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ASSESSMENT OF CALCIUM CARBIDE RIPENED SWEET ORANGE (*CITRUS SINENSIS* L. OSBECK) ON THE BIOCHEMICAL PARAMETERS OF THE WISTAR RATS

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ABSTRACT

The aim of this study was assessment of calcium carbide ripened sweet orange (*citrus sinensis*) on the Biochemical parameters of the Wistar rats. Twenty four (24) adult Wistar rats weighing between 126.9- 213.3 g was used for this study. The experimental Wistar rats were grouped into three and were allowed to acclimatize for two weeks at libitum. Five (5) ml/kg of natural and calcium carbide ripened Pineapple fruit juice were administered orally. At the end of the four weeks feeding period, the rats were sacrificed through cervical dislocation. Blood was collected by cardiac puncture, using 5ml syringes and 23G needles into blood sample containers for biochemical analysis using the standard biochemical methods. The biochemical parameters analyzed were ALP, AST, ALT, total bilirubin, total protein, albumin, creatinine, urea, total cholesterol and lactate dehydrogenase levels in the experimental animals and were compared with the control and natural ripened sweet orange fruit juice groups. The mean values of ALT, AST, ALP, total bilirubin, urea, lactate dehydrogenase and creatinine levels of calcium carbide ripened sweet orange fruit juice were significantly lower when compared to the natural ripened sweet orange fruit juice group. While, albumin, total protein and total cholesterol levels of the calcium carbide ripened fruit juice group were slightly higher than the natural ripened sweet orange fruit juice group. There was a significant difference of all the biochemical parameters except albumin and total protein at ($P < 0.05$). Sequel to the findings of this study, we can conclude that there is need for the requirement for institutional and legislative supports as well as policies to stop the utilization of harmful chemical compounds such as calcium carbide in the ripening of sweet oranges and other fruits.

KEYWORDS: Calcium carbide, Sweet orange (*Citrus sinensis*), Biochemical parameters, Wistar rats, Human health.

INTRODUCTION

Ripening is a biochemical process through which fruits attain desirable flavor, colour, tissue softening and other physiological properties. It is associated with changes in the composition of a fruit, especially the conversion of starch to sugar.[1] During ripening, there is increased rate of respiration and emission of ethylene gas. Ethylene promotes the conversion of fruits' starch to sugar and hence regulates the ripening process. Fruit ripening is a natural process in which the fruit goes through various chemical changes and gradually become sweet, flavoured, coloured, gets soft and become palatable.[2] In recent time, this process has been facilitated to meet the growing demand for fruits due to increasing awareness of their nutritional and health benefits.[3] Many marketers and farmers in most developing countries including Nigeria ripen their fruits with chemicals such as calcium carbide (CaC_2).[3]

Citrus sinensis (L. Osbeck) or sweet orange originated from south East Asia, but is consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system is one of these fruits commonly affected by artificial ripening practices. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health.[4]

Citrus is widely grown in Nigeria and many other tropical and subtropical regions.[5] In terms of volume in production, citrus ranks after banana as the world second fruit crop with more than 108 million tons.[6] Sweet

orange (*Citrus sinensis* L. Osbeck) commonly called orange is a member of this family and a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium.[7] With the ever-increasing demand in consumption of this fruit as dietary source of minerals, vitamins and dietary fiber, farmers who are not able to meet up with these demands tend to use Calcium carbide an artificial fruit ripening agent to facilitate immediate ripening fruits to make them presentable and appealing to man without considering the side effects to human health. Calcium carbide has carcinogenic and neurological disorders properties. It can result in tingling sensation, numbness and peripheral neuropathy. If pregnant women consume fruit ripened with carbide, the children born could develop abnormalities.[8]

The use of calcium carbide is not only toxic to consumers, but it is also harmful to those who handle it. It affects the neurological system, resulting in headache, dizziness, mood disturbances, sleepiness, mental confusion and seizures on a short-term basis, while in the long-term it can cause memory loss and cerebral oedema.[8] This work therefore, sets out to examine the effect of calcium carbide on biochemical parameters of wistar rats.

MATERIALS AND METHODS

Experimental Design

This is an experimental study designed to investigate the Biochemical parameters of the First Filial Generation from the Wistar rats fed with naturally ripened and Calcium Carbide induced ripened sweet orange (*citrus sinensis)* fruits.

Fruit and Calcium Carbide Collection

Mature unripe sweet orange (*citrus sinensis)* fruits were plucked off from the sweet orange (*citrus sinensis)* plant in Yenagoa, Bayelsa State. The fruits were divide into two groups, one group was kept and allowed to rip at normal room temperature and the second category was induced to ripe with calcium carbide at the Histology Laboratory, Bayelsa Medical University, Yenagoa, Bayelsa State. Calcium carbide was bought at Swali Market, Yenagoa, Bayelsa State. 10gram of Calcium carbide was placed in a bowl and 5ml of water was used to dissolved it in a closed metal bucket containing 1kg of the fruit rapped with black nylon and was allowed for two days (48 hours) for ripening. After ripening, sampled fruits were washed and juiced.

Preparation of sample

In this study, 600g of both the naturally ripened and calcium carbide ripened sweet orange (*citrus sinensis)* fruits were peeled separately and blended in an electric blender with 350 ml/1L of distilled water. The juice was filtered with a clean fine sieve and was poured into clean bottles and labeled (CaC_2 ripened sweet orange (*citrus sinensis)* fruits juice and naturally ripened sweet orange (*citrus sinensis)* juice) then, stored in a refrigerator for further usage.

Experimental Wistar Rats

Twenty four (24) adult Wistar rats (12 male and female of each sex) weighing between 126.9- 213.3 g was used for this study. The experimental Wistar rats were grouped into three and was allowed to acclimatize for two weeks (fed with grower mash with clean water) at libitum then, different dosage of the fruit juice were administered orally based on their body weight. They were kept in standard environmental condition in the animal house of the Bayelsa Medical University; following the guidance of National Research Council, Guide for the Care and Use of Laboratory Animals, 2011.[9]

Administration of samples

LD_{50} was done using Lorke (1983).[10] Method for administration of samples.

Group 1: Normal control group of 8 rats (4 males and 4 females) received normal water and feeds only as placebo.

Group 2: Treatment Group.[1] of 8 rats (4 males and 4 females) received 5 ml/kg naturally ripped sweet orange (*citrus sinensis)* juice.

Group 3: Treatment Group.[2] of 8 rats (4 males and 4 females) received Calcium Carbide ripened sweet orange (*citrus sinensis)* juice. The treatment lasted for four weeks.

Blood sample collection

The animals were observed in their cages for clinical symptoms daily and at the end of the four weeks treatment, the rats were sacrificed under chloroform anesthesia and blood was collected by cardiac puncture, using 5 ml syringes and 23G needles into blood sample containers. The blood was allowed to stand for 2 hours to coagulate and was centrifuged for 10 minutes at 2000 rpm and the supernatant (Serum) carefully collected for biochemical analysis.

Biochemical analysis

Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined following the principle described by.[11] while the alkaline phosphates (ALP) were carried out according to the method described by.[12] to assess liver function. Renal function was assessed by measuring plasma creatinine (CREA) levels and blood urea was assayed following the method of.[13,14]

In order to assess the synthetic function of the liver, total protein (TP), Total bilirubin and albumin (ALB) concentrations were determined according to the principles based on the Biuret reaction.[15,16] and bromocresol green reaction.[17] respectively. Total cholesterol (TC) and Lactate dehydrogenase concentrations were estimated following the method described by.[18]

Analysis of Data

Data collected from this study was analyzed as Mean ± Standard Error of Mean (SEM). Significant difference among the groups were determined as P<0.05 by two-way ANOVA, using Statistical Analysis Program for Social Sciences (SPSS 22.0 Version).

RESULTS

Biochemical parameters

Table 1 shows the Body Weight of Animal with the natural and calcium carbide ripened sweet orange fruit juice. Table 2 shows the effects of natural and calcium carbide ripened sweet orange fruit juice on Biochemical Parameters of wistar rats in 5 ml/kg body weight. The biochemical parameters analyzed were ALP, AST, ALT, total bilirubin, total protein, albumin, creatinine, urea, total cholesterol and lactate dehydrogenase levels in the experimental animals and were compared with the control and natural ripened sweet orange fruit juice groups. The mean values of ALT, AST, ALP, total bilirubin, urea, lactate dehydrogenase and creatinine levels of calcium carbide ripened sweet orange fruit juice were significantly lower when compared to the natural ripened sweet orange fruit juice group. While, albumin, total protein and total cholesterol levels of the calcium carbide ripened fruit juice group were slightly higher than the natural ripened sweet orange fruit juice group. There was a significant difference of all the biochemical parameters except albumin and total protein at ($P < 0.05$).

Table 1: Body Weight of Wistar rats in grams.

Group	Control	Natural sweet orange (*citrus sinensis)* fruit juice	CaC_2 ripened sweet orange (*citrus sinensis)* fruit juice
Mean ±SEM	214.30±10.53	184.53±19.53	174.28±17.35

Table 2: Biochemical results of CaC_2 ripened sweet orange (*citrus sinensis)* fruit.

Biochemical parameters	Control	Natural sweet orange (*citrus sinensis)* fruit juice	CaC_2 ripened sweet orange (*citrus sinensis)* fruit juice
AST[u/l]	50.73 ± 2.09^{X}	69.15 ± 0.95^{Y}	67.45 ± 1.65^{Z}
ALT[u/l]	31.10 ± 0.30^{A}	50.10 ± 2.30^{B}	47.30 ± 0.90^{C}
ALP[u/l]	70.60 ± 2.0^{M}	94.35 ± 2.95^{N}	87.20 ± 1.40^{O}
CREATININE[mg/dl]	0.62 ± 0.02^{D}	0.82 ± 0.03^{E}	0.79 ± 0.03^{F}
UREA[mg/dl]	15.50 ± 0.30^{Q}	27.15 ± 1.35^{R}	26.50 ± 0.70^{S}
TOTAL BILURIBIN[mg/dl]	0.34 ± 0.02^{J}	0.67 ± 0.0^{1K}	0.58 ± 0.02^{L}
ALBUMIN[g/dl]	4.50 ± 0.10^{A}	4.20 ± 0.20^{A}	4.35 ± 0.15^{A}
TOTAL PROTEIN[g/dl]	8.50 ± 0.10^{C}	7.20 ± 0.40^{C}	7.60 ± 0.20^{C}
TOTAL CHOLESTEROL[mg/dl]	73.00 ± 3.40^{U}	101.20 ± 2.60^{V}	103.50 ± 4.70^{W}
LACTATE DEHYDROGENASE[u/l]	151.00 ± 2.40^{X}	208.80 ± 2.40^{Y}	207.55 ± 8.65^{Z}

Mean±SEM. The Means with Different superscript alphabets in the same row indicates significant difference at 95% confidence level (p<0.05).

DISCUSSION

The use of calcium carbide for fruit ripening has been known for many years and has been banned in many countries due to the associated health risks. However, there is a dearth of robust scientific data on the investigation of calcium carbide residue in fruits marketed in different parts of the world. This could partly be attributed to the lack of suitable analytical methods for detecting and determining calcium carbide residue in suspected fruits. This study presented evidence demonstrating the use of calcium carbide and natural ripened sweet orange fruits on biochemical parameters. The biochemical parameters analyzed were ALP, AST, ALT, total bilirubin, total protein, albumin, creatinine, urea, total cholesterol and lactate dehydrogenase levels in the experimental animals and were compared with the control and natural ripened sweet orange fruit juice groups. The liver enzymes concerned with amino acid metabolism are determined to investigate liver disease and myocardial infraction.[19] Increases in the serum levels of AST, ALP, ALT and total bilirubin serve as reliable indices of assessment of damage due to the parenchymatous cells of the heart and liver respectively.[20] Since the various liver function tests in the calcium carbide ripened sweet orange fruit fed group in this study were significantly lower when compared with natural ripened fruit fed group, it is possible that prolonged consumption of such calcium carbide ripened sweet orange fruits beyond the experimental duration may result in the heart and liver diseases. These findings are in agreement with.[21]

Creatinine and urea are tests performed to investigate kidney function. Creatinine is a formed cyclic derivative of creatin and closely filtered out by the kidney and with no re-absorption. If the filtration of the kidney is deficient, creatinine blood levels rise up. Higher levels of creatinine indicate a falling glomerular filtration rate and as a result a decreased capability of the kidneys to excrete waste products.[22]

In the current study, the significantly low creatinine and urea levels is an indication that the kidney was not yet adversely affected by the exposure of the rats to calcium carbide ripened orange fruits juice. This also agrees with the work of two researchers who used ethyphon as a toxicant in rabbit diet for two weeks.[23] It is again possible that prolonged consumption of calcium carbide ripened sweet orange fruits beyond the experimental duration could lead to kidney malfunction. Lactate dehydrogenase (LDH) is an important enzyme of the anaerobic metabolic pathway. It belongs to the class of oxidoreductases. It is ubiquitously present in all tissues and serves as an important checkpoint of gluconeogenesis and DNA metabolism. Conditions that can cause increased LDH in the blood may include liver disease, anemia, heart attack, bone fractures, muscle trauma, cancers and infections.in the present study, LDH levels in calcium carbide ripened sweet orange fruit levels were observed to be lower compared to the natural ripened fruit juice fed group. Therefore, at the experimental duration and the dosage used in this study, the calcium carbide ripened fruit juice fed diet does not pose any heart problems. Plasma albumin was higher in rats fed on calcium carbide ripened sweet oranges. This may probably be due to inadequate breakdown of protein thus making protein inaccessible to the cell hence subsequent elevated albumin level. Accordingly, the rats may have experienced protein malnutrition.[21]

In the present study, total cholesterol and total protein levels of were higher in rats fed on calcium carbide induced sweet oranges. This agrees with findings of research on European rabbit exposed to another toxicant known as ethephon used in ripening fruits.[23]

CONCLUSION

In this research, the biochemical parameters analyzed were ALP, AST, ALT, total bilirubin, total protein, albumin, creatinine, urea, total cholesterol and lactate dehydrogenase levels in the experimental animals and were compared with the control and natural ripened sweet orange fruit juice groups. The mean values of ALT, AST, ALP, total bilirubin, urea, lactate dehydrogenase and creatinine levels of calcium carbide ripened sweet orange fruit juice were significantly lower when compared to the natural ripened sweet orange fruit juice group. While, albumin, total protein and total cholesterol levels of the calcium carbide ripened fruit juice group were slightly higher than the natural ripened sweet orange fruit juice fed group. It is again possible that prolonged consumption of calcium carbide ripened sweet orange fruits beyond the duration of feeding in this study could result cardiovascular diseases and kidney malfunction. Sequel to this, there is then the requirement for institutional and legislative supports as well as policies to stop the utilization of harmful chemical compounds such as calcium carbide in the ripening of sweet oranges and other fruits.

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