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A Study of the Hepatoprotective effect of *Garcinia kola* Water Extract in Amodiaquine and Artesunate treated Rats.

E.O. Ajani,* P. D. Shallie,* B. O. Adegbesan, B. A. Salau,** A. Akinpelu***.**
*Department of Biochemistry, Faculty of Basic Medical Sciences,
**Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne.

Correspondence to:
E. O. Ajani
immaanbisi@yahoo.com

SUMMARY

The effect of kolaviron, a water extract of *Garcinia kola* on hepatotoxicity induced by the antimalarial drug, amodiaquine was investigated. The effect was compared with that of artesunate, another antimalarial agent. Thirty (30) adult male rats divided into six (6) groups were used in the study. Groups D, E and F were treated with 100mg/Kg of the extract twice daily for the first one week and 200mg/Kg/day for the subsequent three (3) weeks. Amodiaquine (10mg/Kg/day) was administered orally for four (4) days into rats in groups A and E while rats in groups B and F were treated with artesunate (5mg/Kg/day for four {4} days). Group C was treated with normal saline and kept as test control. All the rats were sacrificed after 4 weeks treatment period. Blood was withdrawn by cardiac puncture while the liver was removed, homogenized and used for both biochemical and histological analysis. Treatment with amodiaquine resulted in increase in relative liver weight while this increase was reversed by pre-treatment with *Garcinia kola* extract. Amodiaquine treatment also resulted in significant increase in liver postmitochondria lipid peroxidation, while pretreatment with *Garcinia kola* significantly decrease the MDA value from the 62.53±0.60 unit/g tissue (prior to *Garcinia kola* administration) to 44.43± 2.16unit/g tissues. Treatment with artesunate neither caused a significant change in the relative liver weight nor in the lipid peroxidation value. Amodiaquine treatment also resulted in significant increase in serum ALT and AST activities and a subsequent decrease in these parameters in the liver, whereas, the observed changes were reversed with pretreatment with *Garcinia kola* extract. Treatment with artesunate and pretreatment with *Garcinia kola* prior to artesunate administration did not alter these parameters significantly. Histological examinations of the liver slices correlated with the observations in the serum and liver. The present study indicates that *Garcinia kola* is effective in preventing the hepatotoxic effect due to prophylactic administration of amodiaquine. It also emphasized that prophylactic treatment with artesunate does not cause a significant alteration in liver function.

Key Words: Garcinia kola, hepatotoxicity, amodiaquine, artesunate, lipid peroxidation

INTRODUCTION

Amodiaquine (Camoquine, Parke – Davis) is a 4-aminoquinoline derivative similar to chloroquine and has been widely used both in the prophylaxis and treatment of malaria (1,2). Following serious toxicity associated with its use as prophylaxis, it was withdrawn by the World Health Organization (WHO) from the list of drugs for the treatment of malaria during 1990 – 1996 but later re- instated in 1996 (1). Aggranulocytosis and liver damage have repeatedly been reported as adverse reactions (ADR's) to amodiaquine (2). The 19th expert committee on malarial reported that amodiaquine could be used for treatment if the risk of infection out weigh the potential for ADR's but still did not recommend amodiaquine as first line treatment (3).

In recent years, increasing attention has been given to therapies combining antimalarial drugs with

different mode of actions. Amodiaquine has been tested in clinical trials in combination with artesunate or sulfadoxine/pyrimethemine with very encouraging results (4). Artesunate is a water soluble hemisuccinate derivative of dihydro – artemisinin. Artemisinin is a sesquiterpene lactone isolated from *artemisia annua*, a herb that has traditionally been used in china for the treatment of malaria (5). Amodiaquine in combination with artesunate is now one option that is recommended by the WHO for use in malarial control programmes. Artesunate and other related artemisinin derivatives have been widely used with no reports of any serious adverse reactions. Neurotoxicity has however been observed in animal studies but not in human. Cardio – toxicity has also been observed following administration of high doses (6).

Garcina kola is a large forest tree found throughout West and Central Africa. The seeds are eating as refreshing past time in Nigeria and are known to contain high content of biflavonoid compound. The seeds known as "bitter kola" or "false kola" are believed to possess aphrodisiac properties and are used for the treatment of catarrh and abdominal colicky pain (7). Considerable experimental evidence has been adduced in support of the antihepatotoxic efficacy of kolaviron in animals against such hepatotoxicants as paracetamol (8), carbon tetrachloride, thioacetamide, galactosamine, phallodine, and ethanol (9,10). In the traditional medicine of Nigeria, *Garcina kola* is employed in the treatment of various diseases including hepatitis (11).

Consequently, in this study, we investigated the effect of pretreatment of animal with *Garcina kola* on the liver histology, lipid peroxidation and some liver enzymes when the antimalarial agents, amodiaquine and artesunate are separately administered.

MATERIALS AND METHOD.

Plant materials

Garcina kola seeds were obtained locally in Sagamu market and certified by the plant science department, Olabisi Onabanjo University, Ago – Iwoye, Nigeria. The peeled seeds (7.5kg) were then sliced, pulverized with an electric blender and dried at 40°C in a Gallenkamp drying oven

Preparation of *Garcina kola* extract

The method of Iwu *et al* (1990) (12) was used in the extraction of *Garcina kola* seed. The powdered seeds were extracted with light petroleum ether in a Soxhlet for 24hours. The defatted dried mare was repacked and extracted with acetone. The extract was concentrated and diluted twice its volume with water and extracted with ethyl acetate (6 X 300ml). The concentrated ethyl acetate fraction gave a yellow solid called kolaviron. Appropriate dose dilutions were made by suspending the extract in 0.9% Nacl solution while normal saline was administered into the control group.

Treatment of Animals

Thirty (30) healthy male albino rats weighing between 150 – 220g were used in the study. They were all obtained from the animal house, physiology department, University of Ibadan, Nigeria and were acclimatized for a week. The rats were randomly distributed into six (6) groups. Amodiaquine (10mg/kg/ day) was administered orally into group A for 4days. Group B was treated with artesunate (5mg/kg/day) orally for 4days. Rats in group C were treated with normal saline while those in group D were treated with *Garcina kola* extract (200mg/kg/day) for 4weeks. Rats in group E and F were treated with *Garcina kola* extract (100mg/kg) twice daily for the first one (1) week and 200mg/kg once daily for the next 3weeks. In the second week of treatment with *Garcina kola*, amodiaquine (10mg/kg) and artesunate (5mg/kg) was administered orally into group E and F rats (respectively) continuously for 4days. Feed and clean drinking water were supplied *ad libitum*. All animals were sacrificed after 4weeks. Blood was withdrawn by cardiac puncture while the livers were quickly removed.

Chemicals

Amodiaquine and Artesunate, were obtained from sigma (St. Louis, USA). All other reagents were of analytical grade and obtained from both Sigma and BDH (Poole, Darset, U.K).

Preparation of Liver Homogenate

The liver, after washing in 4.1% KCl solution was dried and weighed. It was then homogenized in 4 volume of isotonic phosphate buffer, pH 7.4 and centrifuged at 9000g for 20min to obtain the post mitochondrial supernatant fraction.

Preparation Of Serum

Serum was prepared by centrifugation of the blood at 3000g for 10min.

Histopathology

This was carried out according to the method describe by Farombi *et al* (10). Liver fragments were fixed in 10% neutral formolsaline for 18hours and then dehydrated in ethanol 50% for 2hours, 70% for 2hours, 90% for 2hours and then absolute ethanol (100%). The tissue was then embedded in paraffin for 48hours. The hepatic section 6μm thick were then dried in oven, stained with haematoxylin and eosin and then observed under a light microsope.

Biochemical Analysis

Lipid peroxidation was assessed by measuring

the thiobarbituric acid reacting substances (TABRS) formation (13). Alanine amino transferase (ALT) and aspartate amino transferase (AST) enzyme activities were determined using combination of the methods of Mohun and Cook (14) and Reitman and Frankel (15).

Statistics

All results were analyzed by two – tailed student's t – test (16). P values of less then 0.05 were considered statistically significant.

RESULTS

Presented in table 1 below is the result of amodiaquine and artesunate treatment on body and liver weight prior to and without pre – treatment with *Garcinia kola* extract. Compared with the control, the liver of the Amodiaquine treated rat showed a significant increase in liver weight than the control and all other groups. When pretreated with Kolaviron, rats administered with Amodiaquine did not show any significant difference in the relative liver weight compared to the control. The relative liver weight was however significantly lowered compared to that of rats treated with amodiaquine alone. The relative liver weight of rats treated with Artesunate does not differ significantly from the control group nor was it different from the liver weight of rats' pre – treated with *Garcina kola* extract prior to administration of artesunate. The weight of the liver of rats treated with *Garcina kola* extract alone was also not different from that of other groups but significantly lower than that of rats treated with amodiaquine alone.

Presented in Table 2 is the effect of treatment on rat postmitochondria lipid peroxidation. The MDA (mg/ gram tissue) for the amodiaquine treated rats was significantly higher 62.53± 0.60 than the control group 26.78±0.93 and also higher than all other groups.

Table I: Effect of Pretreatment with *Garcinia kola* on Total body and Final Liver Weight of Albino Rats

Group	Treatment	Initial Body Weight (g)	Final Body Weight (g)	Terminal Liver weight (g)	Final Liver weight as % of Final Body Weight
A	Amodiaquine (10mg/Kg/ day for 4days)	156.2±6.2	214.8±4.1	9.17±0.31[a]	.29±0.20[a]
B	Artesunate (5mg/Kg/ day for 4days)	170.7±4.3	229.9±5.5	7.67±1.01[b]	3.37±0.21[b]
C	Normal control (Normal saline)	162.8±8.5	232±6.4	6.30±0.71 [b]	2.70±0.62[b]
D	*Garcinia Kola* (200mg/Kg/ day for 4 weeks)	180.3±6.6	214.7±5.1	6.60±0.52 [b]	3.02±0.22[b]
E	*Garcinia Kola* (200mg/Kg/ day for 4 weeks +Amodiaquine	174.7±4.7	190.5±4.2	5.71±0.40 [b]	, 2.99±0.16[b]
F	*Garcinia Kola* (200mg/Kg/ day for 4 weeks + Artesunate, 5mg/ Kg/ day for 4 days)	144.3±2.8	190.5±5.3	6.15±0.31 [b]	3.15±0.42[b]

Note
* Values are mean ± SD,
* Number of subjects (n) = 5
* Values in the same column with the same superscript are not significantly different from each other. (P>0.05)

Table II: Effect of Pretreatment With *Garcinia kola* On Rat Post Mitochondria Lipid Peroxidation.

Group	Treatment	TBARS (x10^5 mg MDA/g Tissue)
A	Amodiaquine (10mg/Kg/day for 4days)	62.53 ±0.60[a]
B	Artesunate (5mg/Kg/ day for 4days)	30.59±3.24[b]
C	Normal saline	26.78±0.93[b]
D	*Garcinia Kola* (200mg/Kg/ day for 4 weeks)	29.24±1.41[b]
E	*Garcinia Kola* (200mg/Kg/ day for 4 weeks + Amodiaquine, 10mg/Kg/ day for 4 days)	44.43±2.16[c]
F	*Garcinia Kola* (200mg/Kg/ day for 4 weeks + Artesunate, 5mg/ Kg/ day for 4 days)	30.65±1.59[b]

Note:
* Values are mean ± SD
* Number of subjects (n) = 5
* Values in the same column with the same superscript are not significantly different from each other. (P>0.05)

Pretreatment with *Garcinia kola* reduced significantly the MDA level in rats treated with amodiaquine, the value obtained (44.43± 2.16) was however significantly higher than the control value. Treatment with artesunate did not significantly alter the MDA value. Table III is the result of treatment on the serum ALT and AST activities. Following administration of amodiaquine, a significant increase in ALT activity (20.02± 0.60 (U/L)) and AST activity (18.56±1.09(U/L) was observed compared with the control groups (11.26±0.53U/L) and (11.30±0.52U/L) respectively. The activity was also higher than those of rats in groups treated with Artesunate. Pretreatment with *Garcina kola* reduced significantly the ALT and AST activities from the increased caused by Amodiaquine treatment, however the observed activities 15.22± 0.86(U/L) (ALT) and 15.76± 0.48(U/L) (AST) were significantly higher than the pretreatment values (control groups). When treated with Artesunate, rats used in this study showed a slight increase in serum ALT and AST activities (12.50±0.59 and 12.06±0.59U/L respectively) from the control, these increases were however not significant. Pretreatment with *Garcinia kola* extract prior to artesunate administration also results in slight increase in the activities of these enzymes (compared with the control groups) the increase was however not significant (P>0.05) The serum ALT and AST activities of rats treated with *Garcinia kola* extract alone were not significantly different from the control group. Table IV show the effect of treatment on the liver ALT and AST activities. The result indicates that the ALT and AST activities were lower in the liver of Amodiaquine treated rats (8.88±0.35 and 9.55±0.23(U/L respectively) compared to the control group (12.36±1.11 and 11.56±1.21U/L respectively). Pretreatment *with Garcinia kola* extract raised the ALT and AST activities in the liver (10.50± 0.58 and 10.55± 0.11U/L respectively), to an observed activity that was not significantly different from the control group and group treated with artesunate. No significant difference was however observed in the activities of the enzyme in the liver of rats treated with artesunate or prior to or without the administration of the extract.

The result of the histopathology study is shown in fig 1A – F. Visible lesions, cell necrosis, apoptosis, collapsed endothelium of the central vein as well as dilated sinusoid were observed to be permanent in the liver of rats treated with Amodiaquine alone. Some of this observed feature apart from apoptosis and necrosis were also observed in all other groups but were not permanent.

Table III: Effect of Pretreatment With *Garcinia kola* Extract on Serum Alanine Amino Transferase and Aspartate Amino Transferase (ALT) and (AST) Activities

Group	Treatment	ALT (U/L)	AST (U/L)
A	Amodiaquine (10mg/Kg/day for 4days)	20.02±0.60[a]	18.56 ±1.09[a]
B	Artesunate (5mg/Kg/ day for 4days)	12.50±0.59[b]	12.06±0.59[b]
C	Normal saline	11.26±0.53[b]	11.30±0.52[b]
D	*Garcinia kola* (200mg/Kg/ day for 4 weeks)	11.36±0.78[b]	10.62±1.39[b]
E	*Garcinia kola* (200mg/Kg/ day for 4 weeks + Amodiaquine, 10mg/Kg/ day for 4 days)	15.22±0.86[c]	15.76±0.48[c]
F	*Garcinia kola* (200mg/Kg/ day for 4 weeks + Artesunate, 5mg/ Kg/ day for 4 days)	11.92±0.60[b]	13.39±0.65[bd]

Note
* Values are mean ± SD * Number of subjects (n) = 5 * Values in the same column with the same superscript are not significantly different from each other. (P>0.05) * U/L= Unit/litre of blood

Table IV: Effect of Pretreatment with *Garcinia kola* on Liver Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) Activities

Group	Treatment	ALT (U/L)	AST (U/L)
A	Amodiaquine (10mg/Kg/day for 4days)	8.88 ±0.35[a]	9.55 ±0.23[a]
B	Artesunate (5mg/Kg/ day for 4days)	12.84±1.50[b]	11.68±1.27[b]
C	Normal saline	12.36±1.11[b]	11.56±1.21[b]
D	*Garcinia kola* (200mg/Kg/ day for 4 weeks)	12.56±0.72[b]	11.14±0.96[b]
E	*Garcinia kola* (200mg/Kg/ day for 4 weeks + Amodiaquine, 10mg/Kg/ day for 4 days)	10.05±0.58[b]	10.55±0.11[b]
F	*Garcinia kola* (200mg/Kg/ day for 4 weeks) + Artesunate, 5mg/ Kg/ day for 4 days	11.96±0.34[b]	10.98±0.26[b]

Note
** Values are mean ± SD * Number of subjects (n) = 5 * Values in the same column with the same superscript are not significantly different from each other. (P<0.05) *U/L= Unit/litre of blood*

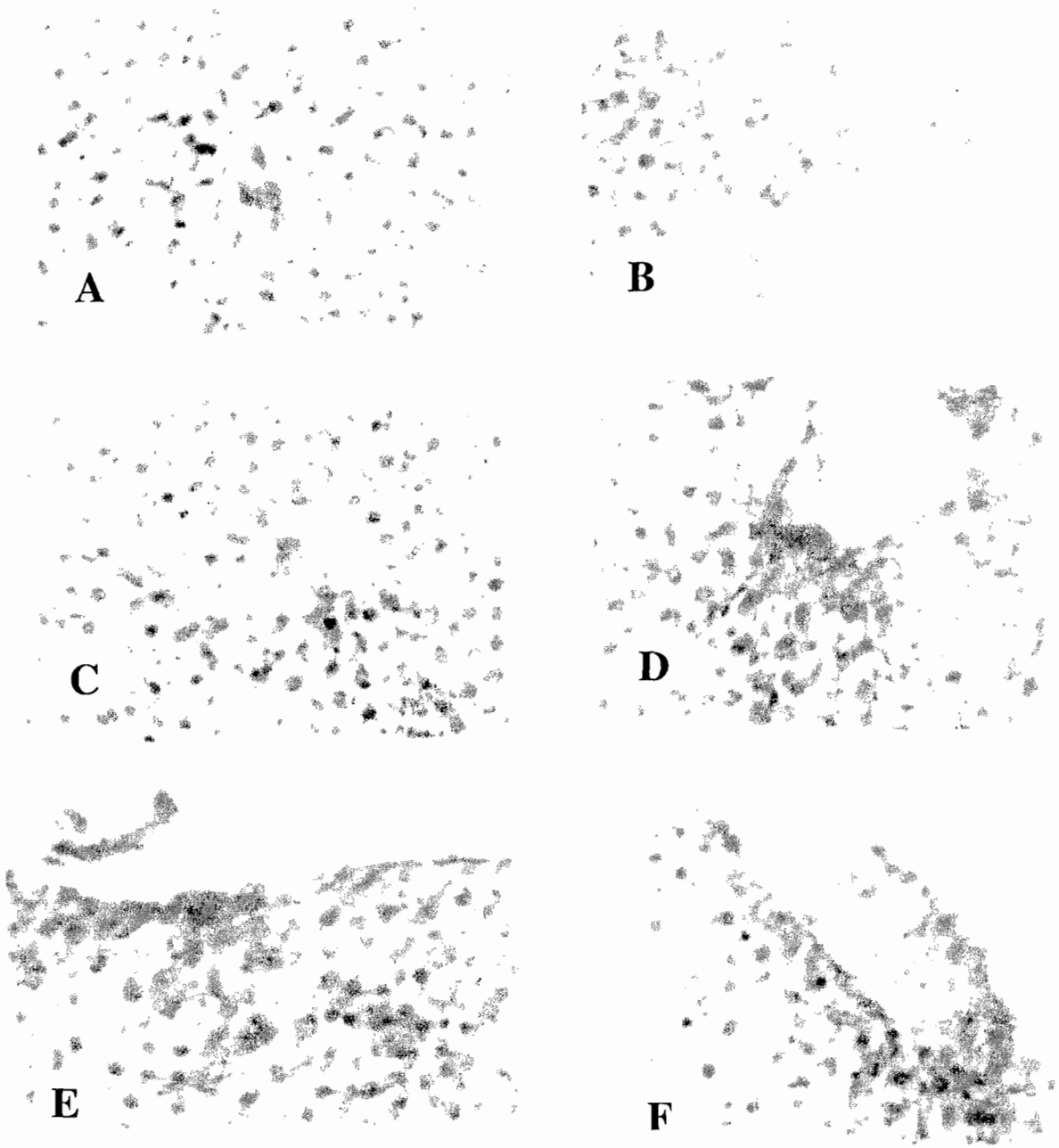

Fig 1: Histology section of liver A, Amodiaquine ; B, Artesunate; C, Normal saline; D, *Garcinia kola*; E, Amodiaquine + *Garcinia kola* and F, Artesunate + *Garcinia kola* treated rats. Apoptosis and necroses were permanent feature in A. Collapsed endothelium was observed in B and F. Kupffer cells was observed in A, B, C, D and E

DISCUSSION

The results of this study emphasize the fact that *Garcina kola* extract has ability to protect the liver against toxic injury and lipid peroxidation resulting from prophylactic effect of amodiaquine administration. This result support previous studies that have demonstrated the *In vivo* hepatoprotective effect of *Garcina kola* against various liver toxicants (8, 9, 10). The result also indicates that though prophylactic amodiaquine administration can result in hepatocellular damage, prophylactic administration of artesunate does not.

The observed increased in the liver weight and relative liver weight when rats were treated with Amodiaquine may be a consequence of alteration in lipid metabolism, which may consequently result in fat accumulation in the liver (17).

That amodiaquine administration for prophylactic purpose can result in liver injury and the hepatoprotective effect of *Garcina kola* in such circumstances is further supported by the result of the ALT and AST activities of the liver and the serum.

Acute hepatocellular injury, apart from other conditions such as myocardial infarction, circulating collapse, acute pancreatitis and infectitious mononucleosis have been reported to result in increase activities of the ALT and AST activities of the serum (15). Previous studies have at several times reported the hepatoprotective effect of *Garcina kola* against various toxicants (18, 19). These findings are further supported by this study.

The result of the histopathological study further support the hepatoprotective action of the *Garcina kola* extract. Cell death has been reputed to take place by at least two distinct processes: apoptosis and necrosis (20,21) and both of these have been reported among other things to be caused by drugs and toxins. Apoptotic cell death has been reported to be as a result of damage to DNA by oxidation or alkylation whereas kupffer cells are activated by the release of cytokines, which may contribute to pathophysiologic process culminating in heopatocyte apoptosis after toxic injury (22). The microscopic study here thus implies that both mechanisms of cell death are involved in hepatotoxic injury caused by amodiaquine treatment. Kupffer cells/ macrophages together with neighbouring hepatocytes, sellate cells and endothelial cells take part in clearing out apoptotic hepatocytes (23). This could explain why there was increased number of kupffer cells in the amodiaquine treated rats.

CONCLUSION

Our study indicates that prophylactic administration of amodiaquine results in liver damage where as this does not occur with administration of artesunate for prophylactic purpose. We also results here that liver damage induced by amodiaquine administration can be reverse by pretreatment with *Garcinia kola*.

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