Effect of Vitamin C against Calcium Carbide Induced Hepatotoxicity

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ABSTRACT

Calcium carbide (CaC_2), a chemical often used by local fruit vendors in Nigeria to stimulate artificial ripening may be harmful to tissues and organs when consumed by man. The aim of the study is to investigate the effect of consumption of fruit ripened with Calcium carbide and ameliorating effect of Vitamin C on the biochemical alterations caused by this chemical in the liver enzymes. Thirty albino rats were divided into three groups. Group A was fed with normal rat chow, group B and C were fed with normal rat chow and plantain chips ripened with Calcium carbide. Animals in group C were also treated with 200mg/kg/day of Vitamin C. They were sacrificed after eight weeks and blood collected for liver enzyme assayed using Reitman and Frankel method. Results obtained showed a statistically significant reduction in weight in experimental group when compared with the control ($P \leq 0.05$). Liver injury was assessed from the activities of liver function diagnostic indices including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities. The result of the study showed that fruits ripened with calcium carbide caused a significant ($P \leq 0.05$) increase in serum ALT (47.20 ± 11.54), AST (48.40 ± 11.17) and ALP (210.80 ± 28.23) activities; an indication of hepatic injury, in comparison with the control group. The indicators of hepatic injury associated with consumption of fruits ripened with calcium carbide were reverted with vitamin C administration only in AST (29.00 ± 2.65) and ALP (148.60 ± 37.16), showing some protective effect of vitamin C against calcium carbide-induced liver injury in rats. The results of this study suggest that there is protective role of vitamin C against calcium carbide-induced liver injury.

Original Research Article

Received: 18th Sept., 2017.

Accepted: 14th Oct., 2017.

Published: 23rd Oct., 2017.

Keywords:

Calcium carbide;
Vitamin C;
Liver enzymes;
Plantain chips.

INTRODUCTION

Fruits play a vital role in human nutrition by supplying the necessary growth regulating factors essential for maintaining normal health (Hayes, 2005; Rossato *et al.*, 2009). They are widely distributed in nature, commercially important and nutritionally essential food commodity. Being a part of a balanced diet, they are one of the best natural food consumed raw. Apart from the consumable part of the fruits, it is important to note that the by-products, such as the fruit peels, could represent precious layers for food, medicinal or cosmetic purposes. The rising demand of fruit safety has inspired researchers about the risk related to the use of fruit contaminated by pesticides, heavy metals or toxins (Dudley,

2004; Ruchitha, 2008). In recent years, there has been considerable research concerning the action of different chemicals on the ripening processes of fruits (Kader, 2002). Calcium carbide is commonly used to induce ripening fruits artificially in many countries because it is cheap and readily available (Block *et al.*, 1992).

Farmers use these processes to harvest their fruits on time before maturation to prevent early damage of ripe fruits. Calcium carbide is known to cause cancer, food poisoning, gastric irritation and mouth ulcers, cerebral oedema and seizures (Per *et al.*, 2007). Secondly, consumption of fruits ripened with calcium carbide can cause alterations in haematological and biochemical parameters (Igbinaduwa and

Aikpitanyi-Iduitua, 2016). Fruit vendors especially in some communities may use carbide gas from CaC_2 to ripen fruits and this could cause serious hazard.

Vitamin C is found in citrus, soft fruits and leafy green vegetables. Kidney and liver are good animal-derived sources of vitamin C (Stangland, *et al.*, 2009). Synthetic vitamin C can be administered orally or intravenously (Padayatty *et al.*, 2004). It is well absorbed efficiently in the small intestine via a saturable active transport mechanism. Reported researches showed that vitamin C has hepatoprotective property; this is linked to its antioxidative property. Vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals (Bashandy and Alwasel, 2011). They reported that vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood hydrogen peroxide and malondialdehyde in liver of carbon tetrachloride intoxicated rats. The ability of vitamin C to prevent Carbon tetrachloride induced hepatotoxicity in rats was also reported by some authors (Ademuyiwa *et al.*, 1994; Kataoka *et al.*, 2012).

In view of the above problems, this study therefore aims to evaluate the protective role of vitamin C against calcium carbide induced hepatotoxicity.

MATERIALS AND METHOD

Acquisition and Acclimatization of Animals

Thirty (30) male albino rats (wistar strain) weighing between 160g to 200g were obtained from the animal house of the Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State. The animals were kept in clean plastic cages under a 12/12hours light/dark cycle and housed in the animal house of the same department. The rats were allowed to acclimatize to the laboratory environment for a period of two weeks before commencement of the experiment. Standard feed and water were provided ad-libitum. All experimental animals were handled in accordance with the US National Institutes of Health Guidelines for the care and use of laboratory animals.

Mature unripe plantain, were purchased from Uli market, Ihiala local government area of Anambra State. Calcium carbide was purchased from main market Onitsha. The fruits were exposed to calcium carbide. The method of exposure was as described by Igbinauwa and Aikpitanyi-Iduitua, (2016). This was done by applying the calcium carbide powder (2g/kg weight of fruit) on the surface of the plantain. It was then placed in a polythene sack and the sack was tied up and covered in a plastic container. After a period of thirty six hours, the plantain was completely ripe. The plantain was carefully peeled and oven dried at 105°C for three hours. After Oven drying, the dried samples were ground into chips and stored in a laboratory cupboard

EXPERIMENTAL DESIGN

After the period of acclimatization, the animals were randomly selected and divided into three groups (A, B, C) with each group comprising ten (10) wistar rats each.

Group A were fed with standard rat chow and water. This is the control group.

Group B were fed with 20g of the ground plantain chips in standard rat chow and water

Group C were fed with 20g of the ground plantain chips in standard rat chow, 200mg/kg of vitamin C and water.

The rats were kept at a room temperature throughout the duration of the study. The administration of feed, ground plantain chips and vitamin C was done daily for period of six weeks after acclimatization. At the end of six weeks, the albino rats were sacrificed after being anaesthetized using chloroform. 5mls of blood was collected by cardiac puncture and transferred into lithium heparinized bottle. The blood samples were spun at 4,000 rpm for 5 minutes for proper separation. The plasma obtained was stored in plain blood containers for biochemical analysis

The plasma were labelled accordingly, stored frozen until needed for the estimation of enzyme activities. The enzyme activities were estimated using the method of Reitman and Frankel (1957). All reagents used were of analytical grade from Randox. The analyses were done using spectrophotometer (Jenway 6305).

Statistical Analysis

Results were presented as mean $\pm$ standard error of the mean (SEM). Statistical Package for Social Sciences (SPSS) software version 16.01 was used for the statistical analysis and data were analyzed with one-way analysis of variance (ANOVA). $P \leq 0.05$ was considered statistically significant.

RESULTS

Table 1: Changes in body weight (g) of Rats during and after Administration.

Weight of Rats	Group A	Group B	Group C
Weight before administration (g)	172.00 $\pm$ 1.94	175 $\pm$ 1.85	170 $\pm$ 1.32
Weight after administration (g)	186.00 $\pm$ 2.85	150 $\pm$ 1.11	168 $\pm$ 1.43
Change in weight (g)	14.00 $\pm$ 1.34	25.00 $\pm$ 0.25	2.00 $\pm$ 0.54
P value	0.06	0.002	0.12

Table 1 showed that there is no statistically significant difference in weight of control (group A) after 4 weeks ($p > 0.05$), while there is statistically significant reduction in weight in experimental group B ($P < 0.05$) after 4 weeks. However, there is no statistical difference in weight of experimental group C which also received oral vitamin C.

Table 2: Activity levels of liver enzymes for various groups of rats fed and treated

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)
Control group (A)	89.80±25.12	15.00±2.24	16.20±3.35
Experimental group (B)	210.80±28.23	47.20±11.54	48.40±11.17
Experimental group (C)	148.60±37.16	29.00±4.47	29.00±2.65
p-value	0.001*	0.000*	0.000*
A vs B (p-value)	0.000*	0.002*	0.000*
A vs C (p-value)	0.061 [#]	0.030 ^s	0.057 [#]
B vs C (p-value)	0.022 ^a	0.006 ^a	0.002 ^a

When the liver enzymes in control group (A) were compared to experimental group (B), there was a statistically significant difference in the levels of the enzymes (indicated *), but when compared with Vitamin C treated group C, changes in ALP and AST were not statistically significant (indicated #), but ALT was significant (indicated ^s). When the two exposed group were compared, there was statistical difference in the levels of the enzymes indicated (^a).

Figure 1: Bar chart showing the levels of ALT and AST in the control group, experimental group and vitamin C treated group.

Figure 2: Bar chart showing the levels of ALP in the control group, experimental group and vitamin C treated group.

DISCUSSION

Fruits are among the sources of numerous vitamins and minerals and hence play important role in human nutrition; and therefore have been recommended for healthy growth. For decades, there has been increasing demand for fruits in our communities. Fruit vendors, in order to meet up with the growing demand; ripen fruits in large quantities by using chemicals such as calcium carbide (Dutta and Dhua, 2004). In this study, the need to investigate the changes that may arise in some liver enzymes following the consumption of calcium carbide -ripened fruits and the protective role of vitamin C came under focus. Since most of the fruits commonly sold in the market may be artificially ripened with calcium carbide, it may lead to deleterious effect in vital organs like kidney and liver (Igbinuwa and Aikpitanyi-Iduita, 2016; Kjuss *et al.*, 2007). In this study, the result of liver enzymes indicated that there were statistically significant increase in the levels of ALT, AST and ALP in the exposed group (B) when compared with the control (group A) (Table 2). However, when the levels of liver enzymes in the two exposed groups (group B and C – vitamin C treated group) were compared, there was a statistical reduction in the Vitamin C treated group. The raised liver enzymes in the exposed groups might be an indication that there may be a disturbance in the activity of the liver even though there was a statistically significant reduction in the vitamin C treated group. This study agrees with the findings that vitamin C normalizes levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, in liver of carbon tetrachloride induced toxicity in rats (Bashandy and Alwasel, 2011). AST and ALT are liver enzymes concerned with amino acid metabolism. They are usually performed to investigate liver disease. Increases in plasma levels of AST and ALT serve as reliable indices of assessment of damage to the parenchyma cells of liver and heart (Edoardo *et al.*, 2003).

CONCLUSION

The observed significant increase in the activities of these enzymes are pointers to calcium carbide-induced toxicity in liver tissues while administration of vitamin C has shown to possess a hepato - protective role against calcium carbide induced hepato-toxicity.

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How to cite this article

Olisah, M.C., Ilechukwu, O.U., Ifemeje, J.C., Ofor, C.C. (2017). Effect of Vitamin C against Calcium Carbide Induced Hepatotoxicity. *Tropical Journal of Applied Natural Sciences*, 2(1): 74-77.
Doi: <https://doi.org/10.25240/TJANS.2017.2.1.12>.

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