

Effects of Calcium carbide Used as a Fruit-ripening Agent on Fruit Toxicity

ABSTRACT

Aims: To provide a comprehensive summary of what has been published about the effects of calcium carbide (CaC_2) as a fruit-ripening agent and to determine the necessity or not to develop awareness among government agencies, policymakers, farmers, vendors, and scientists in order to best address different aspects of artificial fruit ripening issues and to provide more profitable solutions for global health preservation.

Methodology: Scientific information about the effects of CaC_2 published elsewhere was reviewed. Online databases of scientific journals which include Wiley Online Library, Science Direct, PubMed, CAS, CABI, AJOL and Google Scholar were used to select valuable studies.

Results: Most studies have reported the hazardous potential of CaC_2 as a ripening agent. Among other potential effects that were discovered through laboratory investigations are the adulteration of nutritional values of ripened fruits and direct or indirect toxicity in studied living systems such as genotoxicity and cytotoxicity to dividing cells, increase of the cellular oxidative stress, disturbance of the redox balance of the cell, estrogenic disruptions, increase of the white blood cells and Lymphocytes, alteration of hematopoiesis, alteration of sperm cells, a decrease of the fertility rate, weakness of the immune system, etc.).

Conclusion: Overall, this review provides comprehensive information on what is known about the effects of CaC_2 and showed the necessity to discourage its application as an artificial fruit ripening agent through the establishment of laws and regulations for better control of its use in most developing countries.

Keywords: Calcium carbide, fruit, health hazard, ripening agent, toxic

1. INTRODUCTION

Fruits are among the foods with the highest consumption across the world. Their nutritional values are well established, as being a part of a balanced diet. The main interest in fruits lies in their richness in micronutrients such as vitamins, carotene and other metabolites that were reported to play a vital role in human nutrition for maintaining normal health through improving immunity and preventing major diseases and disorders of cardiovascular, digestive and endocrine system [1]. As food, fruits are generally harvested either when mature or when ripe.

“Fruit ripening is the initiation of fruit senescence which is the period when chemical synthesizing pathways is coordinating the process of organ transformation from unripe to the ripe stage to yield an attractive edible, softer, sweeter, nutritious and attractive fruit” [2]. “It is an irreversible phenomenon involving a series of biochemical, physiological, and organoleptic changes which mostly lead to changes in carbohydrate content, changes in color, texture, aroma volatiles, flavor compounds, phenolic compounds, and organic acids” [3]. Fruits are classified as (i) climacteric ones that are harvested at full maturity (e.g.,

mangoes, bananas, guavas, papaya, apple, melon, etc. and (ii) non-climacteric which do not ripe after being harvested (e.g., grapes, pineapple, strawberry, citrus fruits, etc.). As part of a balanced diet, the demand for fruit consumption is significantly growing very fast in most developing African countries due to increased nutrition consciousness.

Nowadays, advancements in science and technology have significantly led to the development of various methods and technics to artificially stimulate this ripening process, mostly to meet the high demand of customers/consumers, especially during the off-season [4]. This includes the use of chemical agents such as calcium carbide, also known as calcium acetylide with the chemical formula of CaC_2 .

However, "scientific reports from several research teams have highlighted the toxic effects of many of these agents including CaC_2 by performing qualitative and quantitative analysis of the presence of ripening agents within the fruit-skin and flesh to understand the relevant health hazard and by analyzing the chemical impact on the food value of artificially ripened fruits" [5]-[7]. "Most were found to change nutritional properties (e.g., heavy metal contamination) of fruits which might consequently lead to serious health hazards to human beings like cancer, skin irritation, diarrhea, liver disease, kidney disease, gastrointestinal irritation with nausea, vomiting, cardiac disturbances, central nervous system depression and cardiac abnormalities etc". [8].

For instance, CaC_2 , a very cheap chemical substance, is one of the most commonly used for ripening fruits like mangoes, bananas, guavas, papaya, apple, melon, etc. Due to its harmful effects on human health, most countries have issued and implemented laws and regulations to discourage or restrict the production, sell and distribution of CaC_2 as an artificial fruit ripening agent [9]. Unfortunately, as highlighted by Islam, Mursalat and Khan [10], most developing countries Niger republic included and its other neighboring francophone countries did not dispose of any of such valuable legislation in order to control or regulate the indiscriminate use of this harmful ripening agent.

Thus, the objective of this study was to review and evaluate the existing published literature on fruit ripening in order to address the scientific understanding of the changes in nutrition values of artificially ripened fruits with calcium carbide (CaC_2) and health related issues, and to make people aware of the right fruit to be chosen for a better life.

2. MATERIAL AND METHODS

The literature review and search strategies were designed to provide an overview of the scientific information about the toxic effects of CaC_2 used as fruit-ripening agent. The search for valuable research papers was carried out through an online database of scientific journals which include Wiley Online Library (<https://onlinelibrary.wiley.com>), Science Direct (<https://www.sciencedirect.com/>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>), CAS (<https://www.cas.org>), CABI (<https://www.cabi.org>), AJOL (<https://www.ajol.info>) and Google Scholar (<https://scholar.google.com/>). Keywords such as 'fruit', 'ripening', 'fruit ripening', ' CaC_2 ', 'artificial ripening agents' and 'toxicity' were used to collect relevant published journals. Criteria were set to screen the search results for relevance in the study. Only scientific journals published in English or French that reported effects of CaC_2 used as ripening agent were considered. All information on toxicities and other effects of CaC_2 were extracted from the exploited literature.

3. RESULTS AND DISCUSSION

A total of 86 articles out of 94 initially were identified through internet database searching. Criteria for study inclusion such as data accessibility, consistency, reliability and uniformity have permitted to exclude eight. Of the 86 journals selected, only 26 fulfill the purpose of this comprehensive review which is to explore the effects of CaC_2 in fruit ripening. Amongst those, there were eighteen studies from Nigeria (70.37%), five from India (18.51%), two from

Bangladesh (50%) and one from Pakistan (3.70%). With respect to the type of effects of CaC₂, 13 studies (48.14%) have reported changes in chemical and biochemical compositions in artificially ripened fruits and the other thirteen (50%) have emphasized on the toxic effects of CaC₂ on various laboratory animal models.

Details on the findings from various research teams followed with well-nourished comprehensive discussions are considerably presented in the present review.

3.1 Effect of CaC₂ in fruit ripening

Treatment of unripe fruits with CaC₂ is known to hasten the ripening process which might lead to softening, flavor and color changes. CaC₂ in contact with moisture may produce acetylene gas which is an analogue of natural ripening hormone ethylene [4]-[6]. However, its use is not well accepted worldwide, certainly due to changes in taste and nutritional values in artificially ripened fruits as reported in many studies. Researchers across the world have investigated the effect of CaC₂ on the nutritional quality of fruits. Among other parameters which determine the quality of fruit, analyses were performed through the analysis of both chemical and mineral compositions of artificially ripened fruits. Fruits as foods with the highest consumption, are relatively good sources of carbohydrates, protein, fat, vitamins, minerals, etc. There are some studies on the chemical, biochemical and mineral composition of fruits from developing countries. Proper understanding of the biochemical and physiological changes during ripening is essential to ensure safe ripe fruit [3], [5], [9].

3.1.1 Implications on proximate analysis

The literature review of most scientific reports from different research groups on the comparison of proximate analysis of different fruit samples has permitted us to best highlight the difference in proximate composition between naturally and artificially ripened fruits.

In terms of crude protein content, a total of eleven studies involving different fruits (banana, mango and pawpaw) were reviewed. In all, artificially ripened fruits with CaC₂ were found to possess lower crude protein contents compared to naturally ripened ones. This was observed in almost all the three types of fruit samples. However, exceptions were found in reports presented by Chukwuma *et al.* [11], Chisom *et al.* [12] and Adeyemi, Bawa and Muktar [13]. This could be due to fruit's types, characteristics and differences in the testing. Protein, a so-called building block of life, is a source of amino acids that contain carbon, hydrogen, oxygen, and nitrogen. Fruits in their nature have low proteins content [14]. Globally, most of the studies have highlighted the significant reduction in protein content in all fruits treated with CaC₂. Proteolysis is a biochemical process in which a protein is broken down partially, into peptide, or completely, into amino acids by proteolytic enzymes. Carl, Edward and David [15], in a published textbook of clinical chemistry have suggested that observed reduction in protein in given substances or materials may be attributed to increased proteolysis. Gbakon *et al.* [16] on the other hand, suggested that the presence of a toxicant could interfere in the synthesis process of protein which may consequently lead to a shift in nitrogen metabolism. Ogbuagu *et al.* [17] in his turn, have suggested that the reduction in protein may also be attributed to protein degradation which may have been initiated by inhibition of ribosomal activity possibly also due to a toxicant presence. Toxicants such as arsenic and phosphorus present in CaC₂ may diffuse into fruit in the course of ripening thus influencing or leading to a decrease in protein content as suggested by many investigators that are cited in the present study.

In terms of lipid content, six studies have reported data on fat content in both naturally and artificially ripened fruits. It can be seen that the fat content of the tested fruits was increased through the artificially ripened ones. Only one study, from Ubuoh, Nwogu and Oporuiche [18] was found to present a decrease in fat content in banana samples ripened with CaC₂.

Globally, these results indicated a significant difference in the fat content of naturally ripened fruits and artificially ripened ones. The fat in fruits is used as an energy source and for protection. Wade and Bishop [19] have also reported “changes in the lipid composition of ripening banana fruits. He found that the content of total lipid in banana fruit pulp tissue remained constant during the climacteric rise induced by the applied ripening agent. The relative proportions of neutral lipid, glycolipid and phospholipid did not change. However, in the phospholipid fraction, there was an increase in the proportion of linolenic acid and a decrease in the proportion of linoleic acid”. Sen, Mishra and Srivastav [20] and Yanez *et al.* [21] suggested that, an increase in the unsaturation of the phospholipid fraction results in increased fluidity in cellular membranes during ripening.

In terms of crude fiber contents, artificially ripened fruits with CaC_2 were found with higher crude fiber content in most of the samples compared to naturally ripened ones. Two studies out of seven were found to report lower fiber contents in artificially ripened fruits compared to naturally ripened fruits [22], [23]. Globally, it seems that CaC_2 contributes to the increase of fiber content in artificially ripened fruits. Fiber was reported to exert effects that go well beyond the regulation of transit. It contributes to the formation of digestive waste to be more regular in the digestive tract [24].

The ash content in artificially ripened fruits was recorded to be higher compared to naturally ripened ones. From the eight studies, only two were not in line as the ash contents in treated samples were found less compared to naturally ripened fruits. This could be related to the initial quality of the fruits or the method used to analyze the samples in the laboratory. Globally, these results indicate a significant difference between naturally ripened fruits and artificially ripened ones. The ripening process of fruits with CaC_2 will cause an increase in the ash content. The high ash content indicates that the fruits may be good sources of minerals [25].

The carbohydrate content of treated fruits with CaC_2 decreased compared to the untreated samples. From the review, the ripening process with CaC_2 was found to significantly affect the carbohydrate value. The most abundant carbohydrates present in a total dry matter of fruits are usually simple sugars such as glucose, fructose, and sucrose in various proportions [26], [27]. Most fruits are rich enough in carbohydrates which are the main source of calories and has an important role in determining the characteristics of food products such as taste, color, and texture [3]. Thus, it could be suggested that eating artificially ripened fruit will let gain fewer calories compared to naturally ripened ones.

The freshness and potential shelf life of fruits is confirmed through moisture content determination. From the reviewed studies, it was found that the artificially ripened fruits with CaC_2 have the highest moisture content compared to untreated ones. This condition could expose the treated fruits to faster decomposition [28], [29]. According to Nuhu, Rabi and Tukur [30], increased moisture content in artificially treated fruit with CaC_2 could be an indication that the ripening agent weakens the fiber of the peel so that moisture is easily absorbed.

3.1.2 Implications on biochemical composition

Fruits in their nature are found significantly rich in carotenoids and vitamins, which are essential bioactive substances known to improve health by preventing the body system from the installation or development of various diseases [31]. From the reviewed studies, only two valuable studies were considered in order to appreciate the implication of artificial ripening agent on the β -Carotene contents that were estimated from different banana samples. The study conducted by Ariyo, Balogun and Solademi [22] which involve banana sample (*Musa* spp., family Musaceae) from Ibadan state (Nigeria), presented a significant increase of β -Carotene content in the artificially ripened samples, when compared to the naturally ripened samples. His result is in accordance with the reports from Himani *et al.* [32] and Fasanya, Olaiya and Karigidi [33] who both reported an increase in the total carotenoid content of

various species of fruits treated with CaC_2 . On the other hand, Igbinaduwa *et al.* [34] and colleagues have reported an increase in vitamin C and β -carotene in naturally ripened fruits compared to artificially ripened ones. For the vitamin C content, results from eight valuable studies were reviewed. Five studies out of eight have reported a decrease in vitamin C content of the CaC_2 ripened fruits, when compared to naturally ripened fruits.

3.1.3 Implications on mineral composition

The maintenance of physiochemical or metabolic processes in the body system will require a significant and well-controlled supply of certain minerals which could be macro or micro-minerals. Are considered essential for life, minerals like potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), fer (Fe), and copper (Cu). While heavy metals like lead (Pb), cadmium (Cd) and arsenic (As) which are less important for the body are mostly considered toxic contaminants [35]. From the reviewed studies, it was found that both naturally and artificially ripened fruits contain elements such as lead (Pb), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), arsenic (As), phosphorus (P), sulfur (S), iron (Fe), potassium (K), sodium (Na), magnesium (Mg), azote (N) and chromium (Cr). However, most of the elements were found with higher proportion in CaC_2 ripened fruits compared to naturally ripened ones [4], [11], [12], [17], [35]-[37]. Most of these elements were known to contribute to a better life. For instance, calcium and phosphorus are known to be very important in the formation of strong bones and teeth, heart function and cell metabolism [38], potassium is known to be essential to help balance body sodium level to keep blood pressure from getting too high as well as playing an important role in heart, muscle and digestive function, iron is known to be necessary for the cure of diabetes, sodium is known to maintain body fluid [39], etc. However, warnings about the hazardous effects on health that may cause most of these elements once in high proportion in the body system were significantly reported as potential risks. "Excessive level of calcium can cause constipation, people exposed to high levels of arsenic can have nausea and vomiting, diarrhea, anemia, and low blood pressure, orally administered phosphorus (as various phosphate salts) in human supplementation is reported to lead to osmotic diarrhea and other mild gastrointestinal effects, including nausea and vomiting have been noted in some studies" [40], [41]. On the contrary, Fenton *et al.* [42] in a meta-analysis study with the objectives to quantify the contribution of phosphate to bone loss in healthy adult subjects, have concluded that there is no evidence that phosphate intake contributes to the demineralization of bone or to bone calcium excretion in the urine.

3.2 Toxic effects of CaC_2

Several studies have reported on the possible toxic effects associated with artificial fruit ripening agents [10]. CaC_2 of industrial grade is reported to be impure as it consists of calcium phosphide (Ca_3P_2) and calcium arsenide (Ca_3As_2) as impurities which are hybrids known to cause health hazards [43], [44]. This therefore raises the interest amongst researchers to further investigate the possible toxicity effects of CaC_2 using different exposure paradigms varying in doses of exposure and models of assessment such as mice, rats, etc. A summary of results extracted from valuable toxicological studies is presented in Table 1. Reported adverse effects of CaC_2 include among others: mutagenicity/genotoxicity and cytotoxicity to dividing cells, increase of the cellular oxidative stress, disturbance of the redox balance of the cell, estrogenic disruptions, increase of the White Blood Cells and Lymphocytes, alteration of hematopoiesis, alteration of sperm cells, decrease of the fertility rate, weakness of the immune system, etc. For example, Alege and Anthony [45] highlighted the mutagenic and toxicity of the CaC_2 on Onion (*Allium cepa* L.) dividing cells. Reported results showed that CaC_2 significantly induces binucleate cells, vacuolated cells, sticky

chromosomes, C-mitosis cells and Anaphase Bridge across the different concentrations which indicate mutagenic and cytotoxic activities of the chemical agent. Various chemicals, used as insecticides or else are reported to cause chromosomal aberrations in plants [46]. Beni, Rajesh and Babu [47] evaluated "the genotoxic effect of chronic exposures to CaC₂ in Wistar albino rats fed orally for 180 days. The chemical was found to cause chromosomal aberrations, micronuclei formation and DNA strand break extensively in the bone marrow and peripheral blood cells". They concluded that the chemical has a potential to cause genomic level of toxicity which may lead to carcinogenic event at a chronic level of exposure. Many chemicals are found to contribute to the release of free radicals within a body system which could negatively deregulate the normal cellular function and though the initiation of detrimental effects on various organs. Now, scientists know that free radicals play a major role in the development of most chronic and complicated diseases such as cancer, cardiovascular disease, etc. Reports from various laboratory based-studies have discovered that free radicals released from CaC₂ may alter the coding of DNA basic information via the interaction with the gene which subsequently result in cancer. Indranil *et al.* [48] and colleagues have conducted a well-controlled study through the exploration of the toxicity associated cellular events in a mammalian cell line (L929 cells) exposed with varying concentrations of CaC₂ for 24h exposure time. A gradual elevation of intracellular ROS has been observed leading to the conclusion that short term CaC₂ exposure may increase the cellular oxidative stress and disturb the redox balance of the cell which then undergoes apoptosis. Exposure of industrial grade of CaC₂ was reported to alter histological architecture of systemic organs. Pataore *et al.* [46] and colleagues have evaluated the effects of exposure of industrial grade CaC₂ on Long Evans rats. Histological analysis of kidney showed the thickening of the lining of collecting tubules with changes in cell structure while lungs were found to be increased moderately in weight, with focal areas of consolidation that was found red-brown to red. However, neither morphological nor structural changes were observed in case of heart, spleen and liver in rats treated with CaC₂. Bini, Rajesh and Babu [49] through preclinical study have evaluated the toxicity on different organs after the exposure of industrial grade CaC₂ to the rats fed for 30 days. Results highlighted the toxic effects of CaC₂ on the internal organs of rats. Changes in some hematological and plasma biochemical parameters in animal models fed on diets containing fruits ripened with CaC₂ were significantly reported by several scientists. Gbakon *et al.* [15] investigated "the changes in several hematological and plasma biochemical parameters in Wistar rats fed on diets containing Mango fruits ripened with CaC₂. Published results showed that CaC₂ ripened mangoes caused significant decrease in Red Blood Cell count, Hemoglobin, Packed Cell Volume and increased the White Blood Cells and Lymphocytes". Depletion in creatinine, protein, cholesterol, potassium and bicarbonate was registered while albumin and bilirubin were significantly found increased. Onwuka *et al.* [50] investigated the activity of juice generated from CaC₂-ripened banana on hematopoiesis in male Wistar rats. CaC₂ ripened banana juices were found to significantly decrease erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins, hematopoietic stem cells and cellular components of blood in a dose-dependent manner. Reduction in packed cell volume, hemoglobin concentration and increase in erythrocyte sedimentation rate; which suggests indication of anemia and red blood cell inflammation. Dike, Cosmas and Lucy [51] recruited thirty male albino mice of isogenic strains which were subsequently exposed to graded CaC₂ for five weeks. Blood cell count by microscopic procedures revealed the presence of lower leucocytes counts and slightly higher lymphocytes in dosed animal with CaC₂ than control. Nwankwo, Osuji and Ubani [52] and colleagues have determined the heavy metals levels of albino rats fed *Musa acuminata* treated with CaC₂. Results showed that the albino rats fed with CaC₂-treated banana have a significant increase in the level of lead and arsenic when compared with untreated ones. For the purpose of reproduction, most male animals produce motile sperm with a tail known as a flagellum, which are known as spermatozoa. "Evidence suggests that a growing number of chemicals adversely affect sperm quality and

reproductive health in men. A number of environmental chemicals were found to adversely impact male reproductive health" [53]-[58]. Adamkovicova *et al.* [59] reported that exposure to cadmium and diazinon at relatively low doses impairs sperm quality and can reduce male fertility. Cadmium and diazinon caused significant changes in sperm morphology with varying effects on motility patterns. Dike and Etsede [60] have investigated the possible inductions of CaC₂ on sperm morphology and viability of the albino mice (*Mus musculus*) after five weeks of exposure. The common abnormalities that were observed included Double Head, Pin Head, Knobbed Head, No Tail and With Hook. Other concerns about the harmful effects of those environmental chemicals were found to affect reproductive organs and systems that mature by regulation of the gonadal hormones [61]. Enitome *et al.* [62] and colleagues for the first time investigated the outcome of ingestion of CaC₂-ripened fruit on some female mice reproductive parameters which were fed for three days. Results show an increase in estrogen level in serum with increased uterus weight. Increased numbers of myometrial cells, presence of secondary follicles and regressing corpus luteal as well as thickened cervix epithelia which were evidence of estrogenic disruptions were also reported. From these evidences, the researchers have suggested that the consumption of fruits ripened with CaC₂ alters the reproductive physiology in female mice. Iyare *et al.* [63] reported the effects of maternal exposure to commercial grade CaC₂ ripened fruits on reproductive development. A significant decrease in fertility rate was seen in the 100g/5kg CaC₂ group when compared to the control. Most have concluded that the reduced fertility could be due to the alterations in levels of reproductive hormones as well as changes in the architecture of the follicles.

Table 1. Toxicity induced by CaC₂

Biological systems	Type of study	Experimentation	Main adverse effects	Warnings	Ref.
Wistar rats	In vivo study	To investigate the changes in several hematological and plasma biochemical parameters in Wistar rats fed on diets containing Mango fruits ripened with CaC ₂	Findings from this study suggest that consumption of CaC ₂ ripened mangoes is causing changes in the hematological and plasma biochemical profiles of consumers; hence that may increase erythrocytes destruction, have suppressive effect on the three major cell lines and interfere with some minerals and vitamins as well as lipid metabolism	Consumption of CaC ₂ may consequently manifest in series of health hazards, lowering the body's ability to resist infection and weakening the whole immunity system when consumed	[16]
Root tip cells of Onion	In vitro study	To evaluate the cytotoxicity potential of CaC ₂ on Onion	The findings indicated that CaC ₂ is mutagenic and cytotoxic to <i>Allium cepa</i> dividing cells	CaC ₂ may not be a safe chemical for use as fruit ripening agent	[45]
Long Evans rats	In vivo study	To evaluate the effects of CaC ₂ in biological system	The histopathological analysis of kidney showed the thickening of the lining of collecting tubules with changes in cell structure while lungs were found to be increased moderately in weight, with focal areas of consolidation that was found red-brown to red; the findings indicate that CaC ₂ may have toxic effects to the rat cells		[46]
Wistar albino rats	In vivo study	To evaluate the genotoxic effect of chronic exposures of CaC ₂ in wistar albino	The results show that CaC ₂ have a potential to cause genomic level of toxicity which may lead to carcinogenic event at a chronic level	This study warns to reinforce the administrative measures against the use of CaC ₂ for fruit ripening	[47]

		rats	exposure	process	
L929 cells	In vitro study	To explore the toxicity associated cellular events in L929 cells	The results show that short term CaC ₂ exposure may increase the cellular oxidative stress and disturb the redox balance of the cell which then undergoes apoptosis	The exposure of CaC ₂ can be associated with severe diseases. The study suggests to stop the uses of CaC ₂ as fruit ripening agent	[48]
Rats	In vivo study	To evaluate the toxic effect of chronic exposures of CaC ₂ in rats	The results show that histological details revealed microvesicular fatty change in liver, corpuscles degeneration in kidney and lymphocytes infiltration in various tissues. In intestine, the mucosal lesion scoring was found high. SOD and CAT activities and GSH level was reduced significantly by CaC ₂ administration. Arsenic and phosphorus detected is above the toxic level: 7.222 and 13.91 mg/dL in CaC ₂ , 1.634 and 6.22 mg/dL in blood and 0.563 and 6.99 mg/dL in liver, respectively	The study suggests that the industrial grade CaC ₂ induce systemic toxicity to rats and the liver is the most susceptible organ	[49]
Male Wistar rats	In vivo study	To investigate the potentials of chemically ripened banana juice in causing the depletion of blood cell in 20 male Wistar rats	This study revealed that chemically ripened banana juices significantly decreased erythropoietin, leukopoietin, thrombopoietin, interleukin-3, prostaglandins (PGE ₂), hematopoietic stem cells and cellular components of blood in a dose-dependent manner. Reduction in packed cell volume (PCV),	The study suggests that chronic consumption of CaC ₂ ripened banana juices induces alteration of hematopoiesis which results to depletion of blood cells	[50]

			hemoglobin concentration and increase in erythrocyte sedimentation rate (ESR); which suggests indication of anemia and red blood cell inflammation were also observed		
Male albino mice	In vivo study	To test CaC ₂ on mammalian model for possible hematological inductions	The study revealed a slight increase in weights of mice exposure to CaC ₂ for 5 weeks. Fewer white blood cells were counted in dosed animals, with slightly higher lymphocytes and few abnormal hypochromic red blood cells in exposed animals than in the control group	CaC ₂ appeared to induce slight immunological inductions on the mammals	[51]
Albino rats	In vivo study	To determine the heavy metals levels of albino rats fed <i>Musa acuminata</i> treated with CaC ₂	The observed results showed that CaC ₂ contains traces of potential toxic elements (heavy metals)	Consumption of carbide ripened bananas is of great public health concern because accumulation of potential toxic elements in the body affects different biological process. Therefore, the use of calcium carbide as a ripening agent should be discouraged	[52]
Wistar albino rats	In vivo study	To evaluate the acute and subacute toxicity of CaC ₂ in Wistar albino rats	The study revealed that the artificial fruit-ripening agents like CaC ₂ cause toxic effects on the internal organs of rats. The subsequent inflammatory response might have weakened the immune system	The study suggests the requisite for urgent measures to regulate the use of harmful synthetic agents in fruit ripening	[57]
Mammalian sperm cells	In vitro study	To investigate possible inductions of CaC ₂ on mammalian sperm morphology and viability	The study revealed that CaC ₂ appeared to induced morphological abnormalities and reduced viability in sperm cells of <i>M. musculus</i>	CaC ₂ appeared to induce morphological abnormalities and reduced viability in sperm cells of <i>M. musculus</i>	[60]

Female mice	In vivo study	To investigated the outcome of ingestion of CaC ₂ -ripened fruit on some female reproductive parameters	This study has shown that consumption of fruits ripened with CaC ₂ negatively alters the female reproductive physiology, accelerates puberty onset and increases serum estrogen levels	Caution must be exercised by fruit sellers in the use of CaC ₂ and policies set in place for strict regulation of its use worldwide	[62]
Rats	In vivo study	To evaluate the possible effects of maternal consumption of banana pulp, force ripened with CaC ₂ , on the offspring	The study revealed that CaC ₂ exposure was related to delayed onset in puberty, decreased serum FSH and a decreased fertility rate in the 100g/5kg CaC ₂ group	Consumption of commercial grade CaC ₂ ripened fruits during pregnancy exposes humans to a significant deleterious effect on puberty onset, and fertility rate of female offspring	[63]

4. CONCLUSION

The availability of sufficient knowledge available to justify the safety of CaC₂ used in ripe fruits was the main impetus for this comprehensive review that provides an (all-in-one) summary of the most important findings showing the nutritional values of calcium carbide-treated fruits are lower than those estimated in naturally ripe fruits. Toxic impurities of calcium carbide which include heavy metals such as arsenic and phosphorus were recorded to be present in most fruits that were treated with the chemical agent. These elements diffuse from the peel to the flesh of fruits, and thus, washing the fruits is not enough measure to reduce the toxic effects of this practice. The referenced toxicological studies in this review have provided preliminary justifications for the possible health effects of CaC₂ in living systems. Thus, contributions of this review of the possible toxic effects of CaC₂ on human are valuable to create awareness of the effect of CaC₂ exposure on human health.

Overall, because of the remarkable increase in the use of CaC₂ as a ripening agent in most developing countries, and reading about its toxicity effects that were reported in most scientific literatures, the use of this harmful agent in ripe fruits should be discouraged. In near future, further toxicological studies based on long term exposure should be envisaged in order to best justify its hazardous effects on health.

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