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The Effects of Carotenoid on Liver Enzymes of Carbon Tetrachloride Induced Hepatotoxicity in Adult Wistar Rats.

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ABSTRACT: *The effects of carotenoid on liver enzymes of carbon tetrachloride induced hepatotoxicity in adult wistar rats were studied. Twenty four wistar rats weighing between 100-200g were used. They were divided into four groups A, B, C and D of six animals each. Group A served as the control and received 0.35ml of distilled water. Group B received 0.41ml of carotenoid; group C received 0.41ml of carbon tetrachloride and group D received 0.41ml of carotenoid + 0.41ml of carbon tetrachloride. The drugs were administered orally using intubation method for a period of twenty one days. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapour and dissected. Liver tissues were removed and weighed. Blood for serum preparation was collected through cardiac puncture. Serum samples were separated from clot by centrifugation at 3000rpm for five minutes using bench top centrifuge. The activities of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were determined using randox kit method. The relative liver weight for group C were significantly higher ($p<0.001$) than the control. The relative liver weight of group B and D increased significantly relative to the control. The group C activity levels were significantly higher ($p<0.001$) than the control, while the group B and D activity levels increased significantly relative to the control. The result of this study shows that no adverse biochemical changes are associated with the use of carotenoid in carbon tetrachloride toxicity.*

KEYWORDS: Carotenoid, Carbon tetrachloride, Liver enzymes, Liver Weight, Wistar rat

I. INTRODUCTION

The liver plays a central role in the metabolism of many drugs and induced hepatic injury is now one of the commonest forms of iatrogenic disease. Indeed, in any patient presenting with obscure liver diseases or unexplained jaundice, the possibility of a drug induced lesion should always be considered. Many of the pathological features such as hepatocellular injury and necrosis, hepatitis both acute and chronic and jaundice by various mechanisms can be reproduced by drugs [11].

Carotenoids are used as antioxidants which help to protect against free radicals that attack molecules of cells. They are fat soluble compounds formally called lipochromes. Their significance is attributed to their well documented antioxidant properties. Their antioxidant effect enables this compounds to play crucial role in protecting organism against damage during photosynthesis [18]. Carotenoids generally cannot be manufactured by species in the animal kingdom so animals obtain carotenoids in their diets.people consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses [10]

In this research work, carbon tetrachloride will be used to induce liver injury in rats. Carbon tetrachloride is a manufactured chemical that does not occur naturally. It is used in the production of refrigeration fluid, propellant for aerosol cans, pesticides, as a clearing fluid, degreasing agent and in fire extinguishers. Exposure of this result mostly from breathing air, drinking water coming in contact with soil that is contaminated with CCl_4. CCl_4 has caused cancer in animals exposed to it as a result of exposure to a very high amount [5, 17, 18]. Epidemiological studies have shown that people with high beta-carotene intake and high plasma level of beta-carotene have a significantly reduced risk of lung cancer. Lycopene and beta-carotene taken along with Vit C and D helps to protect the body against the effect of chemotheraphy and radiation [19].

In toxicity studies, the majority of the aspartate aminotransferase and alanine aminotransferase enzymes measured as indices of drug metabolism are released into the blood stream when cells are damaged or their functions are disrupted. Cell membrane intergrity are accessed by its ability to prevent enzyme leakage is dependent on intrascewllular energy. The cell membrane is therefore impermeable to enzymes as long as the cells are metabolizing normally [15, 20].

CCl_4 has been reported to increase the level of serum aspartate aminotransferase and alanine aminotransferase activities [1]. This increase was significantly ameliorated by anti-oxidants hence the modulation

of the severity of the hepatic damage [4, 21]. This study aims at investigating the effects of carotenoid on liver enzymes of carbon tetrachloride induced hepatotoxicity in adult wistar rats.

II. MATERIALS AND METHOD

2.1 Breeding Of Animals

Twenty four Wistar rats were purchased from the animal house of the Anatomy Department, University of Calabar, Cross River State, Nigeria. They were bred in the experimental Animal house of University of Uyo Akwa Ibom State. They were allowed for a period of five days for acclimatization under normal temperature (27^0C -30^0C) before their weights were taken. They were fed ad libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd. Perspex cages were used to house groups of six (6) animals for routine experiment.

2.2 Drug Preparation

The drugs used for this research work include the following as stated below:

Commercial carotenoid was obtained from Golden Neo-life Diamite (GNLD) Int, Spartan by pharmaceutical contractors Isando Road, Isando, South Africa and purchased from No. 6 Itu Road, Uyo retails outlet, Akwa Ibom State, Nigeria. One Capsule of caroteniod containing 900mg was dissolved in 10mls of distilled water and administered to the animals.

Carbon tetrachloride was obtained from the Department of Biochemistry, University of Calabar, Cross Rivers State, Nigeria.

2.3 Experimental Protocols

The twenty four animals were weighed and allocated into six groups of four animals each. The groups were designated as groups A, B, C, and D. Group A animals served as the control and received 0.35ml of distilled water. The experimental groups B, C and D received different doses of drugs as follows: Group B received 0.41ml of carotenoid. Group C received 0.41ml of CCl_4, Group D received 0.41ml of carbon tetrachloride (CCl_4) and 0.41ml of carotenoid. The drugs were administered once in a day between the hours of 12-3.30pm for a period of twenty one days. The drugs were administered orally using intubation method. After the twenty first day, the animals were weighed and their weight recorded. Twenty four hours after the last administration, the animals were anaestathized under chloroform vapour and were dissected. Blood samples were collected by cardiac puncture using sterile syringes with needles. Blood for serum preparation was collected into sterile plain tubes without an anti-coagulant. Serum samples were separated from the clot by centrifugation at 3,000 rev/m for 5minutes using bench top centrifuge (MSE, Minor, England). Serum samples were separated into sterile plain tubes and were stored in the refrigerator for analysis. All analysis on blood serum samples completed within 24hours of sample collection. Liver tissues were removed from the animals and weighed. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phospotase (ALP) were determined using randox kit method.

III. RESULTS

3.1 Morphometric Analysis Of Body Weights

The result obtained from calculation of initial, final and weight changes of the various groups are presented in table 1.0.

The final body weight for group A (Control), groups B, C, and D showed a statistically significant decrease ($P<0.001$).

The final body weight for group C treated with carbon tetrachloride was significantly higher ($P<0.001$) than the control and other experimental groups (B, C and D) animals. The weight change for group C showed a statistically increase compared with the control and other experimental groups ($P<0.001$).

Table 1.0: Comparison of mean initial and final body weight and weight change in all the groups (A, B, C, and D)

Mean ± SEM given for each measurement)

	GP. A	GP. B	GP. C	GP. D	F-RATIO	PROB.OF SIG.
INITIAL BODY WT.	104.50±4.79	111.75±4.64	164.75±7.63	129.50±8.96	68.230	<0.0001
FINAL BODY WT.	112.50±6.60	121.50±10.66	133.25±8.53	137.75±10.01	30.510	<0.0001
WT. CHANGE	8.00±7.70	9.75±6.50	31.50±15.08	8.25±5.67	16.150	<0.0001

The weight of animals in group C were significantly higher (P<0.001) than group A (Control) and groups B and D before administration. After the administration, the weight of animals in group A (control) and group B and D increased statistically while the group C animals showed a significant decrease (P<0.001) compared to the weight before administration

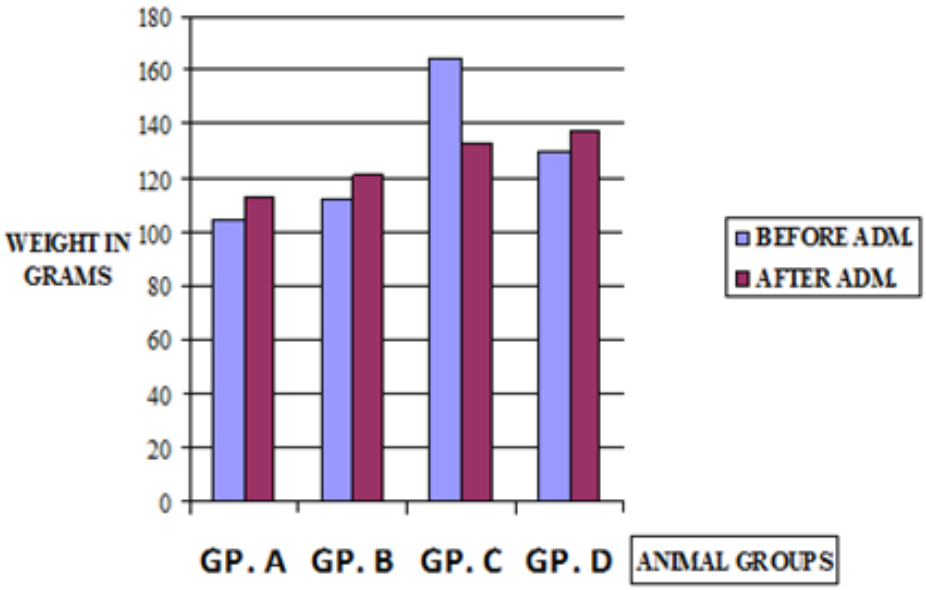

Fig 1.0 Weight of Animal before and after drug administration

3.2 Morphometric Analysis Of Liver Weights

The results obtained from calculations of relative liver weight of the various groups are presented in table 4.3. The relative liver weight for group C (carbon tetrachloride administered) were significantly higher (P<0.001) than that of the group A (control) and other experimental groups (B, and D). The values for groups B and D were similar to the group A (control)

Table 2.0: comparison of mean relative liver weight for group A (control) and experimental groups (B, C and D) (Mean ± SEM given for each measurement)

	GP. A	GP. B	GP. C	GP. D	F-RATIO	PROB. OF SIG.
LIVER WT.	4.79±0.045	4.66±0.161	7.33±0.625	4.72±0.070	53.84	<0.0001

The group C (carbon tetrachloride administered) were significantly higher (P<0.001) than the control group (A) and groups B and D. as shown in Figure 2.0 below

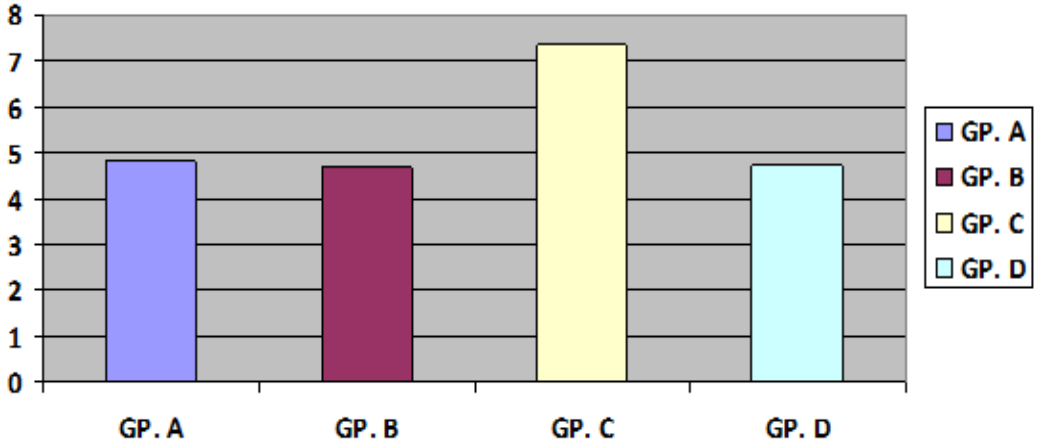

Fig 2.0 Relative Liver Wt. Of Animal Group

3.3 Activities Of Serum Levels Of Aspartate Aminotransferase (Ast), Alanine Aminotransferase (Alt) And Alkaline Phosphotase (Alp)

From the results obtained from calculations of aspartate aminotrasferase (AST), alanine aminptransferase (ALT) and alkaline phosphotase (ALP), there were a significant decrease (P<0.001) in the AST activity levels at all does of the drugs relative to the control (A) except in group C treated with carbon tetrachloride (CCL_4). The group C activity level statistically were significantly higher (P<0.001) than the control (A) and groups B and D.

The alanine aminitrasferase (ALT) activity levels showed a significant decrease (P<0.001) in groups B and D relative to the control (A) except in group C treated with carbon tetrachloride (CCL_4). The alkaline phosphotase (ALP) level in group C were significantly higher than the control group (A) and groups B and D.
The alkaline phosphotase (ALP) activity levels in groups B and D were significantly lower (P<0.001) than the control (A). The alkaline phosphotase activity levels in group C were significantly higher (P<0.001) than the control (A) and groups B and D

TABLE 3.0 : A table showing the activities of serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphotase (ALP)
(Mean ±SEM given for each measurement)

	GP.A	GP.B	GP. C	GP.D	F-RATIO	PROB.OF SIG.
ASPARTATE AMINOTRANSE-FERASE (AST)	78.80±28.55	69.75±13.51	246.66±5.77	68.66±5.77	60.04	<0.0001
ALANINE AMINOTRANSE FERASE (ALT)	50.00±15.74	46.50±11.12	95.33±9.01	41.33±8.08	12.30	<0.0001
ALKALINE PHOSPHOTASE (ALP)	481.39±31.22	397.90±37.83	673±46.75	436.20±161.29	7.58	< 0.0012

IV. DISCUSSION

Carbon tetrachloride has toxicological effect on the liver, kidney and other visceral organs. Studies on the toxic effects of this chemical on the liver have been reported [2]. These reports have all presented carbon tetrachloride as a hepatotoxin.

The results of this study agree with previous researchers that carbon tetrachloride has toxicological effect on the liver enzymes of wistar rats (Rattus norvegicus). These results tend to agree with Ossowka et al, 1996[16] that the effects of carbon tetrachloride toxicity are not easily reversible. It seems variance with Akpanabiatu et al, 2004[3] who postulated that the effects of carbon tetrachloride toxicity on the liver were not long lasting and it was reversible.

It was observed during the studies that generally, the group in which the rats were treated with carotenoid + carbon tetrachloride was able to tolerate carbon tetrachloride in their system much longer. Most of them did not show any sign of enzyme differences in their liver at all. This is similar to the reports by Dinis Oliveira et al, 2006 and Hawazen and Al-Rawi, 2007[9, 12] that substances with anti-oxidant properties such as carotenoid would protect to a large extent against the effects of carbon tetrachloride toxicity.

There were no significant difference (P<0.001) in the serum and tissues levels of AST, ALT and ALP in groups B and D compared with the control as shown in table 3.0. There were significant difference (P<0.001) in the serum and tissues levels of AST, ALT and ALP in group C. compared with the control and groups B and D. These results indicated that carotenoid did not bring about decrease in liver enzyme activity in the liver during the experimental period. Enzyme activities in the serum and tissues are often used as "maker" to ascertain toxic effects of administered foreign compounds to experimental animals [7]. ALP is a membrane bound enzyme [22] while ALT and AST are cytosolic enzymes [6]. These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky and even completely ruptured[8,14]. A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver cells [13].

Observation of the body weight difference in groups reveals gradual increase in weight of animals for the control group A. This could have been physiological as the only substance they were exposed to was water and food. Comparing the results of weight difference reveals severe loss of weight by the carbon tetrachloride exposed group. This is probably as a result of loss of appetite by the animals in the group. The groups that were

treated with carotenoid only and carotenoid + carbon tetrachloride showed increase in weight which is similar to the control group. Carotenoid in this instance functions primarily as a dietary supplement enhancing growth.

The relative organ weights also showed significant differences in groups. There was relative increase in liver weight for the carbon tetrachloride exposed animals compared to the control and carotenoid treated animals. This organ weight increase was irrespective of the fact that there was total body weight loss. This could have been pathological and one may deduce that the increase in liver weight was not growth but inflammation. Antioxidant properties of carotenoid could have been responsible for the control or prevention of inflammation in the groups treated with them.

The animals in group F gives a particularly interesting observation about the dynamics of reactions to the presence of various substances in our systems. On administration of carotenoid respectively to the groups, the animals showed increase in overall body weight similar to that of the control. Administration of carotenoid alone did not cause weight loss to the animals compared with the animals in control group. By this observation one may deduce that administration of carotenoid may boost the tolerance capacity for carbon tetrachloride induced toxicity. Thus, the protective effect carotenoid against carbon tetrachloride induced liver damage recorded in the present study is attributed to their antioxidant properties.

V. CONCLUSION

Carotenoid did not induce adverse alterations in biochemical parameters of serum aspartate aminitransferase (AST), serum alanine aminotrasferase (ALT) and Alkaline phosphotase (ALP) and no histopathological lessons was observed in the liver tissues of the rats. This study has demonstrated the potential ability of carotenoid to protect against carbon tetrachloride induced toxicity in the liver of rats. Rat's tissues are very similar in many aspects to those of human. The findings of this study suggests that carotenoid administered to individuals exposed to carbon tetrachloride poisoning could provide some protection against carbon tetrachloride toxicity and perhaps ameliorate the effects of carbon tetrachloride toxicity on the liver.

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