The Journal of Anatomical Sciences
Email: anatomicaljournal@gmail.com

J. Anat Sci (14) 1

Calcium Carbide Induced Alteration of somebiochemical parameters in the liver of Wistar rat.

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ABSTRACT

Thies changes in some biochemical parameters of maternal and foetal liver induced by consumption of calcium carbide ripened banana in Wistar rats. Unripe bananas were subdivided into four groups; with three artificially ripened using calcium carbide andone ripened via non artificial means. Experimental design consisted of two groups (before and during pregnancy; during pregnancy only). The rats were randomly assigned to a control group which received only food and water and a test group which were fed calcium carbide fora period of 3 weeks before pregnancy. Upon pregnancy, exposure to calcium carbide was continued until day 19. The animals from each group were then sacrificed and blood was collected by cardiac puncture for biochemical analysis. Results obtained showed significant ($p<0.05$) increase in values of AST when groups were compared to positive control group during and before pregnancy. A decrease in ALT levels were observed in all the groups when compared with negative group with no significant difference. ABL, Tbil and Dbil levels showes no significant difference when compared to control group. Consumption of calcium carbide is toxic to the liver of female wistar rats and may be deleterious to human health following long- term exposure.

Keywords: Biochemical, liver enzyme, calcium carbide, banana, wistar rat

INTRODUCTION

Natural fruits make up a complete balanced which plays a major role in maintaining human health and balancing essential hormones during pregnancy (Prasanna, Prabhta and Tharanathan, 2007). According to Cadman (2018), eating fresh ripe fruits during pregnancy ensures both the mother and baby remains healthy. Therefore, fruits are commercially and nutritionally important as they are one of the best natural foods consumed raw.

Ripening is a natural biochemical process in which fruits characteristically become softer, sweet, flavorful and coloured. It can also be stimulated by applying artificial fruit ripening agents like calcium carbide, ethylene gas, among others. In recent times, artificial ripening has become a common practice among farmers and vendors for better consumer satisfaction, and to prevent early damage of ripe fruits (Islan, Mursulat and Khan, 2016).

Although, there are more conventional and safer methods used for artificial ripening, farmers and vendors prefer adopting cheap and unsafe methods, using harmful chemicals like calcium carbide (Siddiqui and Dhua, 2010). Calcium carbide is reported to cause harmful damages such as gastric irritation, mouth ulcers, headache, dizziness, cerebral edema (Per et al., 2007).

It produces free radicals upon ingestion in the human body, which are detrimental to various organs like heart, liver, among others (Phaniendra et al., 2015). The body naturally possesses antioxidants such as carotenoids, Vitamins E and C which help fight free radicals in the body. This imbalance of free radicals and antioxidants leads to oxidative stress which can alter biochemical parameters and destroy cells in the body (Lobo et al.,2010).

Hence, through the generation of oxidative stress, complication during pregnancy such as fetal distress, miscarriage, prenatal mortality may arise (Toboła-Wróbel et al., 2020). Fruits are a good source of antioxidants, but artificial ripening makes the healthy fruit poisonous. This study, is therefore, designed to compare the effect of consumption of calcium carbide ripened banana with naturally ripened banana on the biochemical parameters of maternal and foetal liver in wistar rats.

Materials and Methods

Unripe matured bananas was purchased from Margaret Umahi International Market Abakaliki, Ebonyi State. The fruits were exposed to 500g of calcium carbide using the method of Reena et al., (2018).

Banana treatment with calcium carbide

The fruits were divided into four groups labeled S1, S2, S3, and S4 respectively. Each fruit was subjected to three levels of calcium carbide treatments as follows: 2g, 4g and 6g calcium carbide per kg of fruit to induce ripening; while S1 is the controls (without calcium carbide). Calcium carbide was crushed into small pieces and weighed using analytical weighing balance PW 184 Adam model. The reported method (Rao Sudhakar, 2012), was used in this study to quicken the ripening process of the banana fruits as described.

In this method the fruits were kept in small perforated plastic container and exposed to acetylene gas released from calcium carbide. After 24 hours of exposure, the fruits were left to complete the ripening process at room temperature. Room temperature and humidity readings were recorded daily. Fruit ripening was monitored. Sample S1 (5kg) of the banana was soaked in normal water to form the control group. Sample S2 (5kg) of the unripe banana was dipped in 1% CaC_2 solution (50g/5litre).Sample S3 (5kg) of the unripe banana was dipped in 2% CaC_2 solution (100g/5litre).A market sample treatment, S4 5kg ripened banana fruit was procured from a local market Margret

Umahi International market Abakaliki, Ebonyi State.

Experimental animals

36 matured healthy Nulliparous females adult wistar rats weighing between 150-200g were purchased from animal house, department of anatomy, Faculty of Medicine, Ebonyi State University, Abakaliki. The rats were housed in special clear sided cages with a 12-12hour light: dark cycles and was allowed free access to drinking water and standard rat pellet feed in accordance with the US National Institutes of Health Guidelines for the care and use of laboratory animals.

All findings on animal experimentation were in accordance with international acceptable guideline for laboratory animal use and care by National Institute of Health, 1985; publication No. 8523.

Experimental design

Group 1 consisted of four (4) sub groups with 4 nulliparous albino wistar rats. Treatment lasted for three weeks after which males were introduced into the cages for mating to occur. After successful mating and confirmation of pregnancy, the male albino wistar rats were removed and treatment continues till the 19th day of pregnancy, then it was sacrificed.

Group 2: This group consists of four (4) subgroups also. There was no treatment prior to pregnancy. Treatment commenced immediately pregnancy wasconfirmed. They were sacrificed also on day19 of pregnancy.

The animals were fed with palletized mash and the pulp from the previously ripened banana fruits by CaC2 kept in the refrigerator. The blended bananas were mixed with the rat feed according to the method by Igbinaduwa (2016). At pregnancy day 19, the mother was sacrificed; blood sample was collected first through ocular puncture. Then the maternal and fetal liver was harvested and both maternal and fetal weight noted too. Blood sample was collected in a plain bottle which was allowed to clot before its spinning in other to separate the serum from blood cells, after which the liver parameters were tested. The maternal and fetal liver was harvested and its tissue fixed for histological studies. They were passed through normal histological processes for the slide to be produced.

Biochemical Assay

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), plasma albumin (ALB), direct bilirubin (DBil), total bilirubin (TBil), were determined using assay kits from Randox laboratories, U.K, by adopting the standard procedures described by Tietz*et al.*,1994.

Statistical Analysis

Data for each experiment were expressed as the mean $\pm$ standard error of mean, for data comparison, one-way analysis of variance (ANOVA) was used. $P<0.05$ was taken to be statistically significant using SPSS version 20.

RESULTS

Liver Enzyme Assays of Female Wistar Rats Before and During Pregnancy

Groups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
Negative Control	49.00±4.00	71.00±5.00	4.25±0.15	1.05±0.25	2.20±0.10
Positive Control 1A	$26.00\pm2.00^{\#}$	$33.00\pm3.00^{*}$	$3.80\pm0.20^{\#}$	$1.10\pm0.10^{\#}$	$1.95\pm0.15^{\#}$
1B	$42.00\pm4.00^{\#b}$	$33.00\pm6.00^{*b}$	$4.00\pm0.10^{\#b}$	$0.65\pm0.05^{\#b}$	$2.00\pm0.20^{\#b}$
1C	$46.50\pm12.50^{\#b}$	$29.50\pm3.50^{*b}$	$3.80\pm0.20^{\#b}$	$0.85\pm0.15^{\#b}$	$2.45\pm0.15^{\#b}$
Market Sample (1D)	$58.50\pm9.50^{\#b}$	$48.50\pm7.50^{\#b}$	$3.90\pm0.30^{\#b}$	$0.85\pm0.15^{\#b}$	$1.85\pm0.15^{\#b}$

* and $^{\#}$ denoted significant and no significant difference respectively when compared to negativecontrol at $P<0.05$. ^{b} denoted no significant difference when compared to positive control at $P<0.05$

There was no significant difference in AST, ALB, DBil and TBil when positive control, 1B, 1C and market sample groups were compared to the negative control. Significant difference was observed in ALT when positive control, 1B, and 1C groups were compared to the negative control apart from market sample group which showed no significant difference with the negative control group. This study revealed that AST, ALT, ALB, DBil and TBil showed no significant difference when 1B, 1C and market sample groups were compared to the positive control.

Liver Enzyme Assays of Female Wistar Rats during Pregnancy

roups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
egative Control	49.00±4.00	71.00±5.00	4.25±0.15	1.05±0.25	2.20±0.10
ositive Control 2A	$39.50\pm7.50^{\#}$	$77.00\pm8.00^{\#}$	$4.80\pm0.20^{\#}$	$1.10\pm0.10^{\#}$	$1.70\pm0.50^{\#}$
B	$35.00\pm7.00^{\#b}$	$72.50\pm5.50^{\#b}$	$4.55\pm0.55^{\#b}$	$0.90\pm0.10^{\#b}$	$2.10\pm0.10^{\#b}$
C	$45.00\pm6.00^{\#b}$	$45.50\pm10.50^{\#b}$	$4.50\pm0.50^{\#b}$	$1.00\pm0.00^{\#b}$	$2.15\pm0.15^{\#b}$
Market Sample (2D)	$59.50\pm4.50^{\#b}$	$57.50\pm7.50^{\#b}$	$3.70\pm0.30^{\#b}$	$0.90\pm0.10^{\#b}$	$1.90\pm0.40^{\#b}$

$^{\#}$ denoted no significant when compared to negative control at $P<0.05$. ^{b} denoted no significantdifference when compared to positive control at $P<0.05$

There was no significant difference in AST, ALT, ALB, DBil and TBil when positive control, 1B, 1C and market sample groups were compared to the negative control. This study also revealed that AST, ALT, ALB, DBil and TBil showed no significant difference when1B, 1C and market sample groups were compared to the positive control.

Comparison of each of the Liver Enzyme Assays Between Positive Control Before and During Pregnancy With During Pregnancy Only

Groups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
Positive Control 1A	26.00±2.00	33.00±3.00	3.80±0.20	1.10±0.10	1.95±0.15
Positive Control 2A	39.50±7.50$^{\#}$	77.00±8.00*	4.80±0.20$^{\#}$	1.10±0.10$^{\#}$	1.70±0.50$^{\#}$

* and $^{\#}$ denoted significant and no significant difference respectively when compared to negative control at $P<0.05$

Apart from ALT in which significant difference was observed between positive control groups during pregnancy (group 2A) and before and during pregnancy (group 1A), this study showed (table 3) no significant difference in AST and ALB. No significant difference was also observed in DBil and TBil when both assays were compared betweenboth groups.

Comparison of each of the liver Enzyme Assays Between Rats before and During Pregnancy (1B) with During Pregnancy Only (2B)

Groups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
Group 1B	42.00±4.00	33.00±6.00	4.00±0.10	0.65±0.05	2.00±0.20
Group 2B	35.00±7.00$^{\#}$	72.50±5.50*	4.55±0.55$^{\#}$	0.90±0.10$^{\#}$	2.10±0.10$^{\#}$

* and $^{\#}$ denoted significant and no significant difference respectively when compared to negative control at P<0.05.

From the result (table 4) of this current study, ALT indicated significant difference in the comparison during pregnancy only (2B) and before and during pregnancy (1B). This study showed no significant difference in AST and ALB, DBil and TBil when these assays were compared between both groups (2B and 1B).

Comparison of each of the Liver Assays Between Rats Before and During Pregnancy (1C) with During Pregnancy Only (2C)

Groups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
Group 1C	46.50±12.50	29.50±3.50	3.80±0.20	0.85±0.15	2.45±0.15
Group 2C	45.00±6.00$^{\#}$	45.50±10.50$^{\#}$	4.50±0.50$^{\#}$	1.00±0.00$^{\#}$	2.15±0.15$^{\#}$

$^{\#}$ denoted no significant when compared to negative control at P<0.05.

This study revealed no significant difference as seen in table 5 in ALT and AST when rats in group 2C(during pregnancy only) was compared with rats in group 1C(before and during pregnancy). No significant difference in ALB, DBil and TBil was also observed when these assays were compared between both groups (1C and 2C).

Comparison of each of the Liver Enzyme Assays Between Market sample Before and During Pregnancy (1D) with During Pregnancy Only (2D)

Groups	AST (iu/L) Mean ± SEM	ALT (iu/L) Mean ± SEM	ALB (g/dL) Mean ± SEM	DBil(mg/dL) Mean ± SEM	TBil(mg/dL) Mean ± SEM
Market sample(1D)	58.50±9.50	48.50±7.50	3.90±0.30	0.85±0.15	1.85±0.15
Market sample(2D)	59.50±4.50$^{\#}$	57.50±7.50$^{\#}$	3.70±0.30$^{\#}$	0.90±0.10$^{\#}$	1.90±0.40$^{\#}$

$^{\#}$ denoted no significant when compared to negative control at P<0.05.

Results showed no significant difference in ALT and AST when rats in group 2D (during pregnancy only) was compared with rats in group 1D (before and during pregnancy). No significant difference in ALB, DBil and TBil was also observed when these assays were compared between both groups (1D and 2D).

DISCUSSION

Liver is the main target organ of toxicity because of its role in the body as several compound undergo their first-chain of metabolism there (Abarghoei et al., 2016). Therefore, it is an important organ in research when studying the effects of different chemicals such as calcium carbide deposited in the body. In this current study the need to investigate the alterations/changes that may arise in some of the selected liver enzyme parameters following the consumption of varying concentration of calcium carbide ripened banana came under focus.

Variations in AST and ALT

From the observations made in table 1 (CaC_2 ripened banana fed before and during pregnancy), AST showed a decrease when group 1A (positive control group), 1B and 1C were compared with Negative control group but there was an increase in group 1D but all showed no significant difference ($P<0.05$). Also when group 1B, 1C and 1D were compared with positive control group 1A, it showed a significant increase across the group with no significant difference observed ($P<0.05$). From table 2 (CaC2 ripened banana fed during pregnancy), observations made showed that when positive control 2A, 2B, 2C and 2D were compared with negative control group, AST decreased in group 2A, 2B and 2C and increased in 2D with no significant difference ($P<0.05$), but when group 2B, 2C and 2D were compared with positive control group 2A, there was an increase in 2C and 2D but a decrease in 2B with no significant difference observed ($P<0.05$).

The increased level of AST plasma seen in some of the groups fed with CaC2 ripened banana was mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Concepcion Navaro*et al.*, 1993). The observed significant increase in AST levels of the rats fed with varying concentration of CaC2 ripened banana is yet another pointer to the health risks associated with the use of Calcium carbide in fruit ripening which is also in agreement with previous reports (Goudra and Aradhya, 2007; Igbineduawa and Aikpitanyi-Iduitua, 2016 and Gbakon*et al.*, 2018).

From the observations made table 1 (CaC_2 ripened banana fed before and during pregnancy), ALT showed a decrease when group 1A (positive control group), 1B 1C and 1D were compared with Negative control group but 1A showed a significant difference and other groups showed no significant difference ($P<0.05$). Also when group 1B, 1C and 1D were compared with positive control group (1A), there was a slight increase seen in group 1B and 1D and a decrease in 1C with no significant difference observed ($P<0.05$). From table 2, when positive control group (2A), 2B, 2C and 2D were compared with negative control group, there was a slight increase and decrease seen in 2A and 2B; 2C and 2D respectively, with no significant difference while when 2B, 2C and 2D were compared with positive control group (2A), it observed a significant decrease with no significant difference. Also for the table and observations made, it recorded a decrease of ALT level against the control group which maybe that the liver has not been badly or completely affected, this observation made goes against the findings from Igbineduawa and Aikpitanyi-Iduitua (2016) and Gbakon*et al.*, (2018).

Plasma Albumin

Plasma albumin levels was observed in table 1 to decrease when positive control group 1A, 1B, 1C and 1D that were fed with varying concentration of CaC2 ripened banana compared with the negative control group with no significant difference but there was an increase when group 1B, 1C and 1D was compared against group 1A (Positive control group) with no significant difference as seen in table 1. In table 2, when group 2A (positive control group), 2B, 2C and 2D was compared against Negative control group, it was observed that there

was an inc rease in group 2A, 2B and 2C but a decrease in 2D with no significant difference. When 2B, 2C and 2D was compared against 2A (positive control group), there was a decrease across the groups with no significant figure.

The increased level of plasma albumin observed in group 1(B, C & D) could be adduced to dehydration or probably liver function impairment since the liver is responsible for the metabolism of protein (Cheesbrough, 2006). The hepatic cells are solely responsible for the synthesis of plasma albumin which is very vital in the regulation of the blood pressure, the binding and transportation of the cellular components such as water, cations, hormones, bilirubin, thyroxine as well as drugs (Halsted and Halsted, 1991). Inadequate breakdown of protein by the liver makes the protein inaccessible to cells hence resulting in protein malnutrition and subsequent elevated albumin level.

Variations in Total Bilirubin (TBil) and Direct Bilirubin (DBil)

Bilirubin is a bile pigment formed from the breakdown of heam in the red blood cell. A bilirubin test is used in determining the cause of jaundice, it also helps in diagnosing causes of health conditions like anemia and liver diseases and in the cause of an elevated level of bilirubin, diseases can occur or created. Hyperbilirubin may lead to accumulation of bilirubin in the brain region which leads to neurological disorder (Hossain *et al.*, 2015).

The results from table 1, Direct bilirubin showed a decrease when group 1B, 1C and 1D was compared against 1A with no significant difference. Total bilirubin showed an increase in gp1B and 1C except in gp 1D that showed a decrease with no significant difference. Table 2 showed a decrease in both Dbil and Tbil with no significant difference. This work goes against the findings of previous work done by Gbakon*et al.,* (2018).

Conclusions

Consumption of fruits ripened with calcium carbide can cause varying degrees of alterations in the biochemical liver enzyme parameters of both maternal and foetal liver in Wistar rats.This was observed in the increase in AST levels when group 1 (B and D) were compared with positive control group 1(A). . Thus, it suggests that consumption of fruits ripened with Calcium carbide can lower the body's ability to resist infection and weaken the immune system. It also means that Calcium carbide causes alteration in some vital liver functions.

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