EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 2
276-279
Year: 2023

# EFFECT OF CALCIUM CARBIDE ON TESTIS OF WISTAR ALBINO RATS

**Dr. Gita Yadav*[1], Dr. Shamsher Shrestha[2], Dr. Prabhakar Yadav[3], Dr. Churamani Pokhrel[4]**

[1]Associate Professor, [2]Additional Professor, [3]Associate Professor, [4]Assistant Professor
Department of Human Anatomy, B.P. Koirala Institute of Health Sciences, Nepal.

***Corresponding Author: Dr. Gita Yadav**
Associate Professor, Department of Human Anatomy, B.P. Koirala Institute of Health Sciences, Nepal.

**Article Received on 01/12/2022** **Article Revised on 21/12/2022** **Article Accepted on 11/01/2023**

## ABSTRACT

**Introduction**: Calcium carbide is commonly used as artificial fruit ripening agent. Once $CaC_2$ comes in contact with moisture it releases acetylene gas (Carbide gas) which acts similar to the fruit ripening agent ethylene. Ingestion of acetylene produces radicals which initiate detrimental effects on various organs including all the human system. **Aims & Objectives:** To investigate the effect of calcium carbide on testis via evaluation of gross and histological changes. **Materials and Methods**: Twenty four male healthy wistar albino rats weighing 130-200gm were randomly divided into 4 groups i.e. control group (received normal diet) and group I (normal diet mixed with 0.5gm $CaC_2$), group II (received normal diet mixed with 0.25gm $CaC_2$), group III (normal diet mixed with 0.125gm $CaC_2$). Each group consisting of 6 rats. All groups were treated over a period of consecutive 21 days. On 22$^{nd}$ day all rats were anesthetized by inhalation of ether and scarified by cervical dislocation. Testis were removed and preserved in 10% formalin solution for histological examination. One way ANOVA was used for data analysis at 95% confidence interval .The experiment was carried out at the animal house of department of Human Anatomy. **Results**: $CaC_2$ had no effect on body weight of rats. The weight of rats was increased in all groups. For weight of testis as compared to control group (mean± SD = 1.12 ± .07) the weight of group I (mean± SD = .54 ± .10) decreased but in group II (mean± SD = 1.18 ± .12) and in group III (mean± SD = 1.16 ± .03) there were no as such effect. Histological study of testis showed decrease in diameter of seminiferous tubules of group I (mean± SD = 62.89 ± 14.07) as compared to control group (mean± SD = 105.77 ± 8.69) but no effect on group II (mean± SD = 104.00 ± 5.99) and group III (mean± SD = 114.84 ± 15.37). **Conclusion**: Calcium carbide caused both morphological and histological changes in testis of wistar albino rats.

**KEYWORDS**: Calcium carbide, acetylene, testis, light microscopy and histology.

## INTRODUCTION

Calcium carbide ($CaC_2$) is a grayish black lump of crystalline powder with garlic like odor, which is mainly used to generate flammable acetylene gas, which acts as reducing agent.so also used in steel manufacturing and metal cutting.[1]

Once $CaC_2$ comes in contact with moisture it releases acetylene gas (Carbide gas) which acts similar to the fruit ripening agent ethylene.[2] Normally fruits produce a ripening hormone ethylene that induces the natural process of maturation. This process is artificially accelerated by using different chemicals of which calcium carbide is the commonest. Calcium carbide in contact with moisture produces acetylene which is an analogue of natural ripening hormone ethylene. Acetylene is a highly reactive substance used mainly in welding and allied industries. Industrial grade calcium carbide also contains trace amounts of more toxic arsenic and phosphorous that converts the healthy fruits poisonous.[3]

The south Asian association for regional co-operation (SAARC) countries have wide range of climatic condition with altitude and agroecology suited for a wide diversity of tropical fruits.[4] Fruits plays vital role in human nutrition by supplying the necessary growth regulating factors essential for maintaining normal health.[5] So traders pick green fruits before maturation, ripen artificially to serve in the market earlier than the season for higher profit. Moreover green fruits are transported easily with minimum damage and ripened at the place of retail sell.[6] The rising demand of fruit safety has inspired researchers think about the risk related to the use of fruit contaminated by pesticides, heavy metal or toxins.[5] The different ripening agents include calcium carbide, acetylene, ethylene, propylene, ethrel, glycol and smoke are used by farmer to harvest immature fruits and prevent damage of transportation of ripe fruits, herby enhancing the profits of the sellers.[7]

$CaC_2$ is used as ripening agent for mangoes, bananas, jackfruits, litchis and other fruits also. Packets of calcium

carbide powder are kept in the container of fruits where in contact with moisture, acetylene gas is produced and acts as a ripening agent. In many developing countries[8], calcium carbide is commonly used to artificially ripening the fruit process because it is cheap and readily available. During ripening process, a wide spectrum of biochemical changes takes place, such as chlorophyll degradation, biosynthesis of carotenoids (antioxidants, immune system boosters, anticancer agent), anthocyanin's (powerful antioxidants), essential oil, flavor and aroma components. A similar condition induced by $cac_2$ with help of acetylene gas have good peel color development but poor in flavor[7] which is due to the production of complex mixture of volatile compound ocimene and myrcene and degradation of alkaloids sesquiterpene, which are responsible for flavor and aroma.[8]

SAARC does not have unified policy on fruit cultivation, preservation and distribution within SAARC countries but has banned the use of calcium carbide as an artificial fruit ripening agent. Developing countries like Nepal also have Nepal food regulation 2027, part7; rule no 19 (d) and food law of Nepal (2057).[9] which strongly prohibits the use of carbide gas in fruit ripening. But they are still use for mangoes, bananas, jack fruits, litchis and other fruits.

The application of carbide in commercial practice is important because important impurities such as phosphorous hydride (25 ppm) and arsenic hydride (3 ppm) present in commercial grade calcium carbide could cause health hazards.[10] These hydrides are fat soluble and dissolve in wax layer of fruits.[11] Ingestion of acetylene produces radicals which initiate detrimental effects on various organs and plays important role in aging process, heart disease, stroke and arthritis's.[12] Other toxic effect includes wide range of adverse side effect including all the human system. The toxic effect of $CaC_2$ are food poisoning, gastric irritation difficulty in swallowing, vomiting because of alkaline nature of carbide which usually causes stomach upset and erodes the mucosal tissue in stomach, disrupting the normal intestinal function leading to severe esophageal and gastric damage.[12,13,14]

Similarly, carbide reduces oxygen supply to brain which leads to headache, vertigo, dizziness, delirium, seizure memory loss, cerebral edema and even coma.[15] It causes mouth ulcer, chelitis, grade III mucositis, dry mouth, chronic skin ulcers, eye damage, blindness, stinging pain, watering of eye, inflammation of eyelid, conjunctivitis, opacity and scarring.[16]

Ogbuagu DH and Oritsematosan EJ researched over induction of $cac_2$ on sperm morphology and viability of the Albino mice in 2016 over thirty mice of age 8 weeks of weight 32.5 ±2.0 gram. The animal were divided in 5 groups and treated with $CaC_2$ with graded toxicant concentration (W/W) of 2.5 %, 1.25%, 0.625 %, 0.312% in 4 cages for % weeks and sacrificed. They analyzed the Cauda epididymis and common abnormalities observed were Double head sperm, Pin head sperm, knobbed head sperm, No tail with hook sperm and concludes $CaC_2$ induces morphological abnormalities and reduces viability in sperm cells.[2] So through this study I wanted to see effects of calcium carbide on kidney of albino rat. Due to scarce of such type of study in our setting will help to aware the public regarding the use of calcium carbide and as well as the result of the study can be used as data bank.

## MATERIALS AND METHODS

Twenty four healthy male wistar albino rats weighing 130-200 gram were randomly selected for the study. The rats were acclimatized for 4 weeks before enrolling in the study. The animals were housed in plastic cages and were placed in room at atmospheric temperature and humidity with 12-hour light-dark cycles without any stressful stimuli. All the rats were provided free access to standard mashed rodent diet and water ad libitum.

Calcium carbide was obtained from chamunda importer and exporter (Manufacturer- TYWH, made in china)

After the quarantine period rats were randomly divided into four groups each groups, each consisting the six animals. Control group received normal diet that is120gm Bengal gram. Group I received 120gm Bengal gram mixed with 0.5gm of $CaC_2$. Group II received 120gm of Bengal gram mixed with 0.25gm of $CaC_2$. Group III received 120gm of Bengal gram mixed with 0.125gm/kg of $CaC_2$. All groups were treated over a period of 21 consecutive days. Twenty-four hours after the administration of last doses, on 22 day, rats were anesthetized by inhalation of ether and sacrificed. Testis was taken out by abdominal dissection, weighed and was preserved in 10% buffered formaldehyde solution. After tissue processing slides were prepared by H&E staining and were observed for histological changes.[17]

A minimum of 8 fields for each testis section was examined under light microscopy and oculomicrometers were used for quantitative measurements of parametes like diameter of seminiferous tubules. The diameter of 100 seminiferous tubules was measured and the mean value of each group was calculated.

Statistical analysis was performed by the use of SPSS software (Statistical Package for the Social Sciences). Results were presented as mean values ± standard deviation. The one way analysis of variance (ANOVA) test was used for data analysis and probability values (P) less than 0.05 will be considered statistically significant.

## RESULTS

**Body weight of rats:** The details of body weight before and after experiment are shown in table 1. After experiment when control group body weight is compared with other experimental group the p value is .348 which is >0.05 so statically not significant. The body weight of

all groups is almost equally increased in three week so calcium carbide has no effect on body weight.

**Table 1: Body weight of rat before and after experiment in gram mean± SD.**

| | Before experiment | After experiment |
|---|---|---|
| Control | 132 ± 7.583 | 176 ± 11.14 |
| Group I | 134 ± 6.44 | 181 ± 11.94 |
| Group II | 136 ± .81 | 179 ± 4.54 |
| Group III | 128 ± 5.78 | 173 ± 5.75 |
| P value | .128 | .438 |

**Weight of testis:** After experimental period the weight of testis were found as shown in table 2. As compared to control group the weight of group I decreased but in group II and in group III there were no effect. It means high dose of $CaC_2$ reduces the weight of testis but low dose has no effect on weight of testis.

**Table 2.Weight of testis in gram mean ± SD.**

| | Testis |
|---|---|
| Control | 1.12 ± .07 |
| Group I | .54 ± .10 |
| Group II | 1.18 ± .12 |
| Group III | 1.16 ± .03 |
| P value | **.000** |

**Histological study of testis:** as shown in table 3 the diameter of seminiferous tubules decreased in group I but had no change in group II and group III. It showed that only high dose of calcium carbide affects the diameter of seminiferous tubules but low dose has no effect.

**Table 3: Diameter of seminiferous tubules.**

| | Seminiferous Tubules |
|---|---|
| Control | 105.77 ± 8.69 |
| Group I | 62.89 ± 14.07 |
| Group II | 104.00 ± 5.99 |
| Group III | 114.84 ± 15.37 |
| P value | .000 |

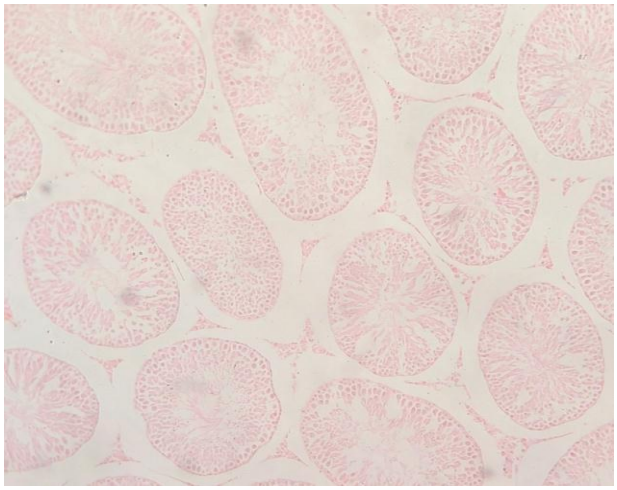

**Figure 1: photomicrograph of rat testis section showing normal histology of control group (100X).**

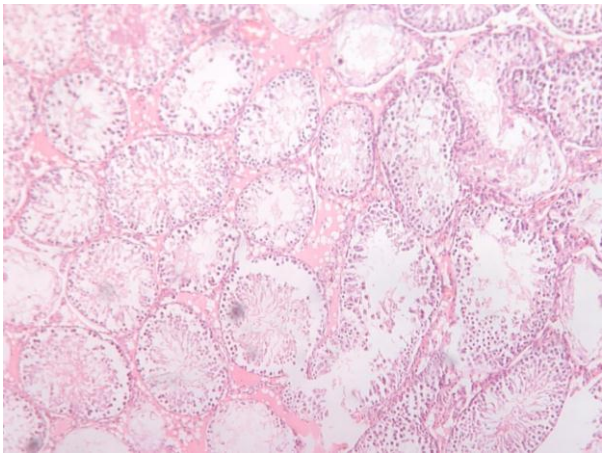

**Figure 2: photomicrograph of rat testis showing changes in cytoarchitecture of group I (100X). Normal cytoarchitecture is disturbed, diameter of seminiferous tubules is decreased and number of spermatids are also decreased.**

## DISCUSSION

This study was done to see the effect of $CaC_2$ on body weight of rat, weight of testis and histological changes on testis. Exposure to $CaC_2$ resulted in body weight gain in all three experimental groups as similar to control group which showed that $CaC_2$ has no effect on body weight the p value was .348 which is >0.05 so statically not significant. Study done by Andrew GS et al (2018) also showed similar effect on body weight.[18]

While comparing control group with experimental groups the weight of testis was decreased in group I and increased in group II and III which were statistically significantly p was value .000. This shows that only high dose affects the weight of testis but low dose have no effect. Histological examination of testis showed decrease in diameter of seminiferous tubules of group I as compared to control group but there was no effect on group II and III. The normal cytoarchitecture of testis was also disturbed.

## CONCLUSION

Calcium carbide caused both morphological and histological changes in testis of wistar albino rats.

## ACKNOWLEDGEMENTS

We are thankful to all members of anatomy department who helped us during this research work.

**Ethical Approval:** Ethical approval was obtained from Institutional Review Committee (IRC/IRB 1460/018) of B.P. Koirala Institute of Health Sciences

**Conflict of Interests:** None

**Funding Support: None**

**Availability of Data and Materials:** All the relevant data will be available upon request through the principal investigator.

**REFRENCES**


1. Hazardous substance fact sheet. NJ Health, August, 2009.
2. Ogbuagu DH, Oritsematosan EJ. Inductions of $CaC_2$ on Sperm Morphology and Viability of the Albino Mice (Mus musculus), 2016; 10(4): 218–22. https://zenodo.org/badge/DOI/10.5281/zenodo.1123709.svg
3. Kader AA. Post-harvest Technology of Horticulture Crops. University of California, 2002; 157. https://irrec.ifas.ufl.edu/postharvest/HOS_5330/Ch4
4. Hossain MF, Akhtar S, Anwar M. Health hazards posed by the consumption of artificially ripened fruits in Bangladesh. Int Food Res J., 2015; 22(5): 1755–60. http://www.ifrj.upm.edu.my/22%20(05)%202015/(3).pdf
5. Olisah M.C., Ilechukwu O.U., Ifemeje J.C., Ofor C.C. Effect of Vitamin C against Calcium Carbide Induced Hepatotoxicity. Tropical Journal of Applied Natural Sciences, 2017; 2(1): 74-77. Doi: https://doi.org/10.25240/TJANS.2017.2.1.12.
6. Fattah SA, Ali MY. Carbide Ripened Fruits- A Recent Health Hazard. Faridpur Med. Coll. J., 2010; 5(2): 37. https://doi.org/10.3329/fmcj.v5i2.6816
7. Patrick I. & Rosemary A. I. Calcium carbide-induced alterations of some haematological and serum biochemical parameters of wistar rats. Asian Journal of Pharmaceutical and Health Sciences, 2016; 1: 1396-1400.
8. Islam MN, Mursalat M, Khan MS. A review on the legislative aspect of artificial fruit ripening. Agric Food Secur, 2016; 5(1): 1–10. https://agricultureandfoodsecurity.biomedcentral.com/articles/10.1186/s40066-016-0057-5
9. Bhurtel S. & Adhikari A.Comparative Study on Ripening Of Banana Using Different Ripening Agents. International Journal of Research, 2017; 04(03).
10. Pokhrel P. Use of Higher Ethylene Genrating Fruits for Ripening as an Alternative to Ethylene. J. Food Sci. Technol. Nepal, 2013; 8(84-86). https://doi.org/10.3126/jfstn.v8i0.11757
11. Amarakoon R, Illeperuma DCK, K.H S. Effect of Calcium Carbide Treatment on Ripening and Quality of Velleicolomban and Willard Mangoesle. Trop Agric Res., 1999; 11(1994): 54–60.
12. Patoare Y, Hossain MI, Islam MN, Chowdhury A, Parveen S, Hossain MM, et al. Effect of calcium carbide on rat tissue. Dhaka Univ J Pharm Sci., 2007; 6(2): 93–8. https://doi.org/10.3329/dujps.v6i2.682
13. Koros K. How Fruits could be Slow Poison, 2013; 11(29). http://www.scienceafrica.co.ke/index.
14. Jindal T. Accidental Poisoning with Calcium Carbide. J Clin Toxicol [Internet], 2013; 3(2): 3–4. https://www.longdom.org/open-access/accidental-poisoning-with-calcium-carbide-2161-0495.1000159.pdf
15. Nepal: People eating carbide-ripened mangoes fall ill. www. 21 food .com /news /detail27823.html. Accessed on 06/06/2010.
16. Edenta C, Okoduwa S, Okpe O. Effects of Aqueous Extract of Three Cultivars of Banana (Musa acuminata) Fruit Peel on Kidney and Liver Function Indices in Wistar Rats. Medicines [Internet], 2017; 4(4): 77. http://www.mdpi.com/2305-6320/4/4/77
17. Jhon D. Bancroft, Alan Stevens. Theory and practical of histological technique. Fourth edition, 47-67.
18. Andrew GS, Simon UT, John AU, Godwin OO, Alexander NI. Studies on changes in some haematological and plasma biochemical parameters in wistar rats fed on diets containing calcium carbide ripened mango fruits. Int J Food Sci Nutr Eng, 2018; 8(2): 27–36.