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Alterations in Some Haematological and Histological Parameters on Consumption of Calcium Carbide-Ripened Banana in Male Albino Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to investigate the changes in several haematological and histopathological parameters in male Swiss albino mice fed on diets with calcium carbide-ripened banana. Fruits were collected from Khahigarh village, located in Raha subdivision of Nagaon district in Assam, and were artificially ripened using calcium carbide. The calcium carbide ripened banana was fed to albino mice, two times in a day for 14 consecutive days. Naturally ripened banana was used as positive control, and treated same way in another group of mice. The results showed that calcium carbide ripened banana induced significant decrease in RBCs count and Haemoglobin concentration; and significant increase in the WBCs. Histopathological observations of liver, kidney

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and testes also revealed the hazardous effect of the chemical. However, no genotoxic effect was observed with the chemical. The results suggest that consumption of banana ripened with calcium carbide can lower the body's ability to resist some alterations of normal functions.

Keywords: Calcium carbide; banana; haematology; histopathology; micronuclei.

1. INTRODUCTION

Fruits are ripened at maturity. It is a natural phenomenon that makes the fruits edible, palatable, and nutritious for the consumers. But Fruits are also ripened artificially in a controlled manner to achieve desired outcome like optimum ripening and better consumer acceptance apart from ensuring a longer shelf life of fruits. Besides, artificial ripening also facilitates the transportation of those fruits which becomes soft and perishable after ripening, as the fruits are transported to distant places in unripe conditions to avoid losses and are artificially ripened at the destination market before sale [1]. Considering the importance of artificial ripening, the FSSAI (Food Safety and Standards Authority of India) has permitted the use of ethylene gas for artificial ripening of fruits, which is the only safe and worldwide accepted method for ripening when done under controlled temperature and relative humidity conditions [2].

Fruits are sources of essential nutrients due to which they have increased demand. Fruit retailers artificially ripen the immature fruits to meet the demand of consumers and make high profit of seasonal fruits [3]. The most frequently applied chemical for this purpose is calcium carbide (CaC_2). However, due to its toxicity, its application in fruit ripening has been banned in many countries [4,5]. Despite its ban, calcium carbide is still being used illegally in artificial ripening of fruits and this has elicited great health concerns.

In contact with moisture, CaC_2 produces acetylene gas [6], which is an analog of ethylene, and is used to ripen fruits artificially [7]. The use of carbide gas or acetylene gas is not permitted for artificial ripening of fruits under Food Safety and Standards Regulations (2011) due to its potential health hazards [8]. Moreover, fruits artificially ripened by CaC_2 also contains traces of arsenic and phosphorus, which are also harmful for human health [6,9]. Acetylene gas may affect the neurological system by inducing prolonged hypoxia [10]. The chronic exposure to acetylene may induce loss of memory [3]. In high concentrations, it could cause loss of consciousness and death [11].

The acetylene gas released from calcium carbide is equally harmful for handlers. There are chances that calcium carbide may leave residues of arsenic and phosphorus on fruits which is harmful for humans. The early symptoms of arsenic or phosphorous poisoning include vomiting, diarrhoea with or without blood, burning sensation of chest and abdomen, thirst, weakness, difficulty in swallowing, irritation or burning in the eyes and skin, permanent eye damage, ulcers on the skin, sore throat, cough and shortness of breath [6]. Consumption of fruits ripened with Calcium carbide causes stomach upset. It can also cause mouth ulcers, gastric problems, diarrhoea and skin rashes. Free radicals from carbide play a major role in the ageing process as well as in the onset of cancer, heart disease, stroke, arthritis and perhaps allergies [12,13,14].

Few laboratorial studies have also reported the toxicological effects associated with consumption of calcium carbide-ripened fruits that include changes in some hormonal parameters, oxidative stress enzymes, haematological indices, and morphologies of ovaries and testes in rats [15,16,17,18,19]. These studies also reported that calcium carbide-ripened fruits could accelerate puberty onset as well as reduce sperm count. Studies showed that there is also a significant risk for CaC_2 handlers [20]. The current study was designed to characterize some possible effects of CaC_2 by taking male mice as experimental model.

2. MATERIALS AND METHODS

2.1 Survey Area

For gathering information about the use of CaC_2, we made a survey based on questionnaires and interviews of the local fruit sellers of Khahigarh village, located in Raha subdivision of Nagaon district in Assam, India.

2.2 Maintenance of Animals

Adult male Swiss albino mice (*Mus musculus*) having body weight 28 ± 2 g and 8–10-week-old were selected from an inbred colony maintained in the animal house of the Department of Zoology, Gauhati University. During the period of

acclimatization (two weeks), the animals were fed on synthetic pellets specified for mice-feed and had continuous access to water. They were maintained under standard conditions with a 12-hour light and dark period and controlled room temperature (24±4°C).

2.3 Experimental Chemical

Calcium carbide (CaC_2), also known as calcium acetylide, is a chemical compound, mainly used in the production of acetylene and calcium cyanamide. It is colourless when pure, but black to greyish-white in colour otherwise, with slight garlic-like odour. Industrially it is prepared from a mixture of lime and coke. In India, CaC_2 is popularly known as "masala'" or "carbide" and is used as a ripening agent (though banned in many countries). In contact with moisture, CaC_2 produces acetylene gas which is an analogue of natural ripening hormone ethylene and quickens the ripening process. Industrial grade calcium carbide also contains trace amounts of more toxic arsenic and phosphorous that converts the healthy fruits poisonous.

2.4 Treatment

The mice were randomly divided in to three groups namely I, II, and III, having six animals in each group. Group I was control group, where the mice were fed with naturally ripen banana and clean water. Group II: where the mice were fed with 50g CaC_2- ripened banana and clean water. Group III: where the mice were fed with 100g CaC_2- ripened banana and clean water. After treatment period, blood samples were collected from the right ventricles of the animals. Then the animals were sacrificed by euthanasia with thiopentone sodium in overdose (100 mg/kg body wt.). On sacrificing the mice, tissue samples were collected and stored properly to perform the experiments.

2.5 Formulation of CaC_2 for Treatment

Banana collected from Khahigrah, were divided into two categories: the first category was left to ripe naturally; the another one having ten numbers of banana was exposed to 100gm CaC_2, to ripe artificially. CaC_2 was purchased from fancy Bazar market situated in Guwahati, Assam. The ripe banana from different categories were blended separately with the help of mortar pestle to obtain smooth paste, to feed orally to the experimental animals. Treatments were carried out daily for two times: at the empty stomach and in the evening at 12 hours interval, for 14 consecutive days. Two different doses, i.e., 50g and 100g CaC_2-ripenend banana per kg body weight of the animals, were formulated to carry out the experiments.

2.6 Haematological Parameters

Haemoglobin concentration (HbC) was estimated by using Sahli's haemoglobinometer [21]. Improved Neubauer haemocytometer was used to count total RBCs and total WBCs [22].

2.7 Micronuclei Test

Micronucleus test as per the method of Fenech [23], was used for toxicological screening to evaluate potential genotoxicity of the test compound.

2.8 Histological Study

For histological study routine eosin-haematoxylin staining procedure was followed.

2.9 Statistical Analysis

The data obtained were statistically analysed using mean ±SE (Standard Error). One-way ANOVA test was used to derive significant differences between means through SPSS.

3. RESULTS

3.1 Haematological Analysis

Total RBCs, WBCs and HbC: In both the treated groups, the total number of RBCs decreases with an increase in the concentration of the formulation of CaC_2 as shown in Table 1. However, the results were significant in high dose received group ($p<0.05$)**.** The results were also significant between the treatment groups ($p<0.05$).

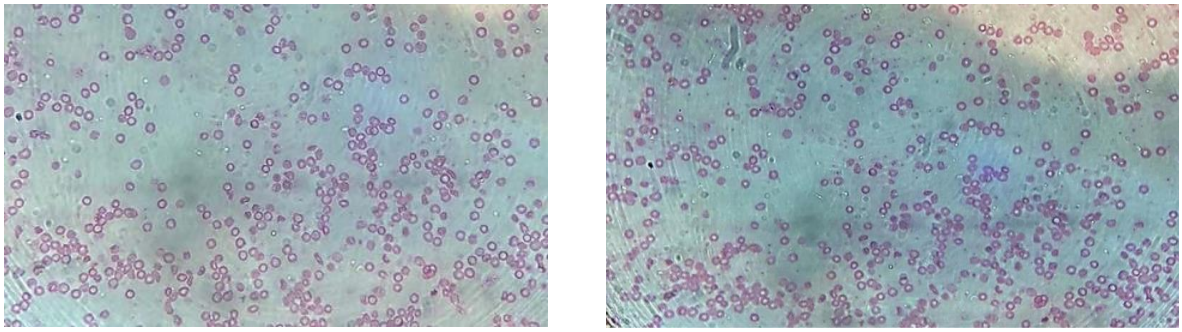
Similarly, the total number of WBCs increases in both the low and high dose received groups, with the increasing concentration of the formulation of CaC_2. However, the results were significant in high dose received group ($p<0.05$). The results were also significant between the treatment groups ($p<0.05$).

The amount of HbC also decreases in both the experimental doses with the increasing concentration of the formulation of CaC_2. However, the results were significant only at the high dose received group ($p<0.001$).

Table 1. Total RBCs, WBCs and HbC in control and 50g and 100g CaC_2-ripenend banana treated albino mice

Experimental group	RBCs (10^6 cells/µl)	WBCs (10^3 cells/µl)	HbC (gm/dl)
control	7.015±0.196	9.025±0.101	12.15±0.189
50g/kg	6.841±0.213[b, c]	9.566±0.364[b, c]	10.93±0.170[a, b]
100g/kg	5.85±0.281[a]	10.80±0.247[a]	10.53±0.199[a, d]

Each value is mean ± SE of six observations (superscript a denotes significant with control at p< 0.05; b denotes not significant with control at p< 0.05; c denotes significant with high dose received group at p< 0.05; d denotes not significant with low dose received group at p< 0.001)



A B

Fig. 1. Showing normal blood cells in both control group (A) and 100g CaC_2-ripenend banana treated group (B)

3.2 Micronuclei assay analysis

No micronuclei were observed in any group of the treated mice (Fig. 1).

3.3 Histological Observation

3.3.1 Histology of liver tissue

The histopathological analysis of the liver of 50g/kg CaC_2-ripenend banana treated animals did not exhibited any change when compared with control liver. However, the liver of 100g/kg CaC_2-ripenend banana treated animal showed dilated central vein, distorted portal vein, with vacuolated large hepatocytes and leucocyte infiltration (Figs. 2,3,4,5).

3.3.2 Histology of kidney

The histopathological analysis of the kidney of 50g/kg CaC_2-ripened banana treated animals did not exhibited any change when compared with control liver. However, the kidney of 100g/kg CaC_2-ripened banana treated animal showed some glomerular structure in raptured condition (Figs. 6,7).

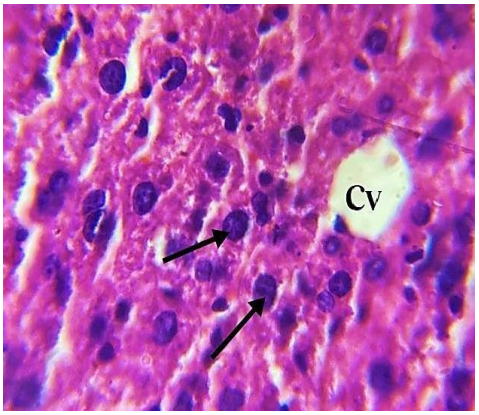


Fig. 2. Control liver tissue showing normal hepatocytes (arrow) and central vein (Cv). (100 X)

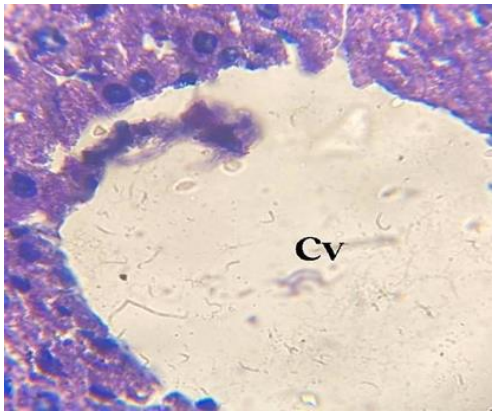


Fig. 3. 100g/kg CaC_2-ripenend banana treated liver tissue showing dilated central vein (Cv) (100 X)

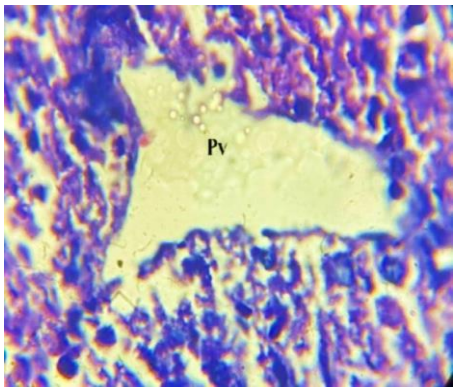


Fig. 4. Control liver tissue showing normal portal vein (Pv) (100 X)

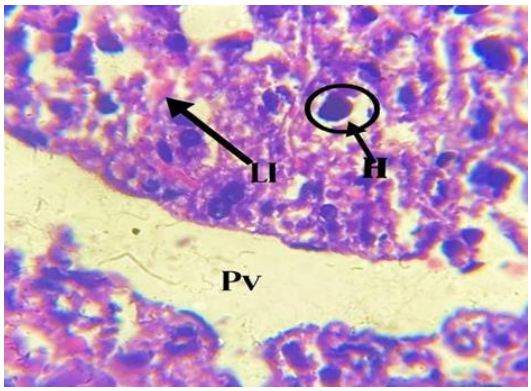


Fig. 5. 100g/kg CaC2-ripened banana treated liver showing distorted portal vein (Pv), vacuolated large hepatocytes (H, black circle) and leucocyte infiltration (LI, arrow) (100 X)

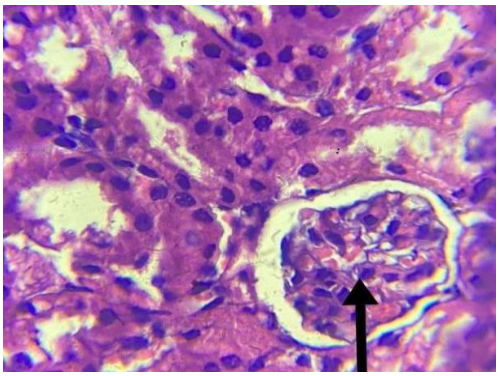


Fig. 6. Control group kidney showing normal glomerular structure (100 X)

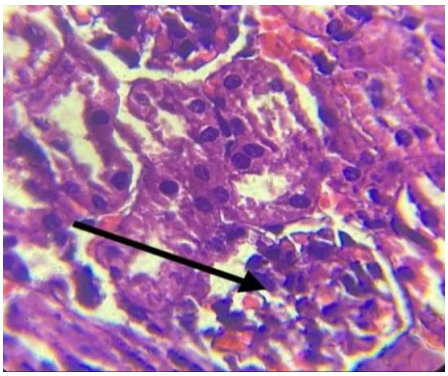


Fig. 7. 100g/kg CaC2-ripened banana treated group (black arrow) showing ruptured glomerular structure (black arrow) (100 X)

3.3.3 Histology of testes

The histopathological analysis of the testes of 50g/kg CaC_2-ripened banana treated animals did not exhibited any change when compared with control testes. However, the testes of 100g/kg CaC_2-ripened banana treated animal showed a lesser number of germ cells in the seminiferous tubules in comparison with the control (Figs. 8, 9).

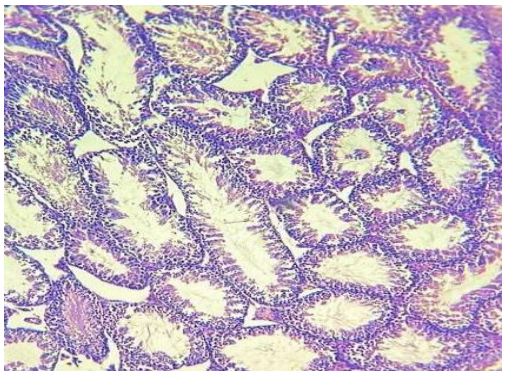


Fig. 8. Control testis showing normal germ cells in the seminiferous cells (black arrow) (10 X)

Fig. 9. Testes of 100g/kg treated CaC2-ripened banana treated group showing lesser number of germ cells in the seminiferous tubule (black arrow) (10 X)

4. DISCUSSION

In the present study the amount of haemoglobin was decreases significantly in both the experimental doses, with increasing concentration of CaC_2 formulation. In both the groups, the total number of RBCs was also decreases significantly with increasing concentration of CaC_2 formulation. Similar findings were reported by Ouma et al. [19]. A decrease in RBCs and Hb of CaC_2-ripened banana treated groups, may be due to several factors including impaired erythropoiesis, accelerated RBC lysis and anaemia [12]. However, in the present study the total number of WBCs was increases significantly in high dose received groups. This result is in agreement with Andrew et al. [24] who also observed significant increase in WBCs. In animals WBC plays a major role in the defence mechanism. Thus, an increase in WBC may be a defence against the invading toxicant.

Micronucleus assay has become one of the most popular methods to assess genotoxicity of different chemical and some physical factors, that induced DNA damage. In the present study no micronuclei were observed in all the treated groups. Hence, the chemical has no genotoxic effects. However, further study is required in this context.

The present study further investigated the effect of CaC_2 through histopathological analysis of the liver, kidney and testes to support haematological analysis. Exposure to high dose, resulted in the damage of the liver. The liver injury was characterized by dilated central vein, distorted portal vein, with vacuolated large hepatocytes and leucocyte infiltration. Histopathological analysis of the kidney of high dose received groups was characterized by some raptured glomerular structure. Similar findings are reported in many studies [18,19,25]. Liver and kidney are essential organs controlling vital physiological and biochemical processes such as homeostasis, detoxification and elimination of lethal metabolites and drugs. Hence, it can be concluded that histological alterations of liver and kidney are due to the effects of CaC_2 and/or its metabolites.

In this study the testes of high dose received animals showed a lesser number of germ cells in the seminiferous tubules in comparison with the control. This finding is consistent with the study of Yadav et al. [26], who reported a change in the normal cytoarchitecture of testes, characterized by decreased diameter of seminiferous tubules and decreased number of spermatids. However, further elaborate study is required to evaluate the effects of CaC_2 on testes.

5. CONCLUSION

The present study reported that fruits ripened by CaC_2 poses some great health hazards. There were several variations in the measured haematological indices and observed histopathological indices, especially for animals in the group that were fed on diets containing high doses of banana ripened with calcium carbide. Thus, the results suggest that consumption of banana ripened with calcium carbide can lower the body's ability to resist some alterations of normal functions. Moreover, future elaborate studies on the effect of calcium carbide ripened fruits on male fertility is also suggested.

Despite the prohibition on sale of fruits that have been artificially ripened, the traders often found selling such ripened products. Although FSSAI has banned the use of CaC_2 for fruit ripening process due to its serious health risks, traders in India have been using calcium carbide freely to ripen fruits in India. Despite the legal prohibition, it is easily available in the market. Therefore, administrative actions should be reinforced more vigorously. If possible, its production must be banned. Due attention should be given to build awareness among traders and consumers, to enforce restrictions on the illicit use of the toxicant for fruit ripening. Above all, natural ways and products should be researched for ripening purpose.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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