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Decrease in activities of cation ATPases and alkaline phosphatase in kidney and liver of artemether treated rats

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ABSTRACT: Artemisinin (qinghaosu) and its derivatives are endoperoxide-containing compounds which have emerged as an important new class of antimalarial drugs. Of all the derivatives, the most abundant in circulation now in Nigeria is artemether. The effects of artemether on the activities of some cation ATPases in selected rat tissues were studied. The drug was administered to rats through intramuscular injection at the recommended dose for a five-day treatment. There was no significant difference observed in the activities of ATPases in the various tissues studied except the kidney where slight decreases were observed, but the Ca^{2+}-Mg^{2+} ATPase activity in the kidney was significantly reduced ($P < 0.05$). On the contrary, there was a significant increase ($P < 0.05$) in Ca^{2+}-Mg^{2+} ATPase activity in the heart. There was a significant reduction ($P < 0.05$) in the alkaline phosphatase activities in the kidney and liver, but a significant increase ($P < 0.05$) in that of the brain while that of the heart was not significantly affected ($P > 0.05$). Artemether may exhibit more toxic effects in the kidney and liver due to their exposure to more concentrations of the drug by virtue of their being the main sites of excretion and metabolism, respectively. Artemether may also inhibit cation transport in the kidney.

Key Words: Malaria; Antimalarial drugs; Artemisinin (qinghaosu); Alkaline phosphatase; Adenosine triphosphatases (ATPases).

Introduction

Malaria has become a major global disease because of the fast spreading resistance to commonly used quinoline antimalarial drugs (Su *et al*., 1997). Among the few alternative drugs available, artemisinin is becoming increasingly important. It was isolated from *Artemisia annua* L., a plant with long history of medical use against malaria (Klayman, 1985). Artemisinin and its derivatives are promising and potent antimalarial drugs which meet the dual challenge posed by drug-resistant parasites and rapid progression of malaria (Dhingra *et al*., 2000). They are all effective against *Plasmodium* parasites that are resistant to the drugs commonly used (Woerdenbag *et al*., 1994). This is due to the fact that these drugs are structurally unrelated to the classical quinoline and antifolate antimalarial drugs, thus there is little or no cross-resistance (Bhisutthibhan *et al*., 1998). They possess a stable endoperoxide bridge to which their antimalarial properties have been attributed (Meshnick, 1998).

Artemether is a β-methyl ether of artemisinin and was first synthesized by the Shangai Institute of Materia Medica, Chinese Academy of Science (You *et al*., 1992). Intramuscular artemether has been clinically evaluated in Africa and registered in some African countries for the treatment of severe and complicated malaria (Salako, 1998) and is the most abundant in circulation in Nigeria.

Some toxic effects of artemisinin and its derivatives have been reported, especially their neurotoxicity at high doses or prolonged exposure (Nosten and Price, 1995; Kamchonowongpaisan *et al*., 1997). A transient heart block in few patients treated with artemether has been reported (Valecha, 1996). Sodium artesunate has been reported to inhibit Na^+-K^+ ATPase in mouse small intestine *in vitro* (Wang *et al*., 1990). No work has been done thus far to show the effects of artemisinin and its derivatives on activities of ATPase *in vivo*. In the present study, we have sought to verify whether a similar inhibitory effect is exerted by intramuscular artemether *in vivo* on cation ATPase and alkaline phosphatase.

Materials and Methods

Adenosine triphosphate (ATP), Tris (hydroxymethyl) aminomethane and bovine serum albumin (BSA) were obtained from Sigma Chemical Company Ltd., Poole, England. Other reagents were of analytical grade.

Young female albino rats (*Rattus novergicus*) used were randomly distributed into two groups, viz. the control group (CG) and the group treated with artemether (TG). The drug was administered at the dose for a five-day treatment (3.2 mg/kg the first day and 1.6 mg/kg on subsequent days). The animals were sacrificed 24 hours after the last dose was given. The liver, kidney, heart and brain of each rat in each group were isolated and their homogenates prepared as described earlier (Balogun *et al*., 1997).

Protein concentrations were determined by the procedure of Gornal *et al*. (1949) using BSA as standard. ATPase activity was assayed spectrophotometrically by measuring the release of inorganic phosphate from ATP essentially by the method of Ronner *et al*. (1977) as modofied by Bewaji *et al*. (1985). Alkaline phosphatase activity was assayed by the method of Bessey *et al*. (1946) as modified by Wright *et al*. (1972). Data were statistically analyzed and differences compared using Student's t-test at $P < 0.05$.

Results

Figs. 1 – 4 show the activities of Mg^{2+}-ATPase, Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase and alkaline phosphatase respectively in the various tissues of both control and artemether-treated rats.

There was no significant difference ($P > 0.05$) observed in the activities of the cation ATPases in the various tissues studied, except in the kidney and the heart. There was little reduction in the activities of the ATPases in the kidney but that of Ca^{2+}-Mg^{2+}-ATPase was significantly reduced ($P < 0.05$). The Ca^{2+}-Mg^{2+}-ATPase activity in the heart was significantly increased ($P < 0.05$). there was a significant reduction ($P < 0.05$) in the activities of alkaline phosphatase in the kidney and the liver but a significant increase ($P < 0.05$) in that of the brain while that of the heart was not significntly affected ($P > 0.05$).

Discussion

The significant reduction ($P < 0.05$) observed in the activities of the enzymes of the kidney and liver may be as a result of their exposure to more concentration of drug by virtue of excretion and metabolism respectively. the reduction observed in the activities of the enzymes might be as a result of the drug interacting with the membranes of the cells thus affecting the enzymes or the drug directly interacting with the enzymes. Since artemisinin and its derivatives have been reported to act through carbon-centred radicals (Posner *et al*., 1995), artemether may cause peroxidation of the lipid bilayer of the membrane (Webster and Nunn, 1988) or a modification of some essential groups (e.g. sulphydryl groups) in the enzymes (Yuan *et al*., 1992), thus reducing the activities of the enzymes.

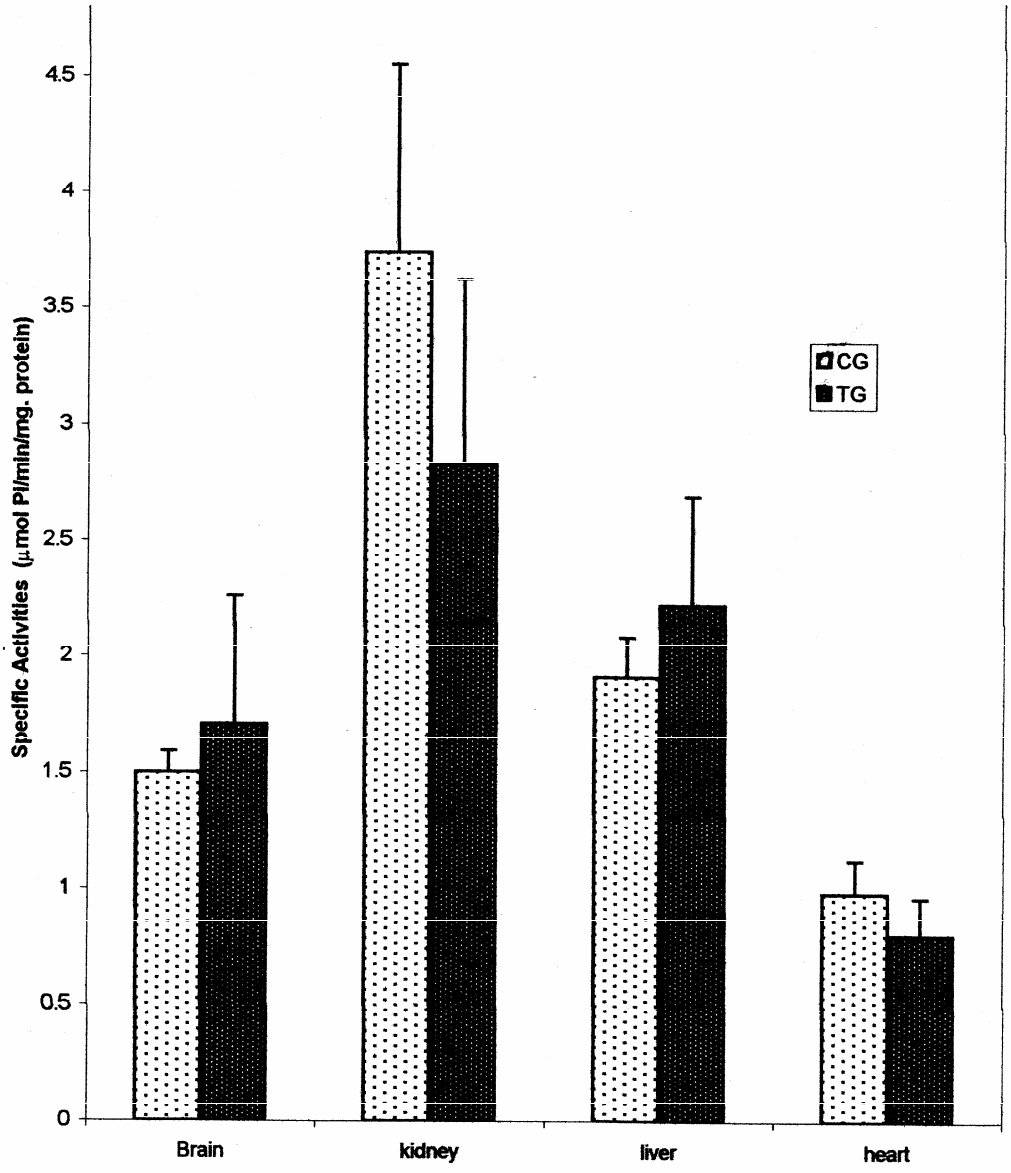

Figure 1: Activities of Mg^{2+}- ATPase in the tissues of rats treated with artemether. Values are given as mean and SEM for a minimum of four determinations. CG, Control group; TG, Treatment Group.

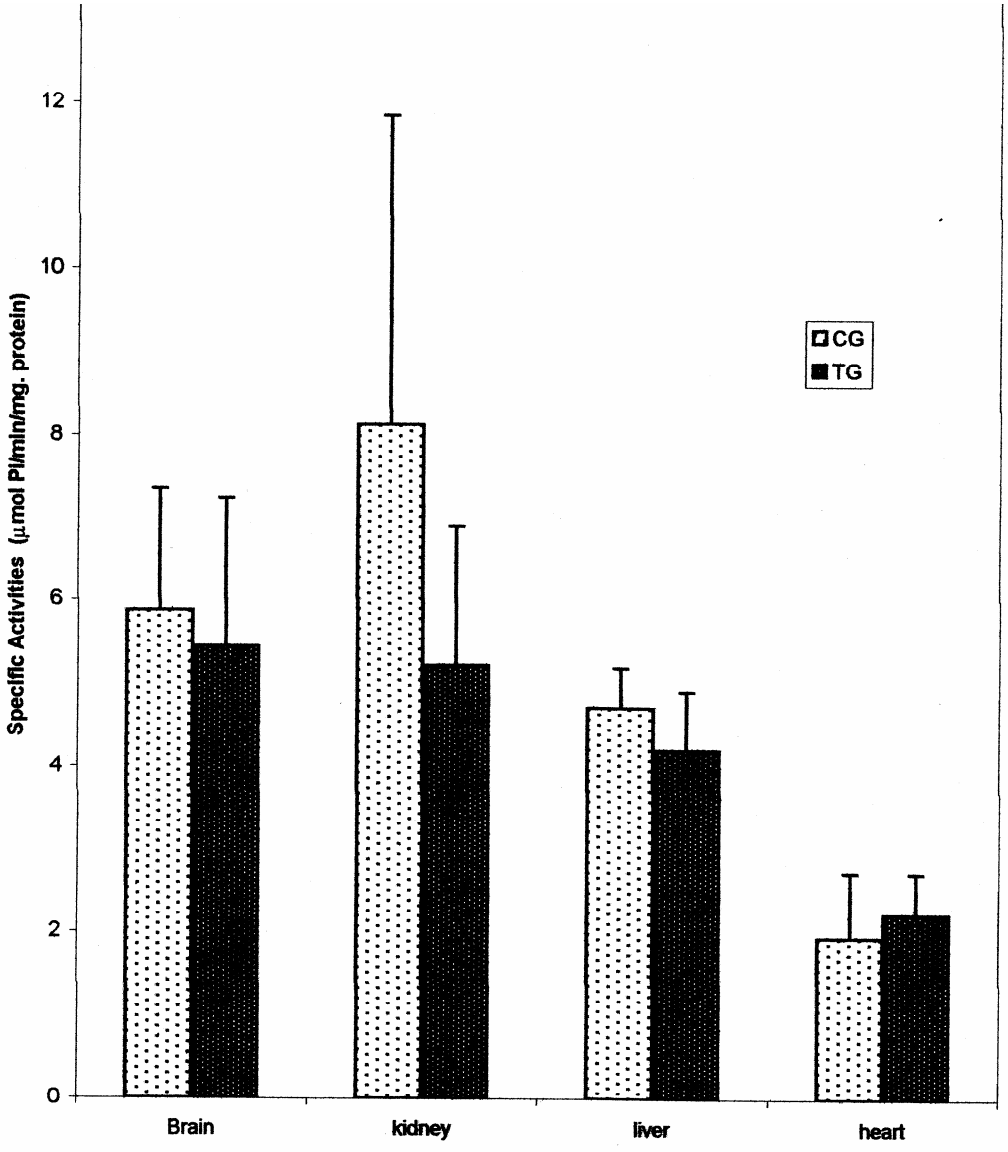

Figure 2: Activities of Na^+-K^+ - ATPase in the tissues of rats treated with artemether. Values are given as mean and SEM for a minimum of four determinations.
CG, Control group; TG, Treatment Group.

The latter alternative may be the major factor responsible for this reduction since artemisinin has been reported to alkylate various proteins *in vitro* (Yang *et al*., 1994). Also, one of the reactions in the two-step mechanism of the antimalarial action of artemisinin and its derivatives is alkylation of specific proteins in the parasite (Kamchonwongpaisan and Meshnick, 1996). Thus the significant reduction ($P < 0.05$) in the activities of the enzyme in the kidney and liver may be as a result of direct interaction of the drug with the enzymes. Nevertheless, the contributory effect of the drug on the cell membrane to the reduction in the activities of these enzymes could be significant. As earlier mentioned, the drug may cause peroxidative damage to the lipid bilayer of the membrane which may lead to the shortening of the fatty acyl chain lengths which in turn could lead to the reduction in the activities of these membrane-anchored enzymes. For example, Ca^{2+}-Mg^{2+}-ATPase activity has been reported to be directly related to the acyl chain lengths of the bilayer lipids (East *et al*., 1984).

The reduction in the activities of Ca^{2+}-Mg^{2+}-ATPase in the kidney of treated rats may lead to an increase in intracellular calcium ion concentration which has been reported to block Na^{+}-K^{+}-ATPase activity (Schatzmann and Burgin, 1978; Schuurmans-Stekhoven and Bonting, 1981). This may also contribute to the reduction, though not significant ($P > 0.05$), observed in the activities of Na^{+}-K^{+}-ATPase and Mg^{2+}-ATPase in the kidney. High intracellular concentration of calcium ion stimulates enzyme activities leading to the formation of phosphatidic acid from the membrane phospholipids via 1,2-diacylglycerol and a phosphorylating kinase reaction (Schatzmann and Burgin, 1978). This may adversely affect the properties of the bilayer lipids that may be needed for optimum activities of membrane-bound enzymes.

The significant increase ($P < 0.05$) observed in the activities of Ca^{2+}-Mg^{2+}-ATPase in the heart and the alkaline phosphatase in the brain may be due to the response of the cellular systems to offset the stress introduced by exposure to the drug. It has been reported earlier that increased activities of various enzymes were observed under various conditions of stress (malomo *et al*. 1993, 1995).

Since artemether has been reported to be neurotoxic at high doses (Nosten and Price, 1995) and to cause transient heart block at the recommended dose (Valecha, 1996), the cellular systems may swing some mechanisms into action to oppose the onset of these. This may account for the rise in the activities of these enzymes in the two tissues. Such mechanisms might have been overcome in the liver and the kidney due to their exposure to more concentrations of the drug.

Alkaline phosphatase and Ca^{2+}-Mg^{2+}-ATPase may be more susceptible to modification by artemether than Na^{+}-K^{+}-ATPase. The activity of a membrane-bound enzyme is a function of the lipid head group, backbone and fatty acyl chains of the bilayer lipids of the membrane (carruthers and Melchior, 1986). Since some regions of the membrane may be richer in some lipid species than others due to their mutual solubility properties, there is the existence of heterogeneous regions or domains of differing lipid composition or physical state in which specific enzymes could exist (Carruthers and Melchior, 1986). Also, certain specialized lipids such as cholesterol preferentially associate with certain classes of lipids (Carruthers and Melchior, 1986). All these differences put together may make one membrane bound enzyme more susceptible to modification than the other. Thus, the regions of the bilayer where Ca^{2+}-Mg^{2+}-ATPase and alkaline phosphatase exist may be more susceptible to modification by the drug than the regions where Na^{+}-K^{+}-ATPase and Mg^{2+}-ATPase exist.

Artemether may have some adverse effects on cellular proteins, especially enzymes, which may explain the few toxic effects already identified. from the results of this study, it can be said that artemether may adversely affect cation transport in the kidney.

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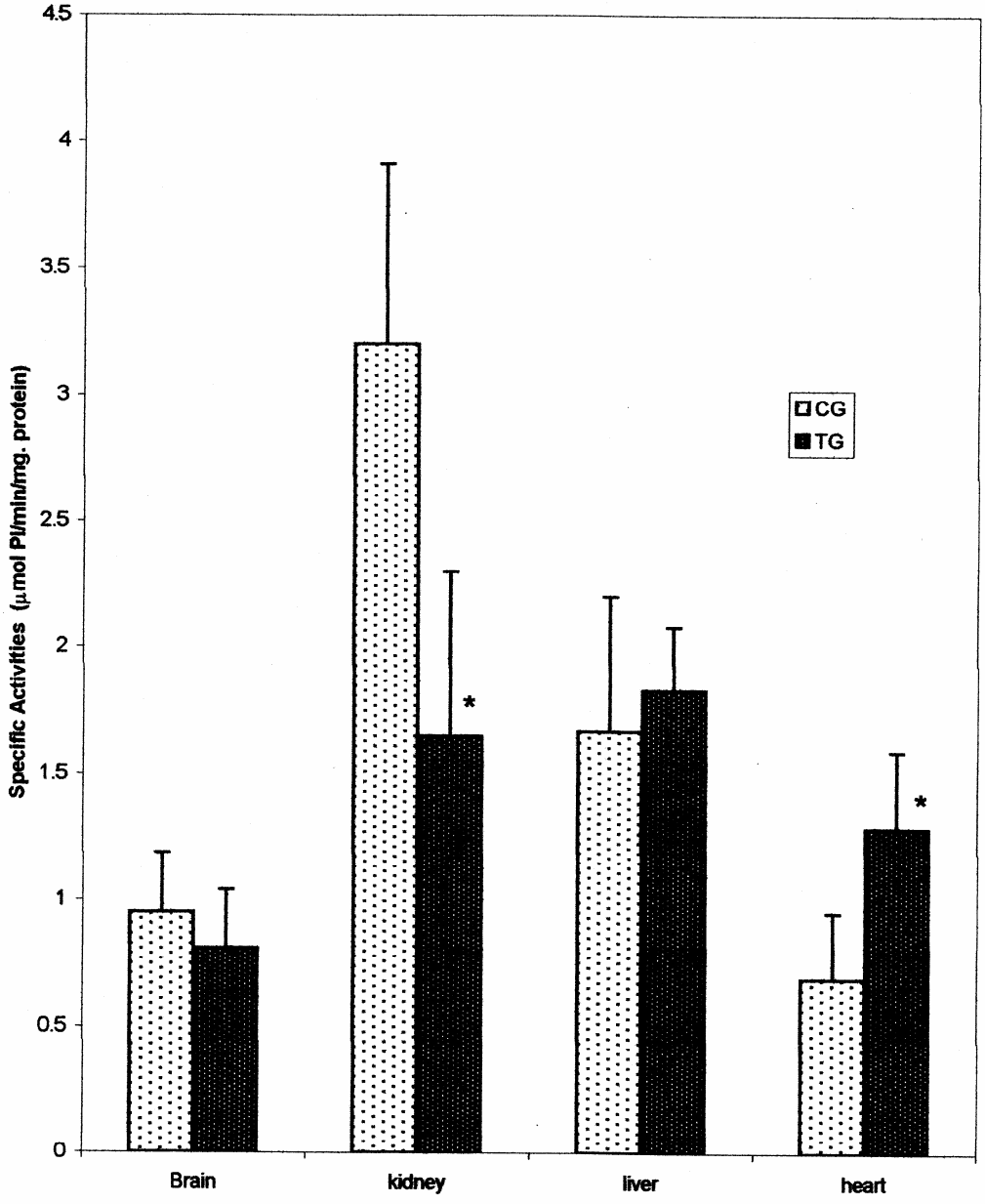

Figure 3: Activities of Ca^{2+}-Mg^{2+} - ATPase in the tissues of rats treated with artemether. Values are given as mean and SEM for a minimum of four determinations. (*Significantly different from Control ($p<0.05$))
CG, Control group; TG, Treatment Group.

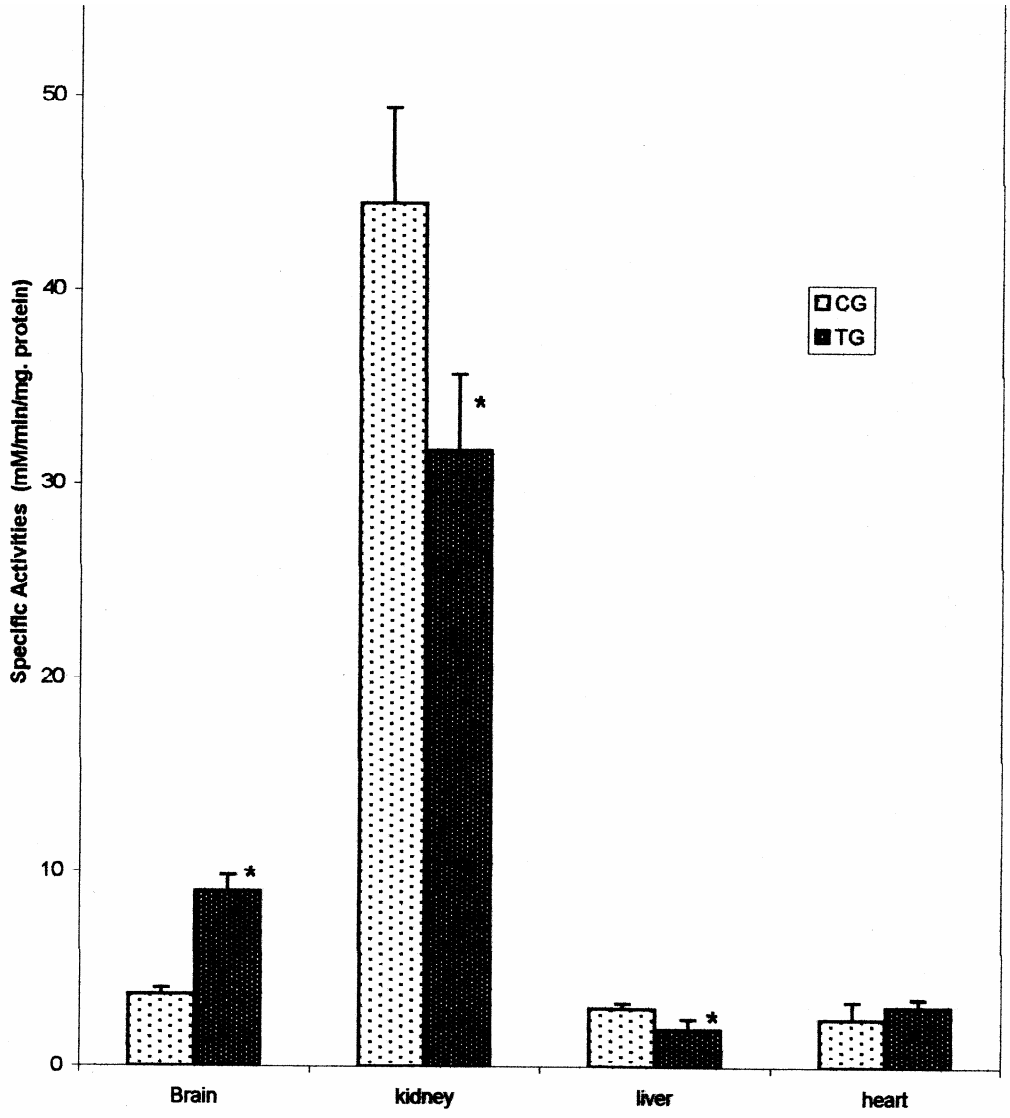

Figure 4: Activities of Alkaline Phosphatase in the tissues of rats treated with artemether. Values are given as mean and SEM for a minimum of four determinations. (*Significantly different from Control ($p<0.05$))
CG, Control group; TG, Treatment Group.

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