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EVALUATION OF NATURAL AND ARTIFICIAL RIPENED PINEAPPLE (*ANANAS COSMOSUS* L. MERR.) FRUITS ON BIOCHEMICAL PARAMETERS OF WISTAR RATS

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ABSTRACT

Fruits are of high significance worldwide due to their nutritional and commercial values. Likewise, they are sources of essential nutrients; fortify diet with vitamins and minerals. Artificial ripening may contaminate the fruit and may cause toxicity. This study was aimed at evaluating natural and artificial ripened Pineapple (*Ananas cosmosus*) fruit juice on the Biochemical parameters of 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 principles of biochemical analysis. The biochemical parameters evaluated were creatinine, urea, albumin, total protein, transaminases (AST, ALT), alkaline phosphatase (ALP), total cholesterol, total bilirubin and lactate dehydrogenase and were compared with the control and natural ripened groups. All mean values of ALT, ALP, Urea, creatinine, Albumin and total cholesterol were lower when compared with the natural ripened group. The concentrations of AST, total bilirubin, total protein and lactate dehydrogenase were observed to be higher when compared with the natural ripened group. Statistically, all biochemical parameters evaluated had significant differences at 95% confidence level ($P < 0.05$), except creatinine, albumin and total proteins. This indicates that, consuming calcium carbide ripened pineapple fruits formulated diets may cause myocardial infarction, hepatic dysfunction and liver and heart diseases, therefore artificial ripening by the use of calcium carbide method should be discouraged.

KEYWORDS: Artificial, Natural, Pineapple (*Ananas cosmosus*), Biochemical parameters, CaC_2, Wistar rat.

INTRODUCTION

Fruits are of high significance worldwide due to their nutritional and commercial values. Likewise, they are sources of essential nutrients, fortify diet with vitamins and minerals, and provide variety to food and make food appetizing. They further account for a substantial fraction of the world's agricultural output, and, some have acquired great economic importance. Fleshy and juicy fruits are used in producing jams, marmalade, ice creams, yoghurt and juices. In Sub-Saharan Africa, agriculture (fruits production a mainstay) accounts for about 32% of gross domestic product (GDP).[1]

At present, various ripening agents are being used in the process of fruit ripening. Naturally ripened fruits are perishable; therefore, various chemicals like ethephon, ethylene, and calcium carbide that are hazardous to human health are being used to ripen and enhance the fruits' shelf-life. Artificial ripening may contaminate the fruit and may cause toxicity.[2] Consequently, these chemicals are responsible for different kinds of health burdens such as weakness, skin disease, kidney failure, cancer, lung failure, dizziness, ulcer, heart diseases.[3] However, these accelerated ripening processes may also alter the fruit metabolism and fruit quality due to varying physiological processes influenced by multiple biological and environmental factors.

Pineapple (*Ananas comosus)* is a tropical plant with an edible fruit; it is the most economically significant plant in the family *Bromeliaceae.* The pineapple is indigenous to South America, where it has been cultivated for many centuries. The fruit is juicy with excellent flavor and taste. Pineapple is now considered to be the third most important fruit crop in world production after banana and citrus.[4] Pineapple contains different vitamins, antioxidants, a proteolytic enzyme, bromelain which helps in the digestion process by breaking down proteins. Bromelain has anti-inflammatory, anti-clotting and anti-cancer properties. Furthermore, it can also interact with other medications. Care should be taken when consuming fresh pineapple with medications (antibiotics, anticoagulants, blood thinners, anticonvulsants, barbiturates, benzodiazepines, insomnia drugs and tricyclic antidepressants) due to the possibility of certain side effects associated with it.[5]

Both natural and chemical ripening procedures are used for pineapples. However, different ripening procedures may have different nutrient values and sensory profiles, influencing consumers' choices.[6] The final fruit ripening stage involves a sequence of biochemical and physiological occurrences prime to changes in texture, color, aroma, and flavor that shape the fruits more palatable, attractive, and tasty.[7] The rising demand of fruit safety has inspired researchers about the risk related to the use of chemically ripened fruits.[8] The present study was therefore conducted to investigate the possible changes in biochemical parameters in wistar rats fed on diets containing natural and artificial (calcium carbide) ripened pineapple (*Ananas comosus)* in Yenagoa, Nigeria.

MATERIALS AND METHODS

Fruit and calcium carbide sample collection

Mature unripe pineapple (*Ananas comosus)* fruits were plucked off from the pineapple (*Ananas comosus)* 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 pineapple (*Ananas comosus)* 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 pineapple (*Ananas comosus)* fruits juice and naturally ripened pineapple (*Ananas comosus)* 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: Feeding Group (1) of 8 rats (4 males and 4 females) received 5 ml/kg naturally ripped pineapple (*Ananas comosus)* juice.
Group 3: Feeding Group (2) of 8 rats (4 males and 4 females) received 5ml/kg Calcium Carbide ripened pineapple (*Ananas comosus)* juice. The Feeding 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 wistar rats administered with natural and CaC_2 ripened pineapple fruit juice and the control. While Table 2 shows the effects of natural and CaC_2 ripened pineapple fruit juice on biochemical parameters of wistar rats at 5 ml/kg dose. The biochemical parameters evaluated were creatinine, urea, albumin, total protein, transaminases (AST, ALT), alkaline phosphatase (ALP), total cholesterol, total bilirubin and lactate dehydrogenase and were compared with the control and natural ripened groups. All mean values of ALT, ALP, Urea, creatinine, Albumin and total cholesterol were lower when compared with the natural ripened group. The concentrations of AST, total bilirubin, total protein and lactate dehydrogenase were observed to be higher when compared with the natural ripened group. Statistically, all biochemical parameters evaluated had significant differences at 95% confidence level ($P < 0.05$). Except parameters like creatinine, albumin and total proteins were not significant at 95% confidence level.

Table 1: Body weight of Wistar rats in grams.

Group	Control	Natural ripened pineapple fruit juice	CaC_2 ripened pineapple fruit juice
Mean ±SEM	214.30±10.53	184.53±19.53	174.28±17.35

Table 2: Biochemical Results of natural and CaC_2 Ripened Pineapple fruits.

Biochemical parameters	Control	Natural ripened pineapple fruit juice	CaC_2 ripened pineapple fruit juice
AST [u/l]	50.73 ± 2.08^{A}	66.40 ± 0.20^{D}	67.60 ± 0.80^{M}
ALT [u/l]	31.10 ± 0.10^{F}	49.10 ± 3.30^{Y}	47.60 ± 1.80^{A}
ALP [u/l]	70.60 ± 2.00^{I}	85.80 ± 2.60^{S}	85.50 ± 0.70^{A}
Creatinine [mg/dl]	0.62 ± 0.02^{Z}	0.75 ± 0.03^{Z}	0.73 ± 0.04^{Z}
Urea [mg/dl]	15.50 ± 0.30^{P}	24.90 ± 0.50^{T}	24.3±1.80R
Total Biluribin [mg/dl]	0.34 ± 0.02^{S}	0.60 ± 0.06^{X}	0.64 ± 0.02^{H}
Albumin [g/dl]	4.50 ± 0.10^{B}	4.40 ± 0.20^{B}	4.05 ± 0.05^{B}
Total Protein [g/dl]	8.50 ± 0.10^{A}	7.80 ± 0.60^{A}	7.90 ± 0.30^{A}
Total Cholesterol [mg/dl]	73.00 ± 3.40^{W}	105.25 ± 6.95^{Q}	95.20 ± 2.40^{A}
Lactate dehydrogenase [u/l]	151.00 ± 2.40^{X}	196.60 ± 1.80^{G}	199.7 ± 1.90^{B}

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

DISCUSSION

The Biochemical results in this study indicated a slight decrease in serum total protein in the rats fed with calcium carbide induced pineapple fruit formulated diet, compared with the control. The reduction in protein may be attributed to increased proteolysis.[19] or probably reduction in the synthesis of protein which maybe occasioned by the presence of a toxicant which may consequently have resulted into a shift in nitrogen metabolism.[20] The observed decrease agrees with the information from emerging data suggesting that toxicants such as arsenic and phosphorus present in calcium carbide may leak into the fruit in the course of ripening.[21]

The plasma albumin level was observed to decrease in the calcium carbide fed group when compared with the control and the natural ripened fed group. This could be adduced that with the induced dosage and the period of feeding calcium carbide does not cause dehydration or liver function impairment since the liver is responsible for the metabolism of protein.[22] The hepatic cells are solely responsible for the synthesis of plasma albumin which is very vital in the regulation of the blood pressure and the binding and transportation of cellular components.[23]

Increase in bilirubin concentration as seen in the calcium carbide ripened pineapple group may be suggestive of a hepatobiliary disorder occasioned by the complete or partial blockage of the bile ducts due to increased tension on the bile ducts owing probably to the presence of toxicants or possibly infections which consequently leads to the accumulation of plasma bilirubin [24, 25]. A bilirubin test is important in establishing the underlying factor for jaundice. Hyperbilirubinema may result in the buildup of bilirubin in the brain which is a risk factor in neurological disorder. Hyperbillirubinaemia is prevalent in parenchymal liver disease and biliary obstruction.[26] In the current study, the highest level of bilirubin was noticed in the rats fed calcium carbide ripened pineapple formulated diet which differed significantly from the control and naturally induced ripening methods. This suggests that calcium carbide increases the level of billirubin more than the natural ripening method which lends support to earlier report of hepatic dysfunction induced by toxicants such as Arsenic found in calcium carbide.[27,28]

Results obtained for creatinine and Urea revealed that no significant difference was found in the calcium carbide induced fed group when compared to the naturally induced fed group. Creatinine and urea are analysis carried out to evaluate kidney function.[29] Creatinine is produced from creatine as a waste product of normal breakdown of muscle tissue which is then filtered out through the kidneys without re-absorption and excreted in the urine. Blood level of creatinine is elevated when this infiltration is deficient, thus creatinine levels are used as a test of renal function.[30] Since no significant difference was observed for both parameters it implies that the kidney was not yet negatively affected by the artificial ripening methods. This report agrees with earlier report.[31,20] who submitted no significant difference in both parameters.

The marker enzymes of hepatic function showed a varied response of the experimental rats. Aspartate transaminase (AST) in the calcium carbide fed group was slightly elevated when compared with the natural ripened fed and control groups and also significant statistically. This finding agrees with previous report of Igbinaduwa and Aikpitanyi-Iduitua,[32] and Gbakon *et al*.,[20]

When compared to the natural ripened fed group, there was a slight decrease in the levels of Alkaline Phosphatase (ALP) and Alkaline transaminase (ALT) was observed in the calcium carbide ripened fed group. ALP are a set of vital metallo enzymes needed for the hydrolysis of ester bond and the cleaving of phosphorus thereby creating an alkaline environment suitable for cellular functions.[33] They are abundant in the hepatic parenchyma, osteoblasts, intestinal mucosa, placental cells and renal epithelium. Significantly elevated activity level of ALP implies liver impairment as reported by Young *et al*.,[34] Similarly, Aspartate Transaminases (AST) and Alanine Transaminases (ALT) are liver enzymes responsible for amino acid metabolism. They are vital enzymes in investigating liver disease and myocardial infarction. Increased plasma activity levels of AST (present in the same amount in the heart, skeletal muscles and liver) and ALT (most prevalent in the liver) are indicative of cardiac and hepatic tissue damage.[35] since damage results in the drainage of these enzymes from intercellular stores into the plasma.[36] therefore they serve as accurate indicators of liver or cardiac injury. The observed decrease in the amount of this enzyme (ALP) in the rats that were fed calcium carbide ripened pineapple formulated diet is an indication that the use of calcium carbide in fruit ripening at the dosage and time period of this study may not necessarily cause liver damage which is in disagreement with previous reports.[37] The study revealed a slight though statistically insignificant increase in AST but it is possible that prolonged consumption of calcium carbide ripened pineapple fruits beyond the four weeks could result to liver impairment.

In this study, there was a decrease in total cholesterol in calcium carbide ripened pineapple when compared with the natural ripened fed group. This agrees with findings of research on European rabbit exposed to another toxicant known as ethephon used in ripening fruits.[38] The decrease in total cholesterol may probably be an indication of slight degenerative changes in the liver.[39] Meanwhile, there was a significant increase in the levels of Lactate dehydrogenase (LDH) in the calcium carbide ripened fed group observed when compared with the control and natural ripened fed groups which can be lead to heart diseases. These findings were in agreements with the studies of Pauline et al.[40]

CONCLUSION

The results obtained in this study indicates that all mean values of ALT, ALP, Urea, creatinine, albumin and total cholesterol of calcium carbide ripened fruit group were lower when compared with the natural ripened fruit group. The concentrations of AST, total bilirubin, total protein and lactate dehydrogenase of calcium carbide ripened fruit group were observed to be higher when compared with the natural ripened fruit group. This indicates that, consuming calcium carbide ripened pineapple fruits formulated diets may cause myocardial infaction, hepatic dysfunction and liver and heart diseases, therefore artificial ripening by the use of calcium carbide method should be discouraged.

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