HEPATO-PROTECTIVE EFFECT OF ARTESUNATE ON CARBON TETRACHLORIDE-INDUCED TOXICITY IN RATS

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ABSTRACT

Purpose: In the present study, the investigation of the protective effect of Artesunate in experimental acute liver damage induced by CCl_4 was carried out. **Methodology:** Forty wistar rats were equally allocated to four study groups. The first group was designated as the distilled water control group (group 1). The second group was given oral CCl_4 (0.75ml/kg body weight diluted 1:1 in between 80) for 3 days (group 2). The third group (group 3) was given oral Artesunate (50mg/kg body weight) for 3 days followed by CCl_4 oral administration with the same dose given to group 2 and the fourth group (group 4) was given oral artesunate (50mg/kg body weight) for 3 days. Body weight before and after the experiment was noted and plasma AST, ALT, ALP and ALB levels were studied as markers of hepatotoxicity. **Results:** Plasma AST level was significantly higher in group 2, in comparison to group 1 ($P < .05$). Also increase in levels of ALT and ALP was noted in group 2 as compared to group 1. ALT, AST and ALP were increased in group 4. **Conclusion:** Observed improvement in plasma AST, ALT and ALP was recorded in group 3 and all these suggested that Artesunate has possible hepatoprotective effect on hepatocellular damage due to toxicity and it could also have hepatotoxic effect when administered in high dose in experimental animals.

Keynote: Artesunate, Carbon Tetrachloride, Plasma Transaminases, Albumin

1.0 INTRODUCTION

Malaria is, and continues to be a major health problem in some part of the world especially in the tropics [1]. Delay in treating this infection may result in rapid deterioration in the patient's conditions, together with the development of a number of life threatening complications [2]. Several drugs and chemical substances have been found very useful in the management of plasmodium infections. In China, *Artemisinins, derived* from leaves of a plant sweet wormwood or sweet Annie (Artemisia annua) were reported to have antipyretic properties more than 1500 years ago. Since the initial discovery, an array of semi-synthetic oil and water soluble derivatives of artemisinin has been developed with variety of formulations entering clinical studies [3].

Artesunate, an artemisinin derivative, is a semi-synthetic drug used to treat malaria, especially chloroquine resistant malaria in Nigeria. They have active metabolites known as quighaosu. These compounds have impressive parasiticidal properties invivo and invitro. They rapidly arrest parasite metabolism and kill malaria parasite more quickly than other antimalarial drugs [4]. Artemisinin is highly crystalline and does not dissolve in polar or non polar solvent; hence it is modified chemically to yield these derivatives: *Artesunate (hemisuccinate), Artemether (methyl ether), Arteethe r(ethyl ether), Artehinic acid, & dihydroartemisinin* [5].

Artesunate is water soluble artemisinin derivative. It is the most widely used member of the artemisinin derivative [6]. Artesunate has the compound name; dihydroartemisinin1, 2-alpha-succinate and has a molecular formular $C_{19}H_{28}O_8$. Artesunate is effective against *Plasmodium falciparum* resistant to other operationally used antimalarial drugs [7]. Artesunate, like most hepatoactive substances shares similar metabolic pathway with every other artemisinin derivatives. Its biotransformation pathways include hydroxylation followed by glucuronidation in the liver [8]. In human and rat liver artemether is finally converted to an active metabolite; DQHS (dihydroquinghaosu) catalysed by human cytochrome P450 isoenzymes [9].

Liver is the major site of intensive metabolic activity. Liver dysfunction as a result of toxic chemicals, certain drugs and environmental pollutants has largely increased in the last few decades [10].

Carbon tetrachloride has been shown to have deterious effect on humans and animals such as rats and such effects have been demonstrated in organs such as the liver and kidney [11]. It is metabolized by the mitochondrial monooxygenase (P450 2E1) system producing an unstable trichloromethyl (CCl_3) free radical

which rapidly converts to trichloromethyl peroxide (Cl_3COO-) [12] leading to the lipid peroxidation and consequently liver damage [13].

However herbal or alternative treatments of many diseases including hepatopathy are increasing in many countries in the world [10]. So many plant extracts have been found useful in the management of ailments such as respiratory infections, inflammations, digestive organ affections and infections, urogenital, renal and hepatic disorders and many other diseases all over the world [14]. Artesunate like other artemisinins have been reported to exhibit activities in pharmacodynamic areas other than malaria, too. Publications have described antiviral, anticancer, immunomodulating and antiangiogenic activities [15]. The various activities of artemisinins, different from their antimalarial activities, can be regarded as scientifically interesting and in part potentially useful, in view of the relative non-toxicity of these derivatives. However, there are differences in the importance and relevance of these findings and thus this study reveal any significant relevance of this drug in the protection of the liver against liver damage.

2.0 MATERIALS AND METHOD

2.1 Materials and chemical

The materials used includes; Wooden cages, wood shavings, rat pellets, water, syringes, oral canula, cotton wool, lithium heparin sample bottles, centrifuge, weighing balance, artesunate drug, carbon tetrachloride solution dissolved in sunflower oil, chloroform, methylated spirit and twin 80.

2.2 Experimental animals and design

Forty wistar strain rats weighing 120g to 220g each were purchased from the animal house of the Biomedical Sciences department. They were housed in cages and kept in the animal house of the department of Biomedical Sciences Osogbo, Osun State Nigeria with an average ambient temperature of 28 to 32^oC with a 12 hour light /dark cycle, and received rat feed (rat pellet) and water *ad libitum* during an acclimatization period of two weeks and also throughout the period of experimentation. The experiment was carried out at the animal house, Department of Biomedical Sciences, Faculty of Basic Medical Science, College of Health Sciences, Osogbo Osun State-Nigeria. The animals were divided into four study groups of 10 rats each as follows.

Group 1: Feeds + distill water.

Group 2: Carbon tetrachloride (CCl_4).

Group 3: Artesunate + Carbon tetrachloride (CCl_4).

Group 4: Artesunate.

2.3 Statistical analysis

Statistically analyzed data used was presented as mean ± SD of (5) determinations. Statement analysis was carved out using one way analysis of variance (ANOVA). Differences were statistically significant at ($P<0.05$).

3.0 RESULT

In the study of the administration of carbon tetrachloride on serum AST, ALT, ALP and ALB in wistar rats. The carbon tetrachloride with artesunate combined and artesunate treated only groups showed significant increased ($P<0.05$) ALT but with no significant difference in artsunate treated only. More so, carbon tetrachloride treated only and carbon tetrachloride with artesunate combined showed significant increased in ALP level and no significant difference in artesunate treated only. Groups treated with carbon tetrachloride only, carbon tetrachloride with artesunate combined and artesunate treated only showed significant increased ($P<0.05$) in AST and ALB levels respectively.

4.0 DISCUSSION

This study showed that there was no significant difference ($P>0.05$) in weight loss or gain of the animals in all the groups at the end of the experiment. The administration of CCl_4 with a dose of 0.75ml/kg body weight orally to group II, induced increase in the activities of both transaminases with AST being significant (figure III&IV) and notable decrease in albumin (ALB) levels (figure IV) and slight increase in alkaline phosphatase level (ALP) when compared with distilled water control group. All these changes in AST, ALT, ALB and ALP levels are indicative of damage to hepatocytes and these findings agree with the work of [16].

There was an observable increase in the levels of AST and ALT which suggest toxicity on the liver cells of the rats upon artesunate (at dose of 50mg/kg) administration as seen in artesunate control group when compared with the distilled water group. This findings study agree with the work of [5], in which artesunate administration caused significant increase in the liver marker enzymes in rabbit and, [17] in which it showed toxicity in liver cells of guinea pigs. They also agree with [6] in which artesunate caused a transient rise in the liver transaminases. The liver cell damage may have been caused by free radicals generated by artesunate, which are also responsible for their antimalarial actions. The deleterious effects may be caused by free radicals produced during peroxide formation. Precisely, the level of hydroxyl and peroxide radicals induced by artesunate treatment may be responsible for the hepatotoxicity to the rats.

Interestingly, compared to CCl_4 toxic groups, there was a notable improvements in the ALT, AST and ALB levels in the groups which were originally treated with artesunate and subsequently with CCl_4 at same dose with artesunate control groups. This reveals a possible protective activity of the drug on hepatocellular damage induced by CCl_4.

However, considering the interesting possibility of a protective activity of the drug as shown above, it can be inferred that the raised liver enzyme activities at dose (50mg/kg) of artesunate administered to the drug control group suggests hepatocellular damage consequent upon the drug administration as a result of overdose of the drug in the line with findings of [17,18] and may not be as a result of hepatotoxicity as earlier thought.

5.0 Conclusion

Considering the results obtained from the analysis of the entire data derived at the course of this study, it can however, be inferred that there is still a possibility that artesunate to a reasonable extent, may have the ability to confer a protective effect against hepatocellular damage that is due to toxicity and that there may be hepatocellular toxicity associated with over dose of artesunate.

6.0 Acknowledgement:

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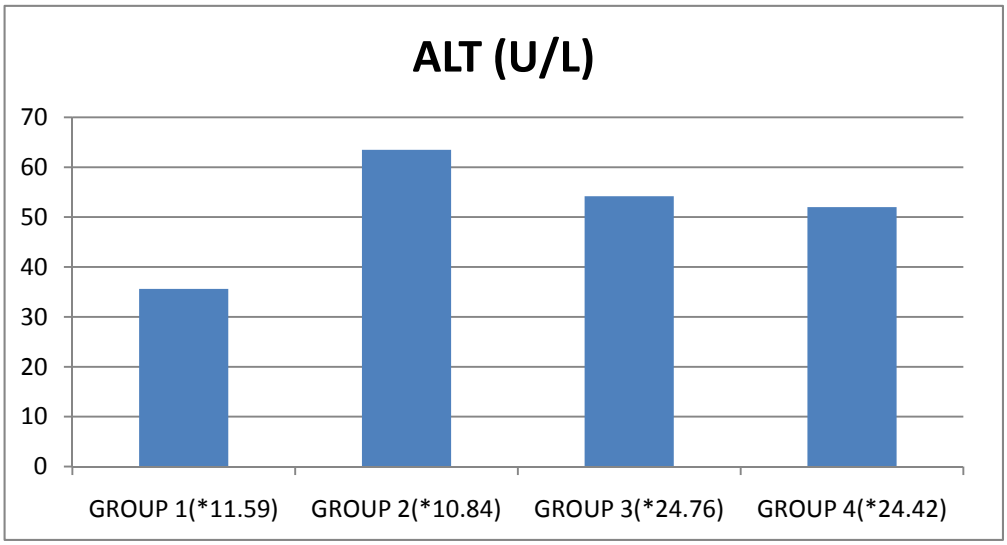

Figure 1 Effect of carbon tetrachloride and artesunate on serum alanine aminotransaminase (ALT) activity.

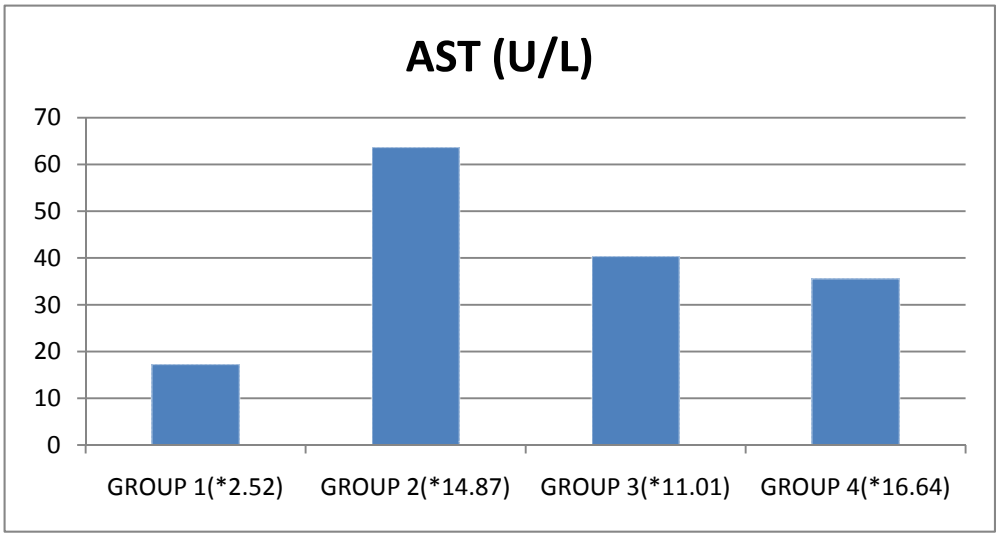

Figure II; effect of carbon tetrachloride and artesunate on aspartate aminotransaminase (AST) activity.

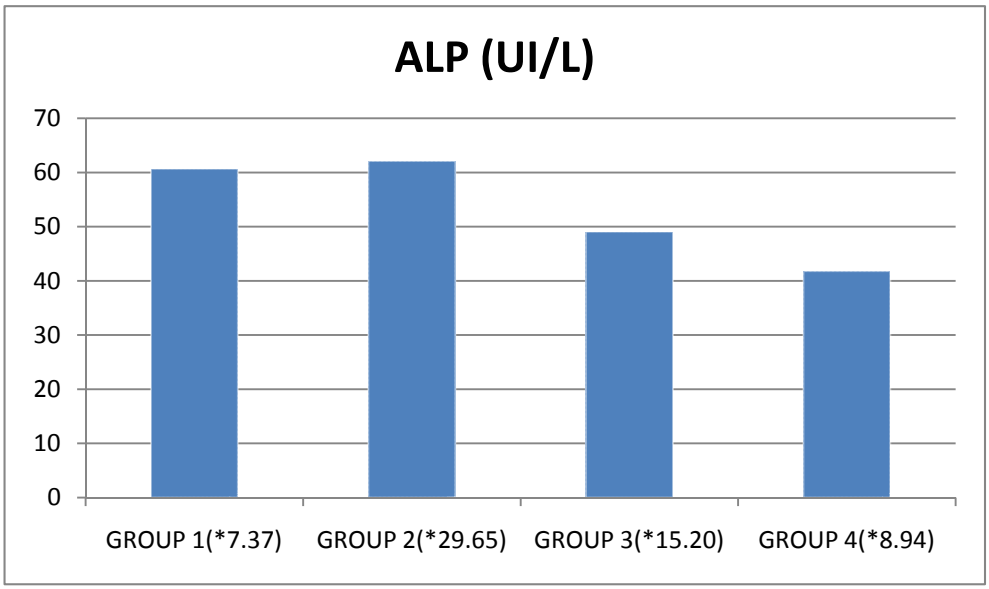

Figure II1; effect of carbon tetrachloride and artesunate on serum alkaline phosphatase (ALP) activity.

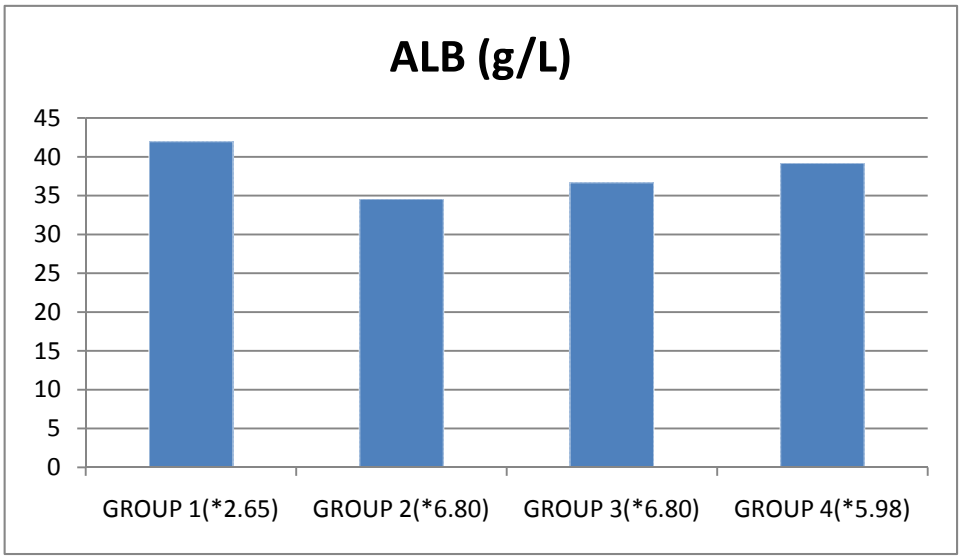

Figure IV: Effect of carbon tetrachloride and artesunate on serum Albumin ALB concentrations