



Protective Potentials of Tomatoes Puree on Inflammation and Neurological Damages Caused by Calcium Carbide Intakes in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author VEN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OBN and EIU, IFC managed the analyses of the study. Authors NAO, EHE, VOG, OO managed the literature searches and references. All authors read and approved the final manuscript.

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ABSTRACT

Calcium carbide (CaC_2) accelerates fruit ripening but has several adverse effects on the fruit. These include making the fruit tasteless, unhealthy, and potentially toxic, leading to health challenges. This study aimed to investigate the nephroprotective and antioxidative effects of tomato puree on albino rats fed calcium-carbide-ripened bananas. Seventy-two albino rats were randomly assigned to six experimental groups, each consisting of twelve rats. The normal control (NOC) group received a standard diet, while the ripened banana control (RBC) group received 120 g/kg of CaC_2 -ripened bananas. The tomato puree control (TPC) group received 400 g/kg of tomato puree, and the vitamin C control (VCC) group was given 100 mg/kg of vitamin C. The treatment groups (RBT & RBV) received CaC_2 -ripened bananas along with tomato puree and vitamin C, respectively. All treatments were administered via oral intubation once daily for six weeks. Blood samples were collected at the 3rd and 6th weeks for biochemical analysis. Rats fed with CaC_2 -ripened bananas exhibited an increase in DOPA levels and a decrease in serotonin levels. Additionally, there was an increase in the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α , indicating neurological impairment. However, administration of tomato puree significantly restored these neurological parameters toward normal levels and resulted in a noticeable reduction in inflammation. This suggests that tomato puree remarkably prevents CaC_2 -induced tissue injury and damage. The findings demonstrate that tomato puree has a protective effect against CaC_2 -induced inflammation and neurological damage by maintaining the activity of the antioxidant defense system and preserving renal architecture, which may be attributed to its bioactive constituents.

Keywords: Antioxidants; calcium carbide; tomato puree; inflammation; neurological damages.

1. INTRODUCTION

Calcium carbide (CaC_2), commonly known as carbide or calcium acetylide, is predominantly used to produce acetylene and calcium cyanamide. Commercial CaC_2 is widely used for welding, desulfurization of steel, and the synthesis of acetylene and cyanamide. Recently, it has also gained attention as a fruit-ripening agent due to the pressure to meet the ever-growing demand for food crops and fruits [1,2]. In contact with moisture, CaC_2 produces acetylene gas, an analog of ethylene, mimicking its function as a natural fruit-ripening hormone. Although CaC_2 accelerates the fruit ripening process [3,4], it has several adverse effects on fruits. In some cases, only the skin (epicarp) of the fruit changes color, while the mesocarp and pericarp remain green, raw, and unripe. When CaC_2 is used on unripe fruits, a larger quantity is often added to ripen them. This can result in fruits that are tasteless, unhealthy, and highly toxic, posing health risks, unlike naturally ripened fruits that play significant roles in human health and nutrition [5,6]. Reports have shown that chemical substances used as artificial ripeners have negative effects on humans, including memory

loss, cerebral edema, prostate issues, and changes in DNA and RNA [7]. It is well established that CaC_2 impacts the sensory system by limiting oxygen supply to the brain, potentially resulting in neural damage. Additionally, evidence indicates CaC_2 's involvement in lung failure, renal failure, dermal diseases, and heart conditions [8,9]. There is a need for detailed studies to elucidate and characterize the negative physiological and biochemical processes affected by CaC_2 in humans to aid in diagnosing exposure and developing pharmaceutical interventions. Previous investigations suggest that oxidative stress and inflammation are involved in CaC_2 -driven organ damage [10]. Therefore, it makes sense that a potent antioxidant and anti-inflammatory agent could mitigate CaC_2 toxicity, as the unsuspecting public is endangered by consuming products treated with CaC_2 . Tomato, the edible berry of the plant *Solanum lycopersicum*, commonly known as the tomato plant [11], plays an important role in nutrition due to its well-established health benefits [12]. Tomatoes (*Solanum lycopersicum* L.) are widely consumed as vegetables and are used in many processed food products, such as sauces,

salads, soups, and pastes [13]. Common nutrients found in tomatoes include vitamins, minerals, fiber, protein, essential amino acids, monounsaturated fatty acids, carotenoids, and phytosterols [14,15]. Tomatoes are also an excellent source of bioactive compounds, commonly known as secondary metabolites [16]. These nutrients perform various bodily functions, including preventing constipation, reducing high blood pressure, stimulating blood circulation, maintaining lipid profiles and body fluids, detoxifying body toxins, and maintaining bone structure and strength [12]. Due to their high concentrations of natural antioxidants, tomatoes can help ameliorate many diseases, especially chronic diseases [16]. These compounds play beneficial roles in inhibiting reactive oxygen species (ROS) by scavenging free radicals, inhibiting cellular proliferation and damage, inhibiting apoptosis, as well as through metal chelation, modulation of enzymatic activities, cytokine expression, and signal transduction pathways [16]. This study was designed to investigate the protective potential of tomato puree against nephrotoxicity and oxidative stress in albino rats fed calcium carbide-ripened bananas.

2. MATERIALS AND METHODS

Albino rats were purchased from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. They were allowed to acclimatize for one week before the start of the experiment. The rats were housed in standard clean cages under controlled room temperatures between 21–25 °C and a 12-hour light/dark cycle. They had access to clean water and were fed a standard rat chow diet.

2.1 Experimental Design

Seventy-two albino rats were randomly assigned to six experimental groups, each consisting of twelve rats. Normal control (NOC) rats were given only feed (rat chow), while the ripened banana control (RBC) group received 120 g/kg of bananas ripened with calcium carbide. The tomato puree control group (TPC) received 400 g/kg of tomato puree, and the vitamin C control (VCC) group was given 100 mg/kg of vitamin C. The treatment groups (RBT and RBV) received CaC₂-ripened bananas along with tomato puree and vitamin C, respectively. All animals had free access to water, and all administrations were conducted via oral intubation for six weeks. After six weeks, all administrations were stopped, and

the animals were fasted for eight hours. Blood samples were collected through cardiac puncture and dispensed into lithium heparin bottles for biochemical analysis.

2.2 Determination of Body and Organ Weights

Changes in general body weight were recorded daily throughout the experimental period. The mean initial and final body weights in grams were calculated at the end of the study.

2.3 Determination of Plasma Dopamine

Concentrations of brain monoamines (serotonin and dopamine) were determined using the method of Kema et al. [17]. Briefly, dopamine was extracted using a cis-diol-specific affinity gel, acylated, and then converted enzymatically. The competitive ELISA kit used a microtiter plate format. The antigen was bound to the solid phase of the microtiter plate, and the derivatized standards, controls, and samples competed for a fixed number of antibody binding sites. After equilibrium was reached, free antigen and free antigen-antibody complexes were removed by washing. The antibody bound to the solid phase was detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction was monitored at 450 nm, and quantification of unknown samples was achieved by comparing their absorbance.

2.4 Determination of Serotonin

Concentrations of plasma serotonin were determined using the method of Kema et al. [17]. Serotonin was acylated and detected using a competitive ELISA kit in a microtiter plate format. Antigens were bound to the solid phase of the microtiter plate. The acylated standards, controls, and samples competed for a fixed number of antibody binding sites. After reaching equilibrium, free antigen and free antigen-antibody complexes were removed by washing. The antibody bound to the solid phase was detected by an anti-rabbit IgG-peroxidase conjugate using Tetramethylbenzidine (TMB) as a substrate. The reaction was monitored at 450 nm, and quantification of unknown samples was achieved by comparing their absorbance with a standard curve prepared from known standard concentrations.

2.5 Determination of Serum Interleukin 1-beta (IL-1β)

The assay for Interleukin 1-beta was carried out using a conventional sandwich ELISA (Enzyme-Linked Immunosorbent Assay) method with 96-well plates coated with antibodies specific for IL-1β [18].

2.6 Determination of Interleukin-6 (IL-6)

The assay for Interleukin-6 (IL-6) was conducted using a similar sandwich ELISA method, with 96-well plates coated with antibodies specific for IL-6.

2.7 Determination of Tumor Necrosis Factor Alpha

The assay for tumor necrosis factor-alpha was conducted using the method of Koch & Gaines [18]. Briefly, a monoclonal antibody specific for TNF-alpha was coated onto the wells of the microtiter strips. Samples, including standards of known TNF-alpha concentrations, controls, or unknowns, were pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for TNF-alpha were incubated simultaneously. After washing, the enzyme streptavidin-HRP that binds the biotinylated antibody was introduced, incubated, and washed again. A TMB substrate solution was then added, reacting with the bound enzyme to generate a colored product. The intensity of this colored product is directly proportional to the amount of TNF-alpha present in the samples.

2.8 Statistical Analysis

Data were analyzed using one-way ANOVA with GraphPad Prism 8.0.2 (263). Post hoc Tukey

tests were used for group comparisons. Data are presented as mean ± standard error of means (SEM), with differences considered significant when $p < 0.05$.

3. RESULTS

The effect of tomato puree on neurotransmitter levels in rats fed calcium carbide-ripened bananas is shown in Table 1. Rats administered calcium carbide-ripened bananas exhibited a significant ($p < 0.05$) decrease in the levels of serotonin and dopamine compared to the normal control group. However, both three-week and six-week administrations of either tomato puree or vitamin C reversed the CaC₂-ripened banana-induced alterations in both neurotransmitters, such that the levels after three or six weeks of intervention were comparable ($p > 0.05$) to those observed in the normal control group.

3.1 Effect of Tomato Puree on Levels of Inflammatory Markers of Rats Fed Calcium Carbide Ripened Banana

Table 3 showed that the administration of calcium carbide ripened banana to albino rats exerted significant ($p < 0.05$) increases in the levels of inflammatory markers (IL-2, IL-6, and TNF-α) about the normal control. However, three weeks and six weeks of administration of either tomato puree or vitamin C significantly reversed the trends of these markers in relation to the group administered calcium carbide ripened banana only. The results also showed no significant ($p > 0.05$) difference in the levels of inflammatory markers in the group administered vitamin C and those administered tomato puree only when compared to the normal control group.

Table 1. Effect of tomato puree on the body weight of rats fed calcium carbide ripened banana

Body weight (g)	Experimental groups					
	NOC	TPC	RBC	VCC	RBT	RVC
Final	130.7±3.7 _a	124.6±4.3 _a	92.0±7.0 ^b	132.8±3.3 ^a	129.8±3.5 ^a	129.6±7.9 ^a
Initial	122.8±6.7 _a	109.5±3.8 _a	121.2±4.1 ^b	119.5±4.5 ^a	116.6±5.7 ^a	120.7±3.2 ^a
Body Weight Change (%)	7.9 (6.4)	15.1(13.8)	-29.2 (24.1)	13.3 (11.1)	13.2 (11.3)	8.9 (7.4)

Values were expressed as mean ± SEM (n = 6). SEM: Standard error of means. Data with different alphabets are significantly different across the group at $p < 0.05$. NOC: Normal control; TPC: Tomato puree control; RBC: Ripened banana control; VCC: Vitamin C control; RBT: Ripened banana and tomato puree; RVC: Ripened banana and vitamin C

Table 2. Effect of tomato puree on neurotransmitters of rats fed calcium carbide ripened banana

Duration of treatment	Parameters	Experimental groups					
		NOC	TPC	RBC	VCC	RBT	RVC
Week 3	DOPA (pg/ml)	19.4±0.9 ^a	22.5±1.4 ^a	31.2±1.0 ^b	24.1±1.1 ^a	19.9±0.5 ^a	20.1±0.8 ^a
	Serotonin (ng/ml)	108.9±6.3 ^a	130.9±8.8 ^a	89.5±5.5 ^b	139.9±11.7 ^a	117.0±6.0 ^a	119.2±7.7 ^a
Week 6	DOPA (pg/ml)	19.3±0.6 ^a	20.0±0.8 ^a	33.9±0.8 ^b	23.7±0.8 ^a	21.1±0.7 ^a	20.2±1.1 ^a
	Serotonin (ng/ml)	119.6±4.0 ^a	132.6±6.4 ^a	84.4±5.7 ^b	130.7±4.4 ^a	106.5±2.4 ^a	93.9±5.2 ^a

Values were expressed as mean ± SEM (n = 6). SEM: Standard error of means. Data with different alphabet are significantly different across the group at p < 0.05. NOC: Normal control; TPC: Tomato puree control; RBC: Ripened banana control; VCC: Vitamin C control; RBT: Ripened banana and tomato puree; RVC: Ripened banana and vitamin C; DOPA: Dopamine

Table 3. Effect of tomato puree on inflammatory markers of rats fed calcium carbide ripened banana

Duration of treatment	Parameters (pg/ml)	Experimental groups					
		NOC	TPC	RBC	VCC	RBT	RVC
Week 3	IL-1β	1.69±0.02 ^a	2.59±0.01 ^a	6.97±0.02 ^b	2.84±0.04 ^a	4.90±0.02 ^b	5.00±0.01 ^b
	IL-6	28.5±1.3 ^a	27.9±1.5 ^a	63.0±4.1 ^b	27.2±1.6 ^a	59.5±0.9 ^b	64.9±3.3 ^b
	TNF-α	23.2±0.7 ^a	22.7±1.0 ^a	57.2±4.0 ^b	25.6±1.2 ^a	46.8±2.4 ^c	43.2±1.7 ^c
Week 6	IL-1β	2.46±0.03 ^a	2.59±0.01 ^a	7.98±0.02 ^b	3.16±0.01 ^a	4.52±0.01 ^c	5.62±0.03 ^c
	IL-6	30.6±1.5 ^a	29.1±0.8 ^a	75.5±2.3 ^b	26.97±0.8 ^a	53.9±1.1 ^c	61.5±1.0 ^c
	TNF-α	22.6±0.6 ^a	22.6±0.9 ^a	70.9±5.0 ^b	25.7±1.1 ^a	43.7±1.6 ^c	44.6±1.5 ^c

Values were expressed as mean ± SEM (n = 6). SEM: Standard error of means. Data with different alphabet are significantly different across the group at p < 0.05. NOC: Normal control; TPC: Tomato puree control; RBC: Ripened banana control; VCC: Vitamin C control; RBT: Ripened banana and tomato puree; RVC: Ripened banana and vitamin C

4. DISCUSSION AND CONCLUSION

Rats administered calcium carbide-ripened bananas showed a significant ($p < 0.05$) decrease in the levels of serotonin and dopamine compared to the normal control group. While there is no study specifically examining the effect of calcium carbide on neurotransmitters related research on radical-generating toxicity supports these findings. For instance, Essam et al. [19] reported that oral administration of tramadol once daily for 28 days significantly ($p < 0.05$) decreased the monoamine neurotransmitters (norepinephrine (NE), serotonin (5-HT), and dopamine (DA)) in streptozotocin-induced diabetes in Sprague-Dawley rats during painful diabetic neuropathy. This result is consistent with the work of El-Baky and Hafez ([20], who reported a significant ($p < 0.05$) increase in plasma monoamine oxidase (MAO), leading to decreased levels of serotonin (5-HT) and dopamine in brain tissue proteins ($p < 0.001$) in tramadol-administered albino rats. The significant ($p < 0.001$) decrease in serotonin levels during calcium carbide toxicity may be attributed to a chronic anabolic deficit caused by a reduction in amino acids in the brain, consequently decreasing serotonin synthesis [21]. The decreases in neurotransmitter levels induced by calcium carbide may also result from its affinity for blocking their uptake [22] and inhibiting their reuptake [23] as well as through interactions with monoamine neurotransmitter receptors [24]. However, both three-week and six-week administrations of either tomato puree or vitamin C reversed the CaC_2 -ripened banana-induced alterations in neurotransmitter levels to the extent that the levels after three or six weeks of intervention were comparable ($p > 0.05$) to those observed in the normal control group (Table 1). This finding correlates with the results of Hanaa et al. [25], who revealed that administration of Cucurbita pepo seed oil resulted in a significant ($p > 0.05$) increase in brain monoamine neurotransmitter levels (dopamine and norepinephrine) after 50 days of rotenone administration compared to the control group. The effect of tomato puree may be attributed to the presence of chemical compounds in the puree that stabilize and protect mitochondrial function by improving mitochondrial membrane potential and reversing decreases in ATP production, in addition to its potent effects against oxidative stress. The increased effect of tomato puree on neurotransmitter content in the brain could also be due to its high concentration of vitamin E [26]. Vitamin E has been shown to

enhance neurotransmitter anabolism in the brain by activating tyrosine hydroxylase, the rate-limiting enzyme for the biosynthesis of neurotransmitters. Adachi et al. [26] demonstrated that the activity of tyrosine hydroxylase was significantly lower in vitamin E-deficient rats compared to controls, indicating that monoamine anabolism in the brains of vitamin E-deficient rats could be impaired and that supplementation with vitamin E could reverse this effect. Additionally, tomato puree may preserve neuronal resistance and potentially recover atrophying neurons [27]. The effect of tomato puree could also be attributed to its antioxidant activity and scavenging capability against various reactive oxygen species generated by tramadol toxicity. Administration of calcium carbide-ripened bananas to albino rats resulted in significant ($p < 0.05$) increases in the levels of inflammatory markers (IL-2, IL-6, and TNF- α) compared to the normal control group. However, both three-week and six-week administrations of either tomato puree or vitamin C significantly reversed the trends of these markers about the group administered calcium carbide-ripened bananas only. Cytokines are biologically active proteins that mediate vital intercellular communication in the immune system and are secreted by various immune cell types. They participate in host defense, inflammatory responses, and tissue repair activities [28]. Pro-inflammatory cytokines are produced by activated macrophages and effector cells that participate in the adaptive immune system, playing a significant role in exacerbating inflammatory processes. Consequently, interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) are critical in fighting infections and cell survival mechanisms. In the current study, CaC_2 supplementation resulted in markedly elevated levels of serum IL-2, IL-6, and TNF- α . Cytokines typically work synergistically with IFN- γ , stimulating the migration of immune cells to infection sites and leading to granuloma development, which is capable of regulating the immune response. In the presence of tomato puree and vitamin C, the CaC_2 -driven elevation of serum IL-2, IL-6, and TNF- α was blocked. Our findings demonstrated that CaC_2 induced an elevation of pro-inflammatory cytokines, indicative of inflammation; however, tomato puree and vitamin C significantly reversed this effect.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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