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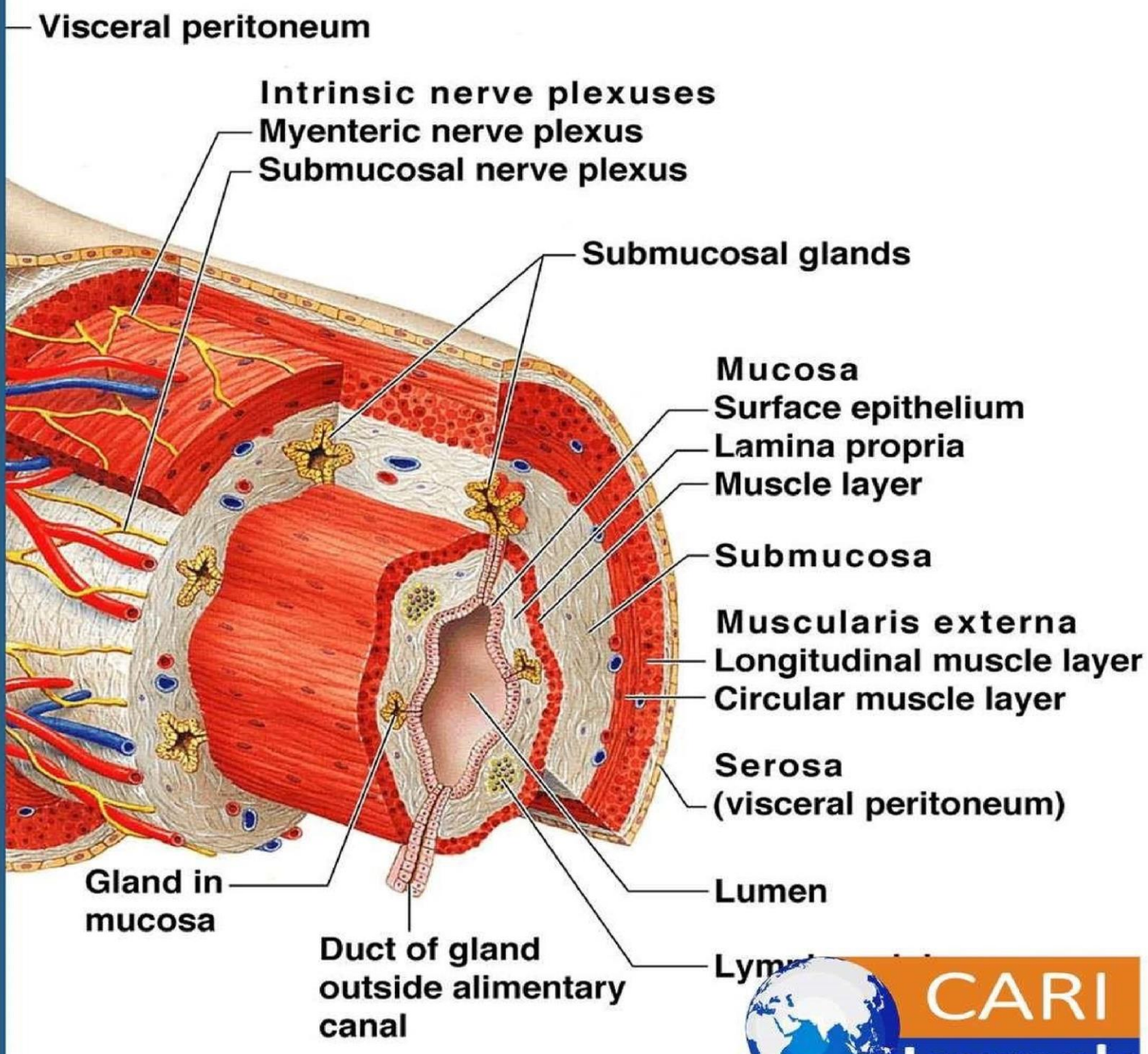

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ASSESSMENT OF THE BIOCHEMICAL PARAMETERS ON THE FIRST FILIAL GENERATION FROM THE WISTAR RATS FED WITH CALCIUM CARBIDE RIPENED ORANGE.

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ABSTRACT

Purpose and Methods: The use of chemicals for fruit ripening is constantly on the increase and inadvertently, such chemicals are consumed unperceived. The aim of the study is the assess the Biochemical parameters of the Pups from the Wistar rats fed with Calcium Carbide induced ripened orange. Unripe mature oranges were gotten for the parent plant at Ogu, Yenagoa, Bayelsa State. The fruits were divided into two; the first group was allowed to ripe at normal room temperature and 1kg of the second oranges wrapped with black nylon were forced to ripe within 48 hours with 10grams of Calcium carbide which was dissolved in 5ml of water in a closed steel bucket. 600g of each groups of the ripened oranges were peeled differently and blended in an electric blender with 350ml/1L distilled water. The juice was filtered with a clean fine sieve and were poured into clean bottles labeled accordingly and stored in a refrigerator for future use. 24 adult Wistar rats [12 male and female of each sex] weighing between 126.9- 213.3g were used. They were acclimatize for two [2] weeks and was fed with standard grower mash with clean water ad libitum. The Wistar rats were grouped into three, [1] Control, of 8 rats [4 males and 4 females] receive normal water and feeds only as placebo. [2] Treatment group I, containing 8 rats [4 males and 4 females] received 5ml/kg of the naturally ripened orange juice [3] Treatment II of 8 rats [4 males and 4 females] received 5ml/kg of the Calcium carbide induced ripened orange juice orally against their body weight for four weeks. The Wistar rats copulates freely during this period. Birthing occurred in the three groups and the Pups were collected weight at birth, at one week and the two week. They were sacrifice at second week and blood sample collected for the Biochemical analysis. Data collected from this study was analyzed as Mean ± Standard Error of Mean [SEM]. Significant difference among the groups was determined as $P<0.05$; by two-way ANOVA; using Statistical Analysis Program for Social Sciences [SPSS 22.0 Version].

Findings and recommendations: The results of this study showed statistically significant increase in the mean AST, ALT, ALP, Creatinine, Urea, Total Biluribin and Lactate Dehydrogenase of the pup of the Wistar rats fed with Calcium carbide forced ripened orange juice in contrast with the control group ($p<0.05$). There was significant reduction in the mean Albumin, Total Protein and Total Cholesterol in the pups from the Wistar rats fed with Calcium carbide forced ripened orange juice when compared with the control group ($p<0.05$). In conclusion, there is nutritional programming of Calcium Carbide forced ripened fruit when consumed by a mother and could cause hepatic and/or extra-hepatic toxicity, renal failure, heart failure, coronary heart diseases of the offspring. Thus; we recommend strongly, the abolishment of Calcium carbide as an agent for fruit ripening.

Key words: *Biochemical Parameters, Calcium Carbide, Forced ripening*.

INTRODUCTION

Fruits provide vital nutrients in human diet by supplying the necessary growth regulating factors such as vitamins, minerals, complex carbohydrates, proteins, lipids and antioxidants essential for maintaining normal health of the human [Hayes, 2005; Rossato *et al.*, 2009] . Calcium carbide is a chemical compound with a formula CaC2. Its main use now is as a source of acetylene. It is a toxic substance banned under the Prevention of Food Adulteration Act. Calcium carbide contains traces of hazardous arsenic and phosphorous compounds. Once dissolved in water, the carbide produces acetylene gas that quickens the ripening process, hence is used in ripening fruits [Halliwell and Gutteridge, 1985]. Marker enzymes' are biochemical parameters associated with health indices, which are always of diagnostic significance in the routine clinical evaluation of the state of health [Ogoun *et al.,* 2022]. The minimal and non- significant increase of ALP, AST and ALT, albumin, creatinine total bilirubin and total protein (5, 10 and 15 mg/kg dosages) of Yoyo Cleanser Bitters when compared with the control had no toxic effect. However, the significant increase in serum levels of urea (10 and 15 mg/kg dosages) and total cholesterol (5 and 10 mg/kg dosages) observed in the treated groups may be due to nephrotoxic and hepato-biliary effects of the herbal formulation which might lead to reduced renal function and hepatobiliary disorder and impaired cholesterol metabolism [Ogoun *et al.,* 2022]. The biochemical indices evaluated were creatinine, urea, albumin, total protein, transaminases (AST, ALT), alkaline phosphatase (ALP), total cholesterol and total bilirubin and were compared with the control groups. All mean values of creatinine and ALT were lower when compared with the control group. Concentrations (10 and 15 mg/kg) of the serum levels of total bilirubin and albumin, ALP (5 and 15mg/kg), AST and total cholesterol (5 and 10mg/kg), total protein (5mg/kg) and urea (5, 10 and 15mg/kg) were observed to be higher when compared with the control. Statistically, there were no significant differences of the evaluated indices at 95% confidence level ($P < 0.05$). [Ogoun et al., 2022].

Biochemical results shows a significant increase of the drug-treated groups of the levels of serum ALP, total bilirubin, total protein, creatinine, albumin, urea, total cholesterol, in comparison with the control group. The results therefore showed that NSAIDs used in this study had toxic effects on vital animal tissues, resulting in hematological disorder, hepatic and renal impairments [Ogidi et al., 2020]. The Levels of total protein in serum increased with age, in contrast, albumin levels decreased [Nistiar et al., 2012]. There is increased amount of sodium ion (Na+), potassium ion (K+) and Urea, and decreased chloride ion (Cl-), bicarbonate ion (HCO3-) and Creatinine (Cr) in the ethanol group. However, there is decrease Na+, K+, Cl-, Urea, HCO3- and Cr in the aqueous group. Though, the increase was not significant. The aqueous and ethanol extracts of Pleurotus tuber regium used in this study showed effects on the Albino Wistar rats, but the aqueous extract showed more beneficial effects than the ethanol extract. Therefore Pleurotus tuber regium extracts can be used as a prebiotic and probiotic material in the maintenance of weight [Oghenemaro et al., 2020] .The aqueous and ethanol extracts of the mushroom has shown that it is of beneficial effects in maintaining a normal sodium, potassium, chloride, bicarbonate, urea, cholesterol and triglyceride, with significant effect on high density lipoprotein (HDL) and low density lipoprotein [oghenemaro et al., 2020]. Silver nanoparticles have found wider and increasing biomedical applications due to their broad antimicrobial characteristics[Adeyemi and Adewumi, 2014]. The administration of AgNPs in rats did not induce major changes in body weight and feed intake. However, this study is evidence indicating that rat tissue biochemical indices were altered following oral exposure to AgNPs. Future studies are ongoing and target the determination of whether the alterations to the biochemical parameters were signs of AgNPs toxicity or not [Adeyemi and Adewumi, 2014]. Administration of AgNPs to rats did not produce significant loss in feed intake and body weight. However, rat exposure to AgNPs caused significant alterations to levels of serum and tissue AST, ALT, and ALP. At the 100 mg/kg AgNPs exposure, rat serum and tissue AST and ALT levels were significantly decreased ($P < 0.05$). In contrast, AgNPs administration elevated ($P < 0.05$) ALP levels in rat serum and tissues. Conclusion. We show evidence that AgNPs administration to Wistar rats altered some biochemical parameters. [Adeyemi and Adewumi, 2014]. Rats treated with peppermint gained more weight ($p < 0.05$) and also decreased the serum concentrations of triglycerides, total cholesterol, LDL, and glucose in T3, T4 and T5 than the other groups ($p < 0.05$). Peppermint extract had a positive effect on body-weight gain and some blood parameters in adult male Wistar rats [Mesbahzadeh et al., 2015].

Alcoholic bitters have been acclaimed to boost sexual function and fertility in animals but there is no reported scientific evidence that evaluated its effects on the normal functioning of the testes. There is significant ($p < 0.05$) increases in protein, cholesterol, testosterone, FSH and LH, as well as in the activity of HMG-CoA reductase, SOD and CAT in all the groups of animals administered the alcoholic bitters, whereas concentration of MDA was significantly reduced ($p<0.05$). Concentration of triglycerides was not significantly different ($p>0.05$) from those of the control animals. Alcoholic bitters enhanced the normal functioning of the testes, the antioxidant enzymes and the release of the reproductive hormones. This may partly explain its use in boosting sexual

function and fertility in male rats [Kayode *et al.*, 2018]. Liver dysfunction reflects the status of heart failure, and previous studies have demonstrated that serum lactate dehydrogenase (S-LDH) levels are increased in patients exhibiting heart failure and liver dysfunction [EN-CI HU *et al.*, 2015]. The Kaplan-Meier survival curves demonstrated that patients with higher S-LDH levels had a significantly lower survival rate than did those with lower S-LDH levels (log-rank test, $P<0.001$).There has been a missing link and paucity of data with regards study of the effect of calcium carbide on nutritional programming. Therefore, this study is aimed at investigating the Biochemical parameters from the pups of the Wistar rats fed with calcium carbide forced ripened fruit.

MATERIALS /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 fruits [oranges.

Fruit and Calcium Carbide Collection:

Mature unripe oranges were plucked off from the Orange plant in Yenagoa, Bayelsa state. The fruits were divide into two groups, one group was kept and allowed to rip at normal room temperatureand 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 [orange] 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 fruits [orange] were peeled separately and blended in an electric blender with 350ml/1L of distilled water. The juice was filtered with a clean fine sieve and was poured into clean bottles labeled [CaC_2 ripened orange juice and Naturally ripened orange juice] then, stored in a refrigerator for further usage.

Experimental Wistar Rats

24 adult Wister rats [12 male and female of each sex] weighing between 126.9- 213.3g 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 Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Administration of samples

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

Group 1: Normal control group of 8 rats [4 males and 4 females] receive normal water and feeds only as placebo.

Group 2: Treatment Group [1] of 8 rats [4 males and 4 females] received 5ml/kg naturally ripped orange juice

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

Birthing

The adult Wistar rats were allowed to mate freely during the acclimatization and treatment period. This period under review, the Wistar rats birthed and the Pups of the three different groups were collected according to the treatment protocols.

The Pups were weighed at birth, at one week, two weeks then sacrificed and blood samples were collected from the three groups for Biochemical analysis.

Biochemical Analysis

The Liver Biochemical parameters are

1. Aspartate Aminotransferase [AST]
2. Alanine Transaminase [ALT]
3. Alkaline Phosphatase [ALP]
4. Total Biluribin
5. Albumin

The kidney parameters are

1. Creatinine
2. Urea

Lipid profile

1. Total Protein
2. Total Cholesterol

Heart

1. Lactate Dehydrogenase

Analysis of Data

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

RESULTS

The data collected for this study was analyzed and the results are presented in the tables below.

TABLE 1: MEAN ADULT WISTAR RAT WEIGHT BEFORE TREATMENT [grams]

GROUP	CONTROL	NATURAL FRUITS	CaC2 RIPENED FRUITS
MEAN VALUE	214.30±10.53	184.53±19.53	174.28±17.35

Mean ±SEM

The mean weight of the control group of the adult Wistar rats is 214.30±10.53. The mean body weight of the parent Wistar rats fed with naturally ripened fruit juice is 184.53±19.53, while the mean weight of the parent Wistar rats fed with Calcium Carcide forced ripened fruit is 174.28±17.35.

TABLE 2: MEAN BODY WEIGHT OF PUPS [grams]

S/N	GROUP	BIRTH	WEEK1	WEEK 2

1	CONTROL	2.10±0.18	11.40±0.50	15.15±0.45
2	TREATMENT WITH NATURAL FRUIT	2.13±0.58	13.65±1.25	28.75±6.25
3	TREATMEN WITH CALCIUM CARBIDE [CaC2]	2.03±0.03	11.96±0.60	16.90±3.60

Mean ±SEM

The mean body weight of the pups from the control group at birth, week one and two is 2.10±0.18, 11.40±0.50, 15.15±0.45. The mean body weight of the pups from the Wistar rat fed with naturally ripened fruit juice at birth, week one and two is 2.13±0.58, 13.65±1.25, 28.75±6.25. while,the body weight of the pups from the Wistar rats fed with CaC_2 at birth, week one and week two is 2.03±0.03, 11.96±0.60 and 16.90±3.60.

TABLE 3: BIOCHEMICAL PARAMETERS OF THE PUPS

BIOCHEMICAL PARAMETERS	CONTROL	NATURAL FRUIT	CaC_2 RIPENED FRUIT
AST[u/l]	52.3 ± 1.33^{A}	72.30 ± 1.16^{B}	76.80 ± 0.66^{C}
ALT[u/l]	25.60±0.69C	42.40 ± 1.25^{B}	40.20 ± 1.10^{B}
ALP[u/l]	70.20 ± 1.21^{D}	102.60 ± 0.91^{E}	98.10 ± 0.66^{F}
CREATININE[mg/dl]	0.49 ± 0.06^{R}	0.62 ± 0.16^{S}	0.730 ± 0.04^{T}
UREA[mg/dl]	15.80 ± 0.52^{M}	17.20 ± 0.69^{N}	18.60 ± 0.84^{O}
TOTAL BILURIBIN[mg/dl]	0.32 ± 0.02^{Q}	0.50 ± 0.06^{R}	3.83 ± 0.50^{S}
ALBUMIN[g/dl]	5.80 ± 0.62^{X}	4.64 ± 0.90^{Y}	4.08 ± 0.05^{Z}
TOTAL PROTEIN[g/dl]	6.62 ± 0.82^{G}	6.31 ± 0.46^{H}	6.12 ± 0.66^{I}
TOTAL CHOLESTEROL[mg/dl]	58.40 ± 0.81^{K}	59.20 ± 1.53^{L}	56.80 ± 0.95^{M}
LACTATE DEHYDROGENASE[u/l]	146.8 ± 3.58^{C}	156.2 ± 1.04^{D}	158.00 ± 0.58^{E}

Keys:

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

The mean values of the Biochemical Parameters such as AST, ALT, ALP, Total Bilirubin, Albumin, Creatinine, Urea, Total Protein, Total Cholesterol, Lactate Dehydrogenase have shown different values between the control group, treatment group one[Natural fruit] and treatment group two [CaC2 forced ripened fruit].

DISCUSSION

Environmental hazards have increased due to human's struggle to meet their daily needs. This has become evident as people quest to hastily maneuver things from their natural form to artificial state. There is the need to showcase clear cut awareness to the populace about the eminent danger of the consumption of these artificial commodities.

Marker enzymes are biochemical parameters associated with health indices, which are always of diagnostic significance in the routine clinical evaluation of the state of health (Ogoun *et al.*, 2022).

It is clear from the results the that AST of the pup from the treatment groups, CaC2 induced ripened fruits is significantly higher than the control as shown in[table 3]. This rise is an indicator of liver disease. Since AST is not specific to only liver function, its increase, is a pointer to hepatic and/or extra-hepatic toxicity. This finding is in consonant with the results of [Ogoun et al., 2022]. AST is found in many tissues e.g, skeletal muscle, heart muscle, and kidney if not all tissues. Rise in AST [enzyme] level are found in approximated 70 percent of patient with Myocardiac Infarction [Hatchett and Thompson, 2002].

From the result of this study, Alanine Transaminase [ALT] serum level tends to higher in the pups of the parent Wistar rat than the pups of the control [table 3]. This upsurge in ALT is an indicator of chronic hepatitis. From the results, there is significant boom in Alkaline Phosphatase [ALP] concentration in the pups from the treatment group with contrast to the control group. This rise in ALP shows there is probable liver disorder in the pup from the rat fed with calcium carbide induce ripened fruit. A rise in ALT is commonly seen in conditions that caused blocked “ducts” such as bile stones or direct damage to the bile ducts. Damage of the liver makes it leaky and AST AND ALT will be released to the blood stream. Total Bilirubin level in significantly higher in the pup of the CaC2 carbide treated Wistar rat than the pup of the Wistar rat fed with naturally ripened fruit and the control group when compared. This calcium carbide induced ripened fruit when consumed is tantamount to cause liver disease due to hyperbilirubinaemia resulting from non-excretion of bilirubin.

The serum level of Albumin as seen in [table 3] tend to lower in the pup of the rat fed with calcium carbide induce ripened fruit which is an indicator of liver cirrhosis. Albumin a is protein synthesized by the liver hepatocytes and it showcase the functional aspect of the liver cell. Low albumin expresses liver dysfunction. Consequently; there is reduction of the Total Protein of the pup from the CaC2 carbide treated Wistar rat and the control group when compared. This showed that CaC2 interferes with the functional mass of the liver.

Kidney functionality was tested with the analysis of the urea and creatinine. In this present study; the creatinine level tend to increase in the pup from the CaC2 carbide treated Wistar and the control group when compared. This inadvertently showed that calcium carbide interferes with the renal functionality leading to kidney disease and failure. Although little increase was also noticed from the pup of the Wistar rat fed with naturally ripened orange. In addition, there is an increase in urea in the pup of the CaC2 carbide treated Wistar rat as compared with the control which is an index of kidney injury. The result of this present study shows that the pup from the CaC2 carbide treated Wistar rat possess lower Total Cholesterol when compared with the control. Very low levels of cholesterol may be associated with an increased risk of cancer, hemorrhagic stroke, depression, anxiety, preterm birth and low birth weight. The most frequent cause of non-cardiac death associated with low total cholesterol is cancer. The results in patients with coronary heart disease is associating low total cholesterol with an increased risk of non-cardiac death [Behar et al, 1997].

Biochemical assessment on the level of Lactate Dehydrogenase was done. The result indicate increase in this enzyme in the pup of the CaC2 carbide ripened juice fed Wistar rat as against the control; exposing Calcium carbide negative effect on the heart and other tissues. Increase level of Lactate Dehydrogenase is a function of heart failure and liver disease. Conditions that can cause this increase are liver disease, anemia, heart attack, bone fractures, muscle trauma, cancers, and infections such as encephalitis, meningitis, encephalitis, intracranial hemorrhage, idiopathic pulmonary artery hypertension and HIV. LDH is the only serum biomarker useful for assessing metastatic melanomas [Jurisic *et al.,* 2015] In malignancy, the growth of tumor cells consumes oxygen more than the supply; thus, hypoxia is quite common.

CONCLUSION

The result of this study on the Biochemical parameters of the pup from the Wistar rats fed of calcium carbide induced ripened fruit[orange] has shown to have reno –toxic effect on the kidneys of the first generation of the Wistar rats. Consequently, The rise in Alanine Transaminase[ALT], AST, ALP, Total Bilirubin and the reduction in Albumin predisposes the fact that, Calcium carbide induced ripened fruit[orange] consumption could cause negative hepatic condition. This results have also revealed that Calcium Carbide induced ripened fruit when consumed could lead to heart failure, coronary heart diseases, and consequently death may rise.

Recommendations.

We therefore, recommend that;

1. Calcium carbide should be stopped as an agent for fruit ripening.
2. Pregnant women should complete avoid eating Calcium Carbide induced ripened fruits
3. Public health practitioners, Government, Multi- national companies should endeavor to enlighten the populace of the eminent dangers associated with fruits ripened with Calcium Carbide.

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