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CYTOTOXIC EFFECTS OF CALCIUM CARBIDE ON SWISS ALBINO MICE

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ABSTRACT

Calcium carbide contains traces of hazardous arsenic and phosphorous. Once dissolved in water, the carbide produces acetylene gas that quickens the ripening process, hence is used for ripening fruits. After ingestion acetylene produces free radicals which initiate detrimental effects on various organs of human body. The aim of the study was to investigate the architectural changes of vital organs in calcium carbide induced Swiss Albino mice and measure the adverse effects of calcium carbide in mice tissues. Twenty-Four (24) mice of 7 days old were bought from the Animal Resource Center, ICDDRB. Mice at 60 days old was randomly used for the experiment and they were divided into four groups: each group consisted of six mice namely group-1 (G1), group-II (G2), group- III (G3) and group-IV (G4), respectively. A concentration of 3g, 8g and 12g/kg body weight of calcium carbide was administered orally once daily for 45 days in each group. Group-I (control group) was supplied with normal foods an *ad libitum* water. On day 45 following administration of calcium carbide in different important organs (stomach, liver, heart, kidney) were collected from the mice of both control and treated groups to investigate the effect of calcium carbide. The weight of the liver was decreased non-significantly in groups 2 and 3, and increased non-significantly in group G3T2 (2.00±0.1544). The weight of the heart was increased non-significantly ($p>0.05$) in group G3T2 and G4T3 but decreased in other groups. Histopathological studies were done in different organs and revealed different microscopic lesions. Kidney showed thickening of the lining of the collecting tubules with change in cell structure and also some glomerulus structure in raptured condition. For many years ethylene has been used as a fruit ripening agent. Recently calcium carbide is getting popular in this regard as it has a faster ripening property. It was concluded that the appropriate recommended dose of calcium carbide for ripening fruits has no significant health hazards effect.

Keywords: Cytotoxic effects, calcium carbide, albino mice

Introduction

Fruits are one of the best natural food sources that are usually edible in raw state, such as apple, bananas, grapes, lemons, oranges etc. Now a day, the fruits are deliberately being contaminated by the hazardous chemicals during storing and ripening for commercial purpose (Cua and Lizada 1989). The pre-treatments of fruits which are intended for better consumer acceptance and marketing facilities is called artificial fruit ripening. The commonly used artificial ripening agents are calcium carbide, acetylene, ethylene, propylene, ethrel (2- chloroethyl phosphonic acid), glycol, ethanol and some other agents (Abeles and Gahagan 1968, Asif 2012).

Once dissolved in water, the carbide produces acetylene gas that quickens the ripening process, hence is used in ripening fruits (Cua and Lizada 1989, Anjum and Ali 2004). In human after ingestion acetylene produces free radicals which initiate detrimental effects on various organs. The free radicals play a major role in the aging process as well as in the onset of cancer, heart disease, stroke, arthritis, and possibly allergies and a host of other ailments. Oxidative DNA damage from active oxygen species such as hydroxyl radical (OH) has been hypothesized to play a critical role in diverse biological processes including mutagenesis, carcinogenesis, radiation damage and cancer chemotherapy (Massalimovet *et al.* 2002). A free radical is a cell killer that wreaks havoc by damaging DNA, altering biochemical compounds, corroding cell

membranes, and destroying cells outright. By definition a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron (Prabha 1997).

Using CaC_2 as ripening agents causes detrimental health problems and damages the tissues of various organs in human body (Freeman 1919). It is impossible to conduct the experimental trial in human being due to toxic effect of CaC_2 as well as it is banned ethically to do any clinical trial on humans. That's why the experiment was performed on Swiss Albino mice as a human model to observe the harmful effect of CaC_2. The researches at cells and tissues level are also very much limited to clear the concepts about toxic effects of CaC_2 on various vital organs of the body (Dudley 2004). So, this experiment was conducted to investigate the cytotoxic effects on tissues of vital organs in CaC_2 induced Swiss Albino mice and the results will be very much helpful for the pathologists as well as researchers also to understand, diagnosis and clarify these results as evidence of CaC_2 toxicity (Mannapovich *et al.* 2017). Thus, the present piece of work is praiseworthy if it was provided the details about architectural and cellular changes of vital organs (Steve 2000). Calcium carbide is one of the major public health concerns in the recent times, the present study was therefore aimed to investigate the histo-architectural changes of vital organs in calcium carbide induced Swiss Albino mice and to measure the toxic dose of calcium carbide for mice tissues.

Materials and Methods

Study area: The experiment was conducted in the laboratory of the Department of Anatomy & Histology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, during May to December, 2018.

Experimental animals: The experiment was carried out on Swiss Albino mice (*Mus musculus*). Twenty-Four (24) mice of 7 days old were bought from the Animal Resource Center, ICDDR, B. The collected mice had neither any developmental disorders, detectable genital diseases nor other diseases that might cause any problem in the experiment or affect the result of the experiment.

Rearing of experimental animals: The mice were adapted at animal rare room Department of Anatomy and Histology, FVMAS, BSMRAU for the period of 60 days before being used for the experiment. The mouse were housed in compartmentalized rectangular plastic cages (9X11X7 inch) wrapped with wire mesh. The cages were kept in well ventilated room at 28±2 ºC and a relative humidity of 70-80% with natural day and light. The mice were cared in proper hygienic conditions, with experimental & normal feeding *ad libitum*. Standard mouse-pellets (collected from ICDDR, B) were used as normal feed. During the whole experimental period, uniformity of the management practices was maintained.

Experimental design: Twenty-four (24) mice at 60 days old were randomly used for the experiment and the mice were divided into four groups, and each group was consisted of six mice which were marked as group-I, group-II, group- III and group-IV, respectively. The group-I was treated as control group (G4T4) and other 3 groups were treated as treatment groups (G1T1, G2T2 and G3T3).

Experimental procedure: A concentration of 3g, 8g and 12g/kg body weight of calcium carbide were administered orally once daily for 45 days to treatment groups with *ad libitum* feed and water, respectively (Morehead and de Chalmot 1996). Group-I (control group) was fed with *ad libitum* foods and water without calcium carbide (Morehead and de Chalmot 1996).

Sample collection: After administration of calcium carbide for 45 days, different important organs (stomach, liver, heart, kidney) were collected from the mice of both control and sample groups to investigate the effect of calcium carbide. The whole experiment was conducted in the laboratory of Anatomy & Histology, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706.

Sample preservation: Immediately after killing, the vital organs were collected as soon as possible with the help of scalpel and scissors avoiding any destruction of the organs. The specimens then were collected and fixed in the 10% formalin solution.

Gross pathology: The postmortem examination in all the cases was performed for the pneumonic mice lung at necropsy, gross tissue changes were observed and recorded carefully and representative tissue sample containing lesions were fixed in 10% neutral buffered formalin for histopathological studies (Booker *et al.* 2008).

Preparation of Harri's Hematoxylin Solution: The hematoxylin and the alum were dissolved in alcohol and in water respectively by the aid of heat. Both the solutions were thoroughly mixed just after removal from heat and boiled as soon as possible. Then mercuric oxide was added slowly after removal from heat. The solution was again heated to simmer and stopped heating when it became dark purple in color. Then the vessel was plunged into a basin of cold water and made it cool. Two to four ml of glacial acetic acid was added per 100ml solution just before use to increase the precision of nuclear stain.

Histopathology: The formalin fixed tissues were trimmed, processed, sectioned and stained as per standard procedure. Specific lesions containing sample from each group were used in histopathological study (Luna 1968).

Photomicrography: Photomicrography was taken using photomicrographic camera (ZEISS AxioCam ERc5s) facilities facilitated by Department of Gynecology, Obstetrics and Reproductive Health of BSMRAU.

Data recording: The data of daily feed intake measure and weakly body weight measure of the mice. After study period gross anatomical data were recorded.

Statistical analysis and data interpretation: Finally, at the end of and histopathological study, all the data were compiled, compared and analyzed for constructive interpretation. Statistical analysis was performed using SPSS (IBM@ Version 21.0, USA). All results are represented as the Means ± S.E.M. For the comparison, one-way analysis of variance (ANOVA) was applied. Differences were considered to be statistically significant when the *p* value was less the 0.05.

Results and Discussion

Effects of Calcium Carbide on body weight gain: Calcium carbide is a toxic component which reduces the body weight gradually. The following figure 1 represented the toxicity of CaC2 on body weight gain. In case of control group (group-I), the body weight gaining rate is higher than the other treated groups. And, the group-II and group-IV showed the lower body weight gaining rate. Analysis of variance of the results revealed that the body weight gaining was varied among the different groups (both control and treated) non- significantly ($p<0.05$) (Table 1). The findings of this study were supported by the other authors (Bhadoria *et al.* 2015).

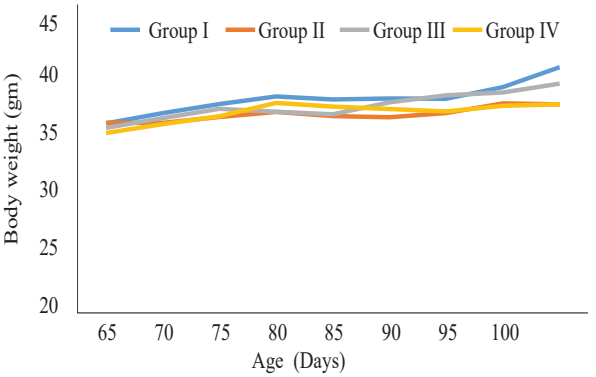

Figure 1. Effects of calcium carbide on body weight of mice.

Effects of calcium carbide on stomach gross anatomical change: The gross anatomical effects of calcium carbide on stomach of mice were presented in the figure 1. Stomach of control group was showed no morphological changes but slight hemorrhage with thin wall of stomach showing in all treated groups. The weights of the stomach (Figure 1) were decreased gradually in all calcium carbide treated feed groups and was lowest in group-IV (G4T3) (0.68±0.099), in comparison to control group (1.49±0.09). It indicated that the high dose of CaC_2 may reduce the organ weight rather than low dose. Subsequently, the lengths of the stomach were increased in all treated groups but highest in the group-III (G3T2) (17.13±0.08) group in comparison to control group (14.7±1.3), and the widths of stomach increased but highest in group-II(G2T1) (8.55±0.04) in comparison to control group (8.25±0.47). Analysis of variance of the results revealed that the differences in the changes of weights, length, widths of stomach among the different groups (both control and treated) were non-significant ($p<0.05$) (Table 1, 2 and 3). These findings were corresponded with the others (Asif 2012, Bhadoria *et al.* 2015).

Effects of calcium carbide on liver of mice: In case of liver swelling were formed on the liver of all treated groups and congestion were also found in all treated groups. The weights of the liver were decreased in groups 2 and 3 and increase in group G3T2 (2.00±0.1544), in comparison to group C (1.44±0.082). Analysis of variance of the results revealed that the differences in the changes of weights of liver among the different groups (both control and treated) were non-significant ($p<0.05$) (Table 1). Histo-pathological lesion was found limited area in the liver of calcium carbide treated mice. In case of treated

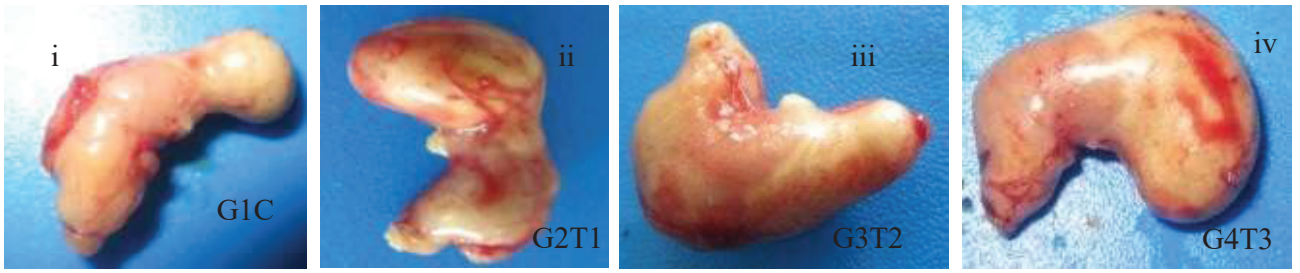

Figure 1 (i-iv). Stomach of mice. Slight hemorrhage with thin wall of stomach.

group there was no significant change but lymphocyte infiltration in the sinusoid, hemorrhage and necrosis were found in case of group G3T2 with massive lymphocyte infiltration in the sinusoid. Bhadoria *et al. (*2015) recorded similar liver swelling lesions.

The gross anatomical effects of calcium carbide on liver of mice are presented in the figure 2. Liver of control group showed no morphological changes but swelling was formed on the liver of all treated groups and congestion were also found in all treated groups. The weights of the liver were decreased in groups 2 and 3, and increase in group G3T2 (2.00±0.1544), in comparison to group C (1.44±0.082). Analysis of variance of the results revealed that the changes of weights of liver among the different groups (both control and treated) were non-significant *p<0.05*) (Table 1). The gross lesions in liver of mice were supported by the other authors Kumar *et al.* (2017)

Histopathologic changes in liver were not seen in control group (Figure 3). In case of treated group there is no significant change but hemorrhage were found in G3T2 & desquamation of villus were found in case of group G2T1 & G4T3 (Figure 4-6) . In case of treated group there is no significant change but lymphocyte infiltration in the sinusoid, hemorrhage & necrosis were found in case of group G3T2 (Figure 5) with lymphocyte infiltration in the sinusoid. The similar histopathologic changes in liver were recorded by kumar *et al.* 2017.

Effects of calcium carbide on heart gross anatomical changes: The gross anatomical effects of calcium carbide on heart of mice are presented in the figure 7. Heart of group C was showed no morphological changes but in group G2T1 & G3T2 showed changes. The heart was slight swollen and in group G4T3 was slight hemorrhaged. The weights of the heart were increased in group G3T2 and G4T3 but decrease in group G2T1 (0.15±0.01) in comparison to C (0.16±0.02). Subsequently, the lengths of the heart decreased in all treated groups and was lowest in group G3T2 (8.4±0.28) in comparison to group C (8.91±0.25). The widths also decreased in all treated groups but it was lowest in group G2T1 (5.5±0.26) in comparison to group C (5.85±0.25). Analysis of variance of the results revealed that the differences in the changes of weights, length, widths of heart among the different groups (both control and treated) were non-significant (*p<0.05*) (Table 1, 2 and 3). Asif 2012 recorded similar gross anatomical lesions in heart.

The histopathological changes were not found in the section of heart of the group C and only showed striated muscle fibers (Figure 8). The intra myocardial spaces were increased in treated groups than the control group.

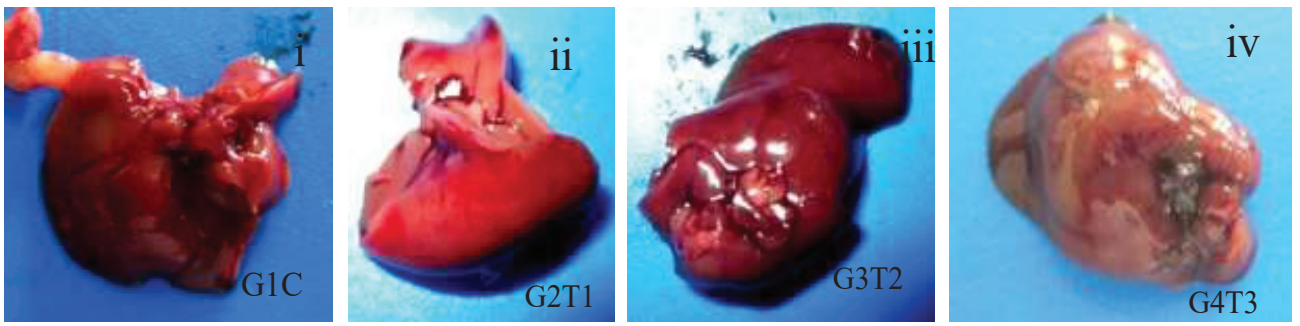

Figure 2 (i-iv). Liver of mice. Swelling are formed on the liver of all treated group (arrow). Congestion in all treated group (red arrow).

Table 1. Weight of stomach, liver, heart and kidney of mice (Mean ± S.E.)

Organ	Group-I	Group-II	Group-III	Group-IV	P Value
Stomach	1.49±0.09	0.89±0.13	0.7±0.8	0.68±0.099	NS
Liver	1.52 ± 0.09	1.38 ± 0.10	1.65 ± 0.09	1.47 ± 0.07	NS
Heart	0.16 ± 0.02	0.15 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	NS
Kidney	0.24 ± 0.02	0.23 ± 0.02	0.25 ± 0.03	0.24 ± 0.01	NS

S.E.= Standard Error
n= 06 samples per group

* = Significant at the level of (p>0.05) NS = Non significant.

Table 2. Length of stomach, heart and kidney of mice (Mean ± S.E.)

Organ	Group-I	Group-II	Group-III	Group-IV	P Value
Stomach	14.7±1.28	15.6±0.78	17.13±0.80	15.5±0.86	NS
Heart	8.91 ± 0.25	8.72 ± 0.32	8.4 ± 0.28	8.8 ± 0.34	NS
Kidney	10.41± 0.33	10.8 ± 0.3	10.64± 0.42	9.85 ± 0.33	NS

S.E.= Standard Error
n= 06 samples per group
* = Significant at the level of (p>0.05) NS = Non significant.

Table 3. Width of stomach heart and kidney of mice (Mean ± S.E.)

Organ	Group-I	Group-II	Group-III	Group-IV	P Value
Stomach	8.25±0.47	8.55±0.36	8.4±0.48	8.33±0.32	NS
Heart	5.85 ± 0.25	5.5 ± 0.26	5.56 ± 0.26	5.76± 0.32	NS
Kidney	6.47± 0.35	6.53 ± 0.3	6.5± 0.36	6.17± 0.51	NS

S.E.=Standard Error
n= 06 samples per group
* = Significant at the level of (p>0.05) NS = Non significant.

Scattered distributed haemorrhages were also found in all treated group. Histo-pathological lesion was found in the heart of calcium carbide treated mice (Figure 9-11). The intra myocardial spaces were increased in treated groups rather the control. Scattered lesions were distributed in all treated groups. The weights of the heart were increased in group G3T2 and G4T3 but decrease in group G2T1 (0.15±0.01) in comparison to group C (0.16±0.02). Analysis of variance of the results revealed that the differences in the changes of weights, length, widths of heart among the different groups (both control and treated) were non-significant (*p<0.05*) (Table 1, 2 and 3). Dudley 2004 and Goonatilake 2008 recorded the similar heart lesions.

Gross anatomical changes of calcium carbide on kidneys: Marked changes were observed in case of kidney only. The gross anatomical effects of calcium carbide on kidneys of mice were presented in the figure 12. Kidneys of group C were showed no morphological change (Figure 13) but both kidneys of all treated group were showed congestion and fragile. The weights of both of the kidneys were decreased in all treated groups but increased in group G3T2 of both kidney and was highest in left kidney of group G3T2 (0.26±0.02) in comparison to left kidney of group C (0.25±0.02).The length of kidneys increased in both kidney but decreased in group G4T3 of right kidney (9.85±0.3) in comparison to right kidney of group C (10.41±0.3). The width of kidneys were increased but decreased in group G4T3 of right kidney (6.17±0.51) in comparison to right kidney of group C (6.44±.35). Analysis of variance of the results revealed that the differences in the changes of weights, length,

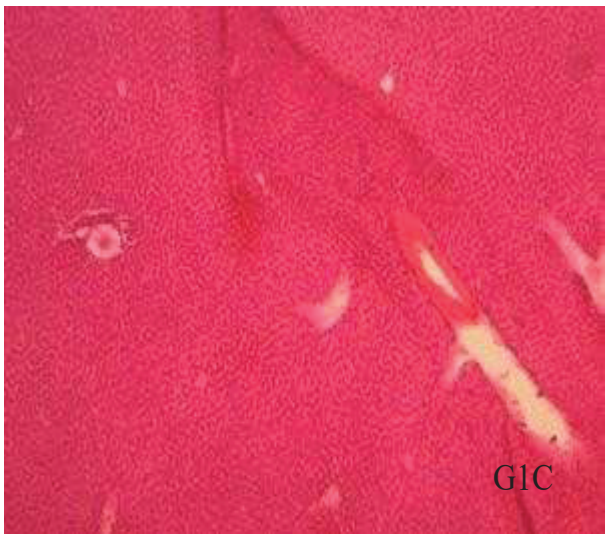

Figure 3. Histology of liver of control group showing normal structure. H&E stain (X100)

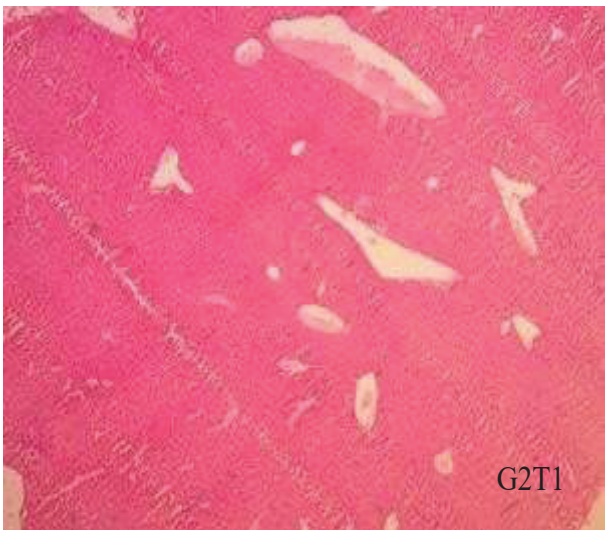

Figure 4. Histology of liver (Group-II), no change were found, H&E stain (X100)

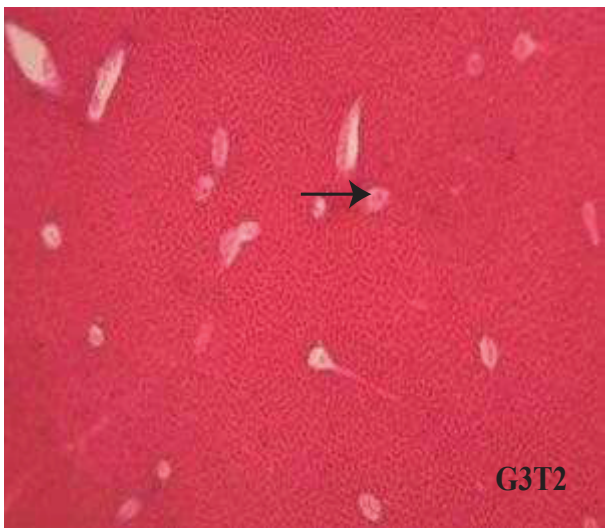

Figure 5. Histology of liver (Group-III), lymphocyte infiltration in the sinusoid H&E stain (X100)

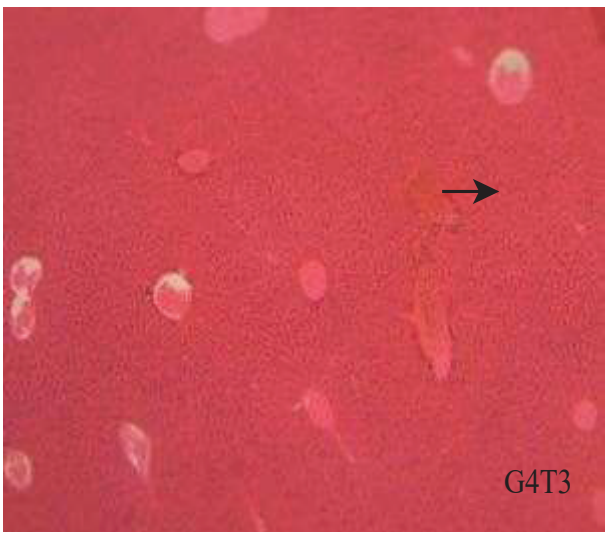

Figure 6. Histology of liver (Group-IV), Arrows showing haemorrhage of liver, H&E stain (X100)

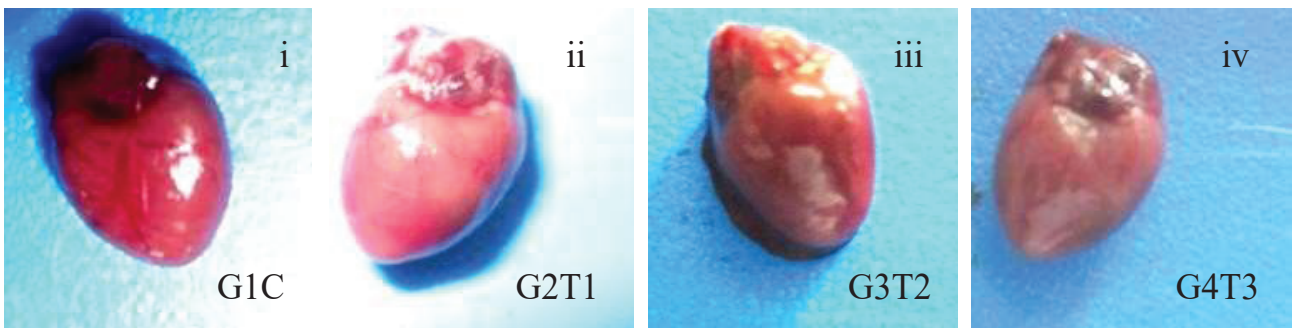

Figure 7. (i-iv). Heart of mice. Group G2T1 & G3T2 showing slight swollen and in case of group G4T3 is Slight haemorrhage.

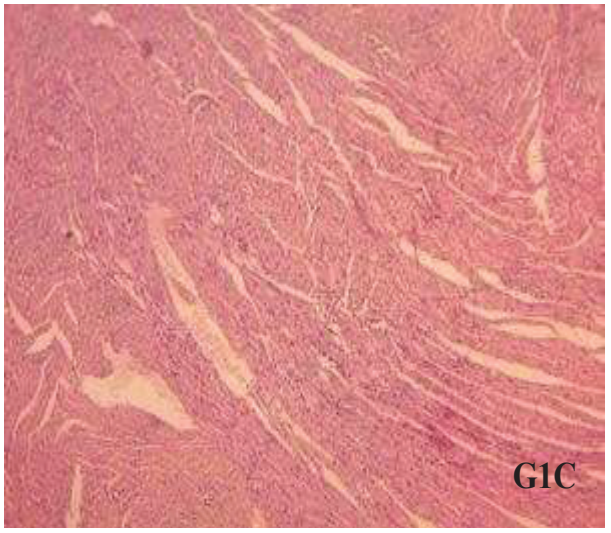

Figure 8. Histology of heart of control group showing normal structure (Group-I), H&E stain (X100)

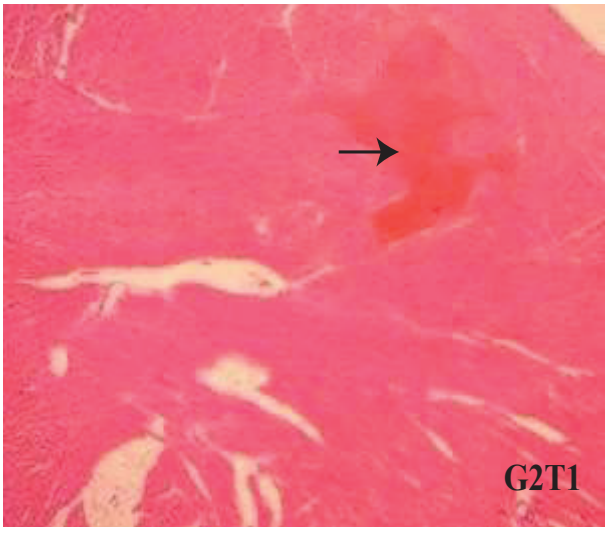

Figure 9. Histology of heart (Group-II), Arrow showing Scattered distributed haemorrhage were also found in heart, stain (X 100)

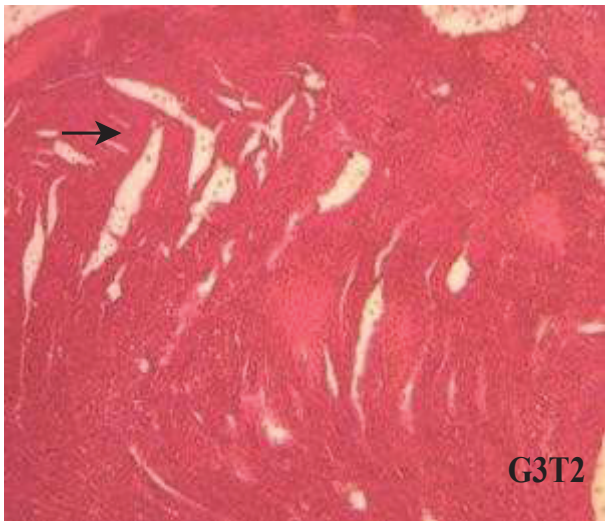

Figure 10. Histology of heart: Arrow indicate intra myocardial spaces were increased (Group-III), H&E stain (X100)

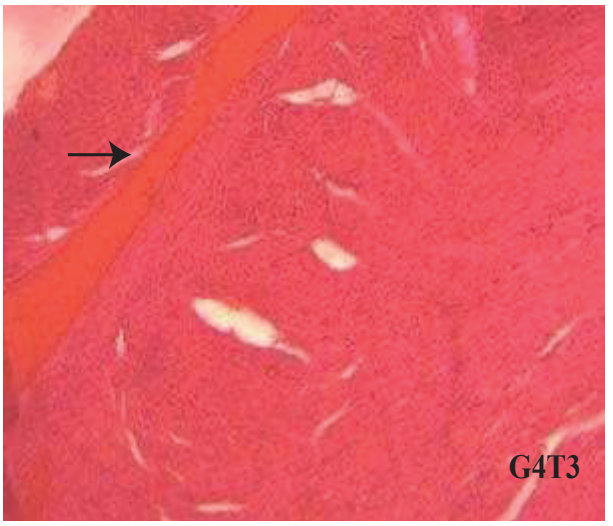

Figure 11. Histology of heart (Group-IV): Arrow showing Scattered distributed haemorrhage were also found in heart, H&E stain (X100)

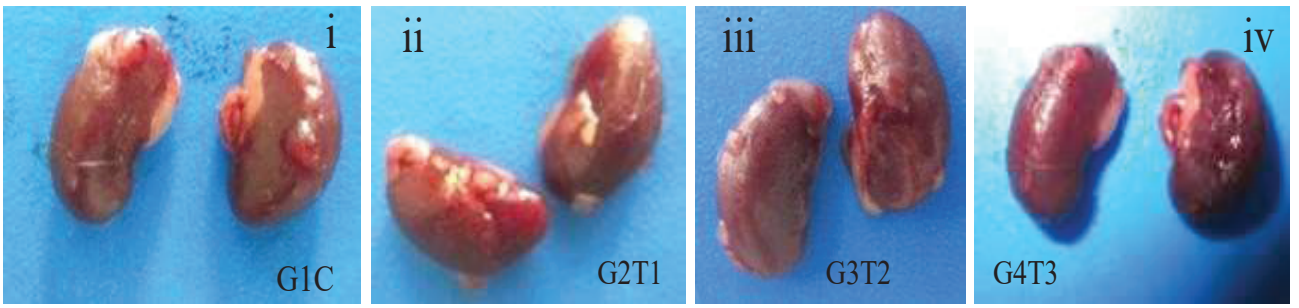

Figure 12 (i-iv). Kidneys of mice: Both kidneys of all treated groups showing congestion and fragile.

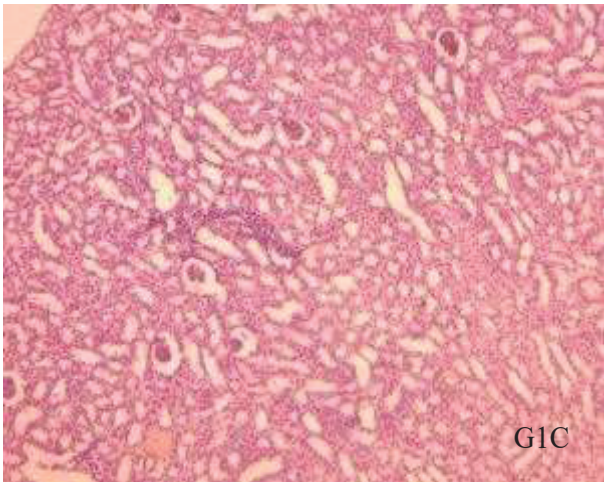

Figure 13. Histology of kidney of control group showing normal structure (Group-I), H&E stain (X100)

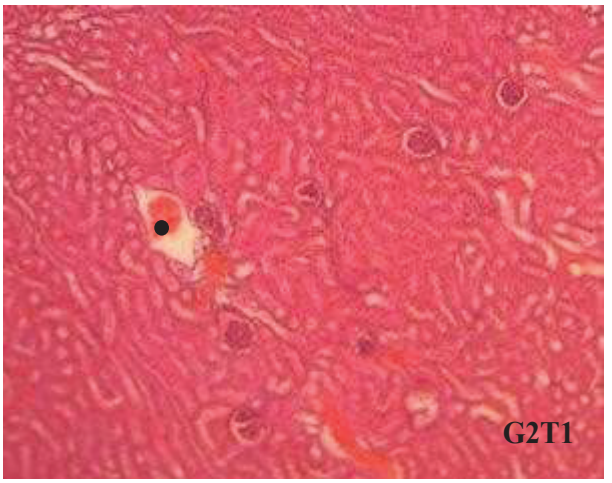

Figure 14. Histology of kidney. star mark indicate focal areas of consolidation that was found red- brown to red (Group-II), H&E stain (X100).

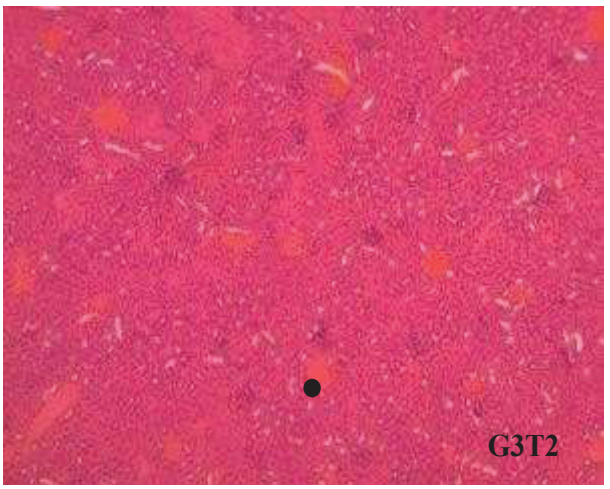

Figure 15. Histology of kidney. star mark indicate focal areas of consolidation that was found red- brown to red (Group-III), H&E stain (X100)

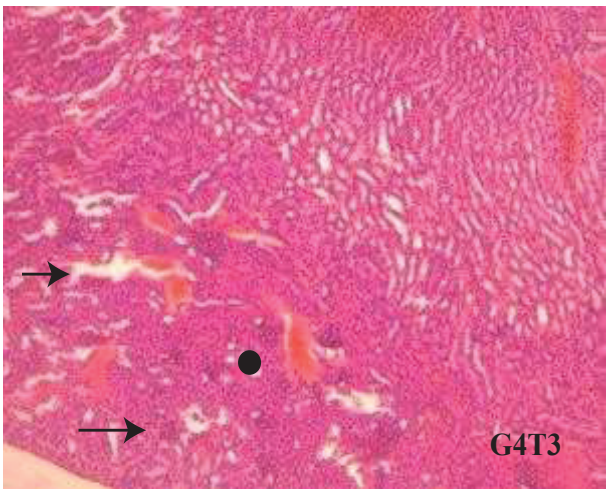

Figure 16. Histology of kidney. star mark indicate focal areas of consolidation that was found red- brown to red, glomerulus structure in raptured condition, (Group-III), H&E stain (X100)

width of kidney among the different groups (both control and treated) were non- significant ($p<0.05$) (Table 1, 2 and 3). Congestion and fragile kidneys were also found with infection of calcium carbide (Nair and Singh, 2003).

The kidneys of calcium carbide treated mice were severely damaged. Both kidneys of all treated group were showed congestion and fragile. The weights of both of the kidneys were decreased in all treated groups but increase in group G3T2 of both kidney and was highest in left kidney of group G3T2 (0.26±0.02) in comparison to left kidney of group C (0.25±0.02). The length were increased in both kidneys but decreased in group G4T3 of right kidney (9.85±0.3) in comparison to right kidney of group C (10.41±0.3). The width of kidneys were increased but decreased in group G4T3 of right kidney (6.17±0.51) in comparison to right kidney of group C (6.44±.35). Analysis of variance of the results revealed that the differences in the changes of weights, length, width of kidney among the different groups (both control and treated) were non-significant ($p<0.05$) (Table 1, 2

and 3).The findings were corresponded with Patoare et.al. 2014 and Goonatilake R. 2008.

Histopathologically, focal areas of consolidation were found as red-brown to red (star mark) (Figure 14, 15 & 16), fibrous thickening of septa and hypertrophy of lining septal cell were also observed in rats of sample group. Histopathological analysis of kidney showed thickening of the lining of the collecting tubules with change in cell structure and also revealed some glomerulus structure in raptured condition. Focal area of consolidation of kidneys were also reported by others (Moissan 1982, Patnaik 2002, Patoare *et al.* 2014).

Conclusion

Calcium carbide is widely used in Bangladesh for ripening different fruits as a cheap alternative to natural plant hormone ethylene. In this study no significant effects of CaC_2 were not found in the liver, heart and kidney of Swiss Albino mice. So, appropriate dose of calcium carbide has no health hazard. High dose of carbide is used on a raw fruit for ripening purposes that results in poor flavor of the fruit and possibly toxic for the human health. Further details study is suggested for measuring the safety level of calcium carbide in human food chain.

Acknowledgement

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