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EFFECT OF AQUEOUS EXTRACT OF GARCINIA KOLA ON SOME LIVER ENZYMES OF RATS WITH CARBON TETRACHLORIDE INDUCED LIVER DAMAGE

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ABSTRACT

The effect of Garcinia kola aqueous extract on the activities (IU/L) of some liver enzymes was determined in serum of rats injected intraperitoneally with carbon tetrachloride at different doses of 0.33, 0.66, 0.99 and 1.32ml. The enzymes studied were Aspartate amino transferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

The 21 day cummulative toxicity study showed a significant increase in the activities of AST, ALT and ALP ($P \leq 0.001$) in serum of rats injected with different doses of CCl_4. The mean values (IU/L) in serum were: for AST 59.00 ± 0.30 without CCl_4 (control) and 107.67±0.87, 154.10± 0.08, 186.12 ± 0.83 and 55.02±0.62 with CCl_4, 0.33, 0.66, 099 and 1.32ml respectively; for ALT 54.0 ±0,15 without CCl_4 (control) and 98.37±0.21, 109.87±0.19,129.40±0.37, and 185.51±3.24 with CCl_4 0.33, 0.66, 0.99 and 1.32ml respectively; for ALP, 54.90± 0.20 without CCl_4 (control) and 65.20±0.09, 85.34± 053, 95.90 ± 2.05 and 177.15 ± 1.24 with CCl_4, 0.33, 0.66, 0.99 and1,32ml respectively). The increase in the activities was significantly higher ($p \leq 0.001$) at higher doses of CCl_4.

The aqueous extract from the seeds of Garcinia kola (w/v) at doses of 20g/50ml, 40g/50ml given orally for 7 days, 14 days and 21 days respectively reduced the activities of three microsomal enzymes in serum of rats injected with different concentrations of CCl_4. The reduction in the enzyme activities was time and dose dependent.

The ALP activity was reduced significantly ($p \leq 0.001$) after 7 days, 14 days and 21 days at different doses of the extract. AST and ALT activities were only significantly reduced at doses 40g/50g and 60g/50ml after 14 days and 21 days. These findings may have possible biochemical significance in the treatment of some liver diseases.

KEYWORDS: Garcinia kola, Carbon tetrachloride (CCl_4), Aspartate amino transferase, Alanine aminotransferase and Alkaline phosphatase.

INTRODUCTION

Garcinia kola (Heckel) is a highly valued ingredient in African traditional medicine. Despite its bitter taste, the seed has been employed in folk medicine for the treatment of several diseases (Hutchinson and Dalziel, 1954, Elekwa 1996). In general, Garcinia species are mostly trees with leathery leaves. The fruit is berry, usually small, with arillate seeds.

Liver diseases were among the first disorders to which serum tests were applied and have proved to be useful in diagnosing diseases of the liver. At present over fifty enzymes are known to exist in the serum or plasma and all these have been known to have abnormal values in patients with hepatic disease (Ukoha, 1998). In the clinical setting, however, aspartate and alanine amino transferases are the two most important amino transferases frequently used for evalution in routine clinical diagnosis and are widely distributed in the body. (Phild, 1994). Alkaline phosphatase is widely distributed in human tissues, including the liver. Alkaline phosphatase measurements are of particular interest in the investigation of hepatobiliary disease. The response of the liver to any form of biliary obstruction is to synthesize more alkaline phosphatase. (McComb, et al 1979). Plant products like kola nut, tea, coffee, are usually taken to stimulate, to excite or increase the functional activity of various organs of the body. Garcinia kola is still being used a lot in the non-conventional medical treatment of certain diseases. Recent observations show that Garcinia kola has featured in both traditional and herbal medicines.

The study is aimed at determining the effect of aqueous extract of G. kola on the activities of the three liver enzymes, AST, ALT and ALP in the serum of rats poisoned with CCl_4, a known hepatotoxin. This would shed more light on the use of Garcinia kola in the treatment of liver diseases.

MATERIALS AND METHODS

Sample Collection and Preparations

1. ANIMALS

About 80 male-Dawley rats about 4 months old and weighing 185±25g obtained from the laboratory animal house, Animal and Environmental Biology and Biochemistry departments, University of Port Harcourt were studied. They were kept under laboratory conditions in cages before and during the experiment and fed with pellet feeds (Livestock Feeds, Nig. Ltd, Ikeja, Lagos), and water ad libitum.

2. A 21-DAY TOXICITY STUDY:

48 rats divided into four (4) groups (2,3, 4 and 5) of 12 rats each were treated intraperitoneally with carbon tetrachloride (mixed with mineral oil 3:1) at doses of 0.33ml, 0.66ml 0.99ml and 1.32ml respectively. This was done daily for a period of 7 days. Another group (Group 1) consisting of 7 rats served as control and did not receive CCl_4. The animals were observed for fatality after each dose for the 7days. They were then kept for another 14 days for daily observation for cummulative toxicity. During this time they were fed with water and their usual food.

At the end of the 21 days, 3 rats from each group (1-5) were sacrificed by decapitation and test samples collected and prepared for analysis.

3. BLOOD COLLECTION AND PREPARATION OF SERUM:

Blood was quickly collected from the rats following decapitation by cardiac puncture into standard sample tubes. The blood was allowed to clot after 45 mins and then centrifuged at 3,000xg for 10 mins at 40^0 C to obtain the

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serum. The serum was centrifuged at 3, 000Xg for 10 mins and the supernatant obtained was stored for analysis.

4. ASSAY OF AST, ALT AND ALP ACTIVITIES:

AST, ALT and ALP activities were assayed according to the method of Deneke (1988). A pack of 30 strips was used. The foil protecting the test area was removed while care was taken not to over –bend the strip. Using the Reflotron Pipette, the sample was drawn up avoiding the formation of bubble. The sample was applied as a drop to the center of the red application zone without allowing the pipette tip to touch the zone. The flap was opened after 15 seconds and the strip placed on the guide until a click was heard. The flap was then closed. The display AST, ALT and ALP confirmed that the test-specific magnetic code has been correctly read. The enzyme activity was displayed in IU/L at 37^0C.

5. PREPARATION OF GARCINIA KOLA AQUEOUS EXTRACT

The seeds of G. Kola (Heckel) were purchased from Port Harcourt Mile 3 market, Rivers State. The testa was peeled off and the white seeds chopped into tiny pieces. They were air-dried and homogenized with an electric blender. The homogenized form was soaked in distilled water for 24 hours. It was first filtered through a white cloth, then with Whatman N0. 1 filter paper. Preparations were carried out twice a week at 100g/250ml distilled water. The concentration was calculated on a weight/ volume ratio.

6. IN VIVO EFFECT OF AQUEOUS EXTRACT OF GARCINIA KOLA ON SERUM AST, ALT AND ALP ACTIVITIES

Activity was determined on the 9 rats, left in each of the groups (1-4) treated with CCl_4. (0.33,0.66,0.99 AND 1.32ml respectively). 50ml each of 20g/50ml, 40g/100ml and 60/100ml of the aqueous extract of G. kola were administered orally to each of the groups for a period of 7, 14 and 21 days. Approximately 7.1ml of the extract was dropped into the mount of the rats per day using syringe to avoid spillage. At the end of 7 days, 3 rats from each group were sacrificed by decapitation; blood sample was collected and treated as already described. Serum, AST, ALT and ALP activities were also determined as already described. The mean AST, ALT and ALP activities were statistically compared with mean AST ALT and ALP activities of these same groups poisoned and treated with the extract.

RESULTS

The results for the 21-day toxicity study is as shown in table 1. Marked increases were observed in the mean activities of the enzymes, AST, ALT, and ALP in the serum of rats administered different doses of CCl_4. The increase in activities were significantly higher in rats treated with CCl_4 ($P \leq 0.001$). The enzyme activities also increased as the CCl_4 dose increased in the order 1.32ml > 0.99m > 0.66ml > 0.33ml. The increase in enzyme activity was also time- dependent.

Table 1: Effect of different doses of carbon tetrachloride (CCl_4) on AST, ALT, and ALP activities (IU/L) in albino treated rats after 7 days.

CCl4 Treatment Groups		Enzyme Activities (IU/L)		
		AST	ALT	ALP
1.	Control	59.52±0.30	54.00±0.15	54.90±0.20
2.	0.33ml	107.67±0.87	98.37±0.21	65.20±0.09
3.	0.66ml	154.10±0.08	109.89±0.19	85.34±0.53
4.	99ml	186.12±0.83	129.40±0.37	95.90±2.05
5.	1.32ml	255.02±0.62	185.51±3.24	177.15±1.24

Values are means (X) ±SD and
n = number of rats =3

Hignest activity was observed as 21 days for the three enzymes. The exposure of the male albino rats to 0.33ml, 0.66ml, 0.99ml and 1.32ml CCl_4 by daily intraperitoneal administration caused no death. The only observed effect was a marked general weakness within the first 7 days. Body weights were not significantly affected.

The results of the *in vivo* effect of aqueous extract of G. koka on AST, ALT, and ALP activities of rats treated with different concentrations CCl_4 are presented in table 2. The mean values of the activities of AST, ALT, and ALP were compared statistically with mean values during the 21 day toxicity study at the different concentration of CCl_4 (table 2). There was an observed slight decrease in the activities of the enzymes, AST, ALT, and ALP in rats that were given 50ml of the aqueous extract orally for 7 days. The only significant difference was seen in the ALP activity ($P \leq -0.001$). All the rats treated orally with different concentration of the extract after 14 days and 21 days evidently exhibited a decrease in the activities of AST, ALT, and ALP.

DISCUSSION AND CONCLUSION

A 21-day toxicity test was carried out in the male albino rats to investigate cummulative toxicity by CCL_4. The effect of aqueous extract of G. Kola on some hepatic enzymes was studied. Since CCL_4 is a model hepatotoxin in rats (Sipes et al 1977, Della Porta et al 1961, Edwards et al 1942) the assay of hepatic microsomal enzymes was important.

Marked increases in the activities of the enzymes AST, ALT, and ALP in serum of rats administered CCl_4 were observed (Table 1). The observed alterations or increase are attributable to CCl_4. Similar elevated levels of serum liver enzymes were recorded by Perez et al (1987) and ATSDR (1989). Smyth et al (1936) and Barnes and Jones (1967) also reported increased hepatic enzyme activities, indicative of liver damage. Repeated or prolonged exposure to 1000 to 1500ppm of CCl_4 inhalation was also observed to have resulted in liver cirrhosis and kidney injury (Torkelson and Rowe, 1978, Sax and Lewis, 1989). They also observed that individuals who recovered from the acute symptoms might suffer form liver and kidney damage. In addition, previous investigators have shown that symptoms increase in severity upon longer exposure (Rondabush et al 1989). This agrees with our findings that the effect of CCl_4 was dose –dependent. Bruckner et al (1986) also reported that liver cirrhosis was observed with the high dose when 1, 10 or 33mg CCl_4 were given to Sprague-Dawley rats in corn oil. Since CCl_4 is insoluble in water, and volatile, the use of mineral oil provided an effective and innocuous vehicle for CCl_4. Previous work by Bruckner at al (1986), Condie et al (1986) showed that corn oil used as solvent/vehicle increased the toxicity of CCl_4.

A slight decrease was observed in the activities of AST, ALT and ALP in rats administered aqueous extract of G.

Table 2: In vivo effect of aqueous extracts of G. kola in carbon tetrachloride (CCL_4) Treated rats after 7, 14 and 21 days administration

CCL4 Treated Sample Groups	Duration in days	Enzyme activities (IU/L)		
2	0	107.67±0.87	98.37±0.21	65.20±0.09
0.33ml	7	105.07±1.49	93.02±2.16	56.50±0.46
	14	101.00±1.16	89.10±2.03	51.00±0.73
	21	99.97±0.05	72.02±2.09	50.60±2.35
3	0	154.10±0.08	109.87±0.19	85.34±0.53
0.66ml	7	142.51±0.33	89.15±0.44	59.09±2.28
	14	142.00±1.30	82.41±2.34	58.05±1.72
	21	129.00±2.15	71.00±1.04	56.40±1.59
4	0	186.12±0.83	129.40±0.37	95.90±2.05
99ml	7	184.12±4.69	115.61±0.66	86.04±2.21
	14	169.11±1.28	100.43±0.44	79.00±0.63
	21	132.21±1.50	88.90±0.62	68.05±1.36
5	0	255.02±0.62	185.51±3.24	177.15±1.24
1.32ml	7	243.00±1.04	153.00±0.80	143.80±1.61
	14	229.11±1.04	123.58±1.59	83.12±1.61
	21	99.97±0.05	72.02±2.09	50.60±2.35

Values are means (X) ±SD
n=3

kola after exposure to CCl_4. The only significant difference was observed in ALP activity ($P \geq 0.001$) after 7 days of administration. All the rats given different doses of the extract for 14 and 21 days exhibited a marked decrease in the AST, ALT, and ALP activities. The reduction in the enzyme activities agrees with the work of Braide (1991) who noted that Kolaviron, a bifflavonoid fraction of extracts of G. Kola when administered intraperitoneally to rats at 500mg/kg caused a significant decrease in liver function induced by treatment with CCl_4 1 hr. after.

The in vivo studies, following intraperitoneal administration of CCl_4 showed that the animals exhibited signs of hepatoxicity. The oral administration of aqueous extract of G. kola caused a reduction in the elevated levels of the hepatic enzymes, AST, ALT and ALP. This result could suggest an involvement of the G. kola extract in the reduction of AST ALT, and ALP activities. The findings of this work therefore suggests that the extract could be reversing the liver cell damage induced by treatment with CCl_4. Thus these findings may be of biochemical significance in the treatment of liver disease.

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