}	3.14 ±0.90^{b}	22.25 ± 0.61^{bc}	29.75 ±0.61^{b}	88.25 ±0.69^{c}

Values for each day along the same column with different superscript letters are significantly different ($P<0.05$)

severe malaria. The first mechanism is the impairment of microcirculation by parasitized erythrocytes[24]. Parasitized erythrocytes have decreased deformability, which results in sluggish blood flow in the microcirculation. Erythrocyte viscosity is also increased because of rigidity of the infected erythrocytes, thus the whole-blood viscosity is increased and contributes further to the slow flow in the microcirculation. The second mechanism is the non-specific effects of infection which include hypovolaemia, intravascular haemolysis, disseminated/intravascular coagulation, endotoxaemia, and cholestatic jaundice[24]. All these may proffer a reason for the increased serum creatinine and urea concentrations in the infected untreated animals. The extract was able to significantly ameliorate these anomalies rather than worsening the conditions.

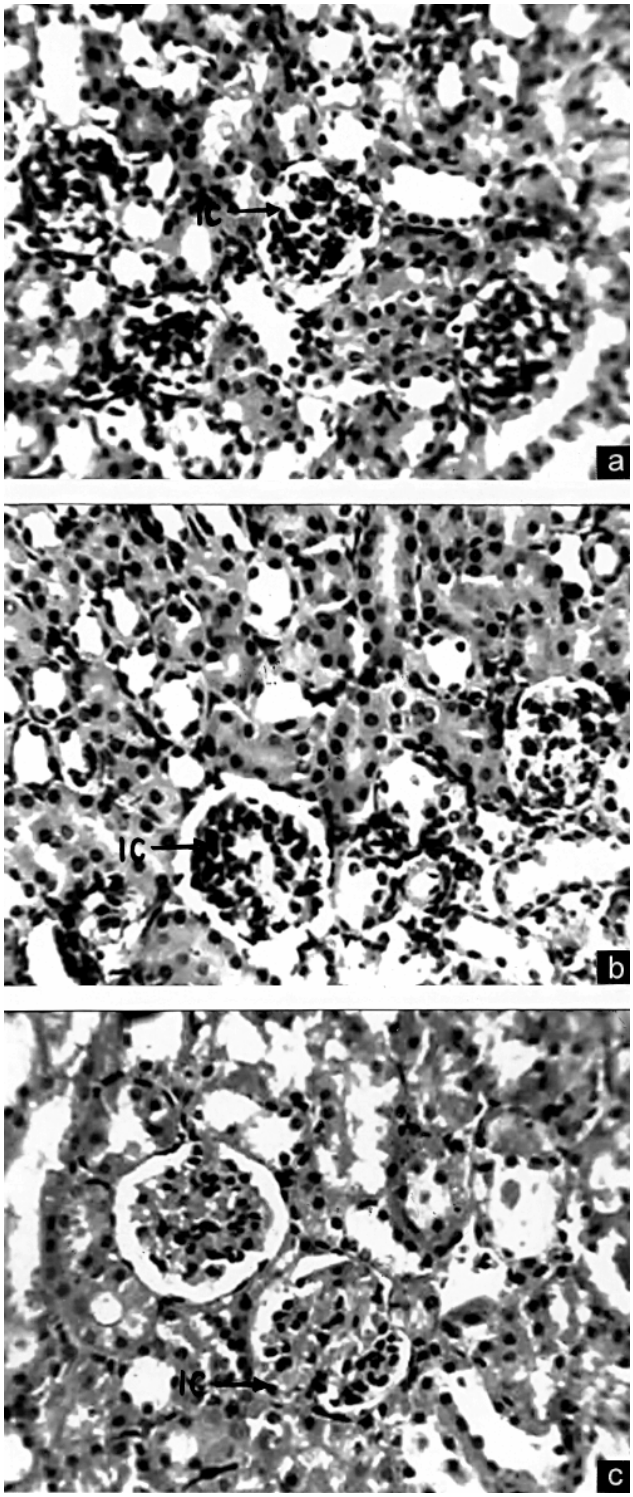

Fig. 1—Photomicrographs of the kidney of infected experimental animals on day 14 ×400). [a = infected untreated group; b = infected extract-treated group; c = infected chloroquine-treated group. Ic = Inflammatory cells].

In clinical practice, an increased serum gamma-glutamyl transferase activity is conventionally interpreted as a marker of alcohol abuse or liver disease[26,27]. However, recent studies have indicated that serum GGT level may be an early predictor for the development of chronic kidney disease[28]. It has been established that oxidative stress plays an important role in the production of renal damage[29-31]. Recently, serum GGT has been proposed as a sensitive and reliable marker of oxidative stress, having a direct proportionality[32,33], thus giving a strong association of serum GGT with the incidence of chronic kidney disease. Thus, increase in serum GGT in infected untreated animals may indicate the pathological state of the kidney in these animals. The extract was able to significantly reverse this condition.

Alkaline phosphatase has been employed to assess the integrity of plasma membrane and endoplasmic reticulum[34]. Results of ALP activity in infected untreated animals in the present study suggest that the integrity of the various membrane systems has been compromised. The observed reduction in kidney ALP activity may lead to less availability of phosphate groups required for oxidative phosphorylation to generate ATP molecules which in turn are used for the phosphorylation of some biomolecules like ethanolamine and choline needed for the synthesis of phosphatidyl ethanolamine and phosphatidyl choline[35]. Inability to synthesize these two major membrane phospholipids may affect membrane fluidity, and may lead to the labilization of the membrane. Therefore the decrease in AST and ALT activities in the kidney of infected untreated animals may result from leakage of the enzymes (being

intracellular enzymes) into extracellular fluids via the altered membrane. Apart from the liver, the kidney is a prominent site of amino acid metabolism. The aminotransferases occupy a central position in the amino acid metabolism because they help in retaining amino groups (to form new amino acids) during the degradation of amino acids and are also involved in the biochemical regulation of intracellular amino acid pool. They also help in providing necessary intermediates for gluconeogenesis. Alterations in their activities may have adverse effect on the amino acid metabolism in the kidney and consequently the intermediates needed for gluconeogenesis. Administration of the extract to infected animals did not complicate these alterations but rather reversed them considerably, comparing favourably well with chloroquine. The cellular inflammation observed in the kidneys of infected untreated animals, as indicated by infiltration of the glomeruli by inflammatory cells, was also appreciably resolved by the extract.

It is noteworthy that the administration of the extract to uninfected mice caused no significant alteration in the parameters studied, both in the serum and kidney compared to control. Also, the extract was able to considerably reverse the alterations in the kidney function indices observed in the infected untreated animals. The results of the present study suggest that the ethanolic extract of *Clerodendrum violaceum* leaves does not adversely affect kidney function at the dose used in traditional medicine but rather enhances it.

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