

Effect of *Cucumeropsis mannii* Seed Oil on Antioxidant Enzymes in the Spleen of Rats Intoxicated with Calcium Carbide

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Abstract

Spleen carries out a vital role in immune response and the regulation of oxidative stress. Calcium carbide, a compound commonly used in acetylene production and fruit ripening has obviously shown harmful effects on human health due to its oxidative stress-inducing properties. *Cucumeropsis mannii*, A specie of African melon, was investigated for its protecting properties against calcium carbide-induced oxidative stress in the spleen of rats. This study aimed to determine the capability of *Cucumeropsis mannii* seed oil (CMSO) to counteract oxidative damage caused by calcium carbide by examining changes in antioxidant enzyme activities in the spleen. The study involved 28 albino rats sectioned into 7 groups, including a normal control, a positive control, a standard control, and four test groups receiving different dosages of CMSO 1.5 mL, 1.5 mL, 2.5 mL and 5.0m L respectively. Results revealed calcium carbide considerably decreased the antioxidant enzyme activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione levels, indicating oxidative stress. Furthermore, treatment with CMSO resulted in significant restoration of enzyme activities, suggesting its antioxidative effect. This study highlights CMSO'S potential as a natural antioxidant in mitigating oxidative stress and preserving spleen health.

Keywords: Immune, Oxidative damage, Acetylene, Fruit ripening.

Introduction

Antioxidants are indispensable molecules that shield and protect cells from the detrimental effects of oxidative stress by counteracting reactive oxygen species (ROS). Superoxide anion and hydroxyl radicals are examples of ROS that can cause substantial cellular damage if not sufficiently neutralized (Smith and Roberts, 2021). The spleen, an organ critical for immune response and surveillance is particularly vulnerable to oxidative stress as a result of high metabolic activities and incessant exposure to blood-borne pathogens and debris (Brendolan and Freschi, 2019). The spleen performs essential functions such as recycling old erythrocytes and storing white blood cells and platelets, which are vital for immune function (Cui *et al.*, 2020). The organ's high metabolic activity and steady contact with blood-borne pathogens and debris make it disposed to oxidative stress. Antioxidants are essential for shielding the spleen from oxidative

damage and upholding its functionality. Oxidative stress has the ability to impair the spleen's capability to filter blood and mount active immune responses by damaging cellular components. The white pulp of the spleen, which contains lymphocytes, is vital for immune response and surveillance. Oxidative damage to these white blood cells can lead to immunosuppression, making the body more susceptible to infections and ailments (Patel *et al.*, 2022). To counteract oxidative stress, the spleen relies on both enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants play critical roles in detoxifying ROS produced during cellular metabolism and immune responses (Garcia *et al.*, 2023). Non-enzymatic antioxidants, including vitamins E and C, flavonoids, and polyphenols, also contribute to the spleen's antioxidant defense by scavenging free radicals and promoting the activity of enzymatic antioxidants

(Huang *et al.*, 2021). Calcium carbide (CaC_2), used for artificial fruit ripening, can introduce detrimental residues such as arsenic and phosphine gas into biological systems. These residues have the ability to contribute to oxidative stress by increasing ROS production, thereby damaging the spleen and compromising its immune functions (Singh *et al.*, 2020). Enduring exposure to calcium carbide and its by-products can exacerbate oxidative stress in the spleen, highlighting the need for effective antioxidant defenses to protect this vital organ (Oguntibeju, 2019).

Cucumeropsis mannii, popularly known as the egusi seed, is native to the Western region of Africa and valued for its oil, which is rich in essential fatty acids and antioxidants. The seed oil contains adequate concentrations of tocopherols and phenolic compounds, which parades substantial antioxidant properties (Adjei *et al.*, 2021). These antioxidants can increase the body's capacity to counteract ROS, thereby protecting or defending cells from oxidative insults. In the spleen, *Cucumeropsis mannii* seed oil has shown ability in improving the activity of antioxidant enzymes, which are essential for maintaining cellular health and function (Nwaogu *et al.*, 2020). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH) are essential for detoxifying ROS in the spleen (Smith and Roberts, 2021).

Therefore, this study is justified due to the significant public health impact of calcium carbide exposure, particularly in regions where it is used as a fruit ripening agent. Calcium carbide releases toxic compounds that pose health hazards. The evaluation of CMSO as a treatment option presents a novel therapeutic strategy in the spleen. Therefore, this research's goal is to fill this knowledge gap by contributing to the scientific understanding of calcium carbide's health effects as well as food safety and the efficacy of CMSO by exploring the therapeutic potentials.

Materials and Methods

Seeds of *Cucumeropsis mannii* were procured from a local peasant farmer in Amaechara located in Abakaliki Local Government Area, Ebonyi State, Nigeria. Taxonomic authentication was performed by a qualified plant taxonomist from Plant Science and Biotechnology Department, Federal University, Oye-Ekiti, Nigeria.

Extraction of CMSO

About ten (10) kilograms of *C. mannii* seeds were de-hulled and carefully sorted to eliminate debris and shells. A manual grinder was meticulously cleaned to get rid of any contaminants and then rinsed with distilled water. Seeds of CMSO were ground four times using a grinder until a fine powder was obtained. The resulting sample was extracted by pressing it on a smooth, slightly inclined stainless-steel surface. Approximately 50 centiliters of lukewarm water were gradually added in small amounts (around four drops at a time) to promote oil release, as water aids in breaking down cell membranes. Once the oil was extracted, most of the added water was immediately decanted. However, water molecules bound to hydrocolloids (such as mucilage and gum) released from ruptured cells were left to settle undisturbed (Dror *et al.*, 2006). The extracted oil was then kept undisturbed in a sealed container for about seven days to allow further sedimentation. After this period, the clarified oil was carefully separated by decantation and transferred into a separate sterilized storage bottle (Hameed and Bahareh, 2012).

Experimental Animals

This study utilized 28 male albino rats, approximately aged 10 weeks, sourced from Ekiti State University, Ado-Ekiti, Nigeria animal house situated in the Department of Biochemistry. Throughout the experiment or research, the rats had continuous access to standard edible rodent feed and clean water, housed in wooden cages within a well-ventilated animal facility at the Federal University in Oye-Ekiti. Following a 14-day acclimatization period under a 12-hour light/dark cycle at room temperature, the rats were used for the study. All procedures adhered to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996).

Experimental Design

The study involved 28 albino rats (approximately 8 weeks old), divided into 7 groups with 4 rats per group. Treatments, including calcium carbide, *Cucumeropsis mannii* seed oil, and ascorbic acid, were administered orally via intubation. Group A received feed and water, group B were administered 100 mg/kg calcium carbide only, group C were administered 100 mg/kg calcium carbide and ascorbic acid, group D received 1.5 mL of CMSO while test groups E, F and G received 100 mg/kg calcium carbide and CMSO at

varying concentrations of 1.5, 2.5 and 5.0 mL orally for a duration of 14 days.

CMSO's dosage in this study was established from our earlier studies (Onodugo *et al.*, 2024), although statistics or data not shown while the dosage of CAC and ascorbic acid was in accordance to the studies of Onodugo *et al.* 2024; Ibeachu and Nwidoobie, 2022.

PREPARATION OF SPLENIC TISSUE HOMOGENATES

About 0.5 g of spleen tissue was excised and homogenized in 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% Triton X-100. The homogenate was centrifuged at 12,000 rpm for 12 minutes at 4°C. The resulting supernatants were carefully collected and stored at -4°C for subsequent biochemical analysis.

BIOCHEMICAL ANALYSIS

The study assessed enzyme activity by measuring the levels of:

1. Superoxide dismutase (SOD)
2. Catalase (CAT)
3. Glutathione peroxidase (GPx)

4. Glutathione (GSH)

These antioxidant enzymes and glutathione levels were evaluated using spectrophotometric methods.

Statistical Analysis

Results presented were analyzed using R correlation coefficient. Data were articulated as mean \pm standard deviation while their mean values were compared using the one-way ANOVA and $p < 0.05$ was considered to be statistically significant.

Results

Effect of CMSO Treatment on Antioxidant Enzyme activity in the spleen of Rats Intoxicated with Calcium Carbide.

The results showed intoxication of rats with calcium carbide significantly ($P < 0.05$) reduced the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione level in CAC group when compared to the normal and standard controls. However, treatment with CMSO significantly ($P < 0.05$) elevated activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione levels (Figures 1-4).

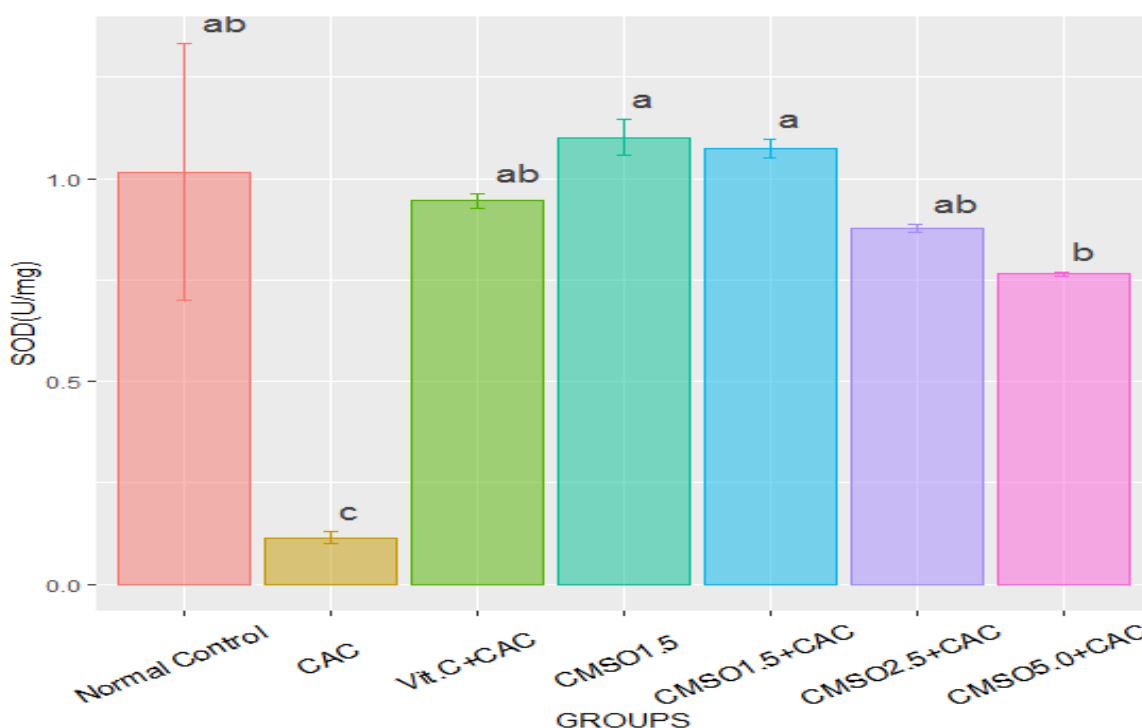


Figure 1: Effects of CMSO treatment on superoxide dismutase activity in the spleen of albino rats intoxicated with calcium carbide. Values are expressed as mean \pm standard deviation ($n = 4$). Bars bearing different alphabetical

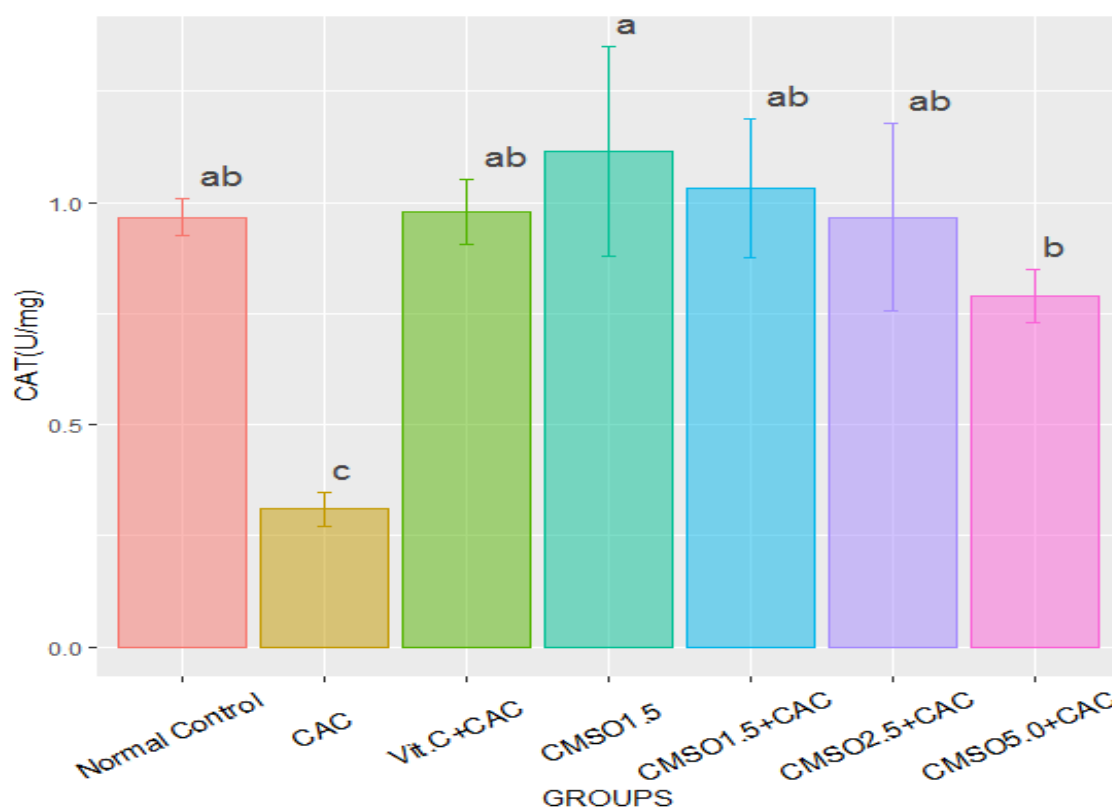


Figure 2: Effects of CMSO treatment on Catalase activity in the spleen of albino intoxicated with calcium carbide. Values are expressed as mean \pm standard deviation ($n = 4$). Bars bearing different alphabetical letters indicate statistically significant differences at $P < 0.05$. Bars with two alphabetical labels are significantly different from any bars lacking both letters. CAC (Calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

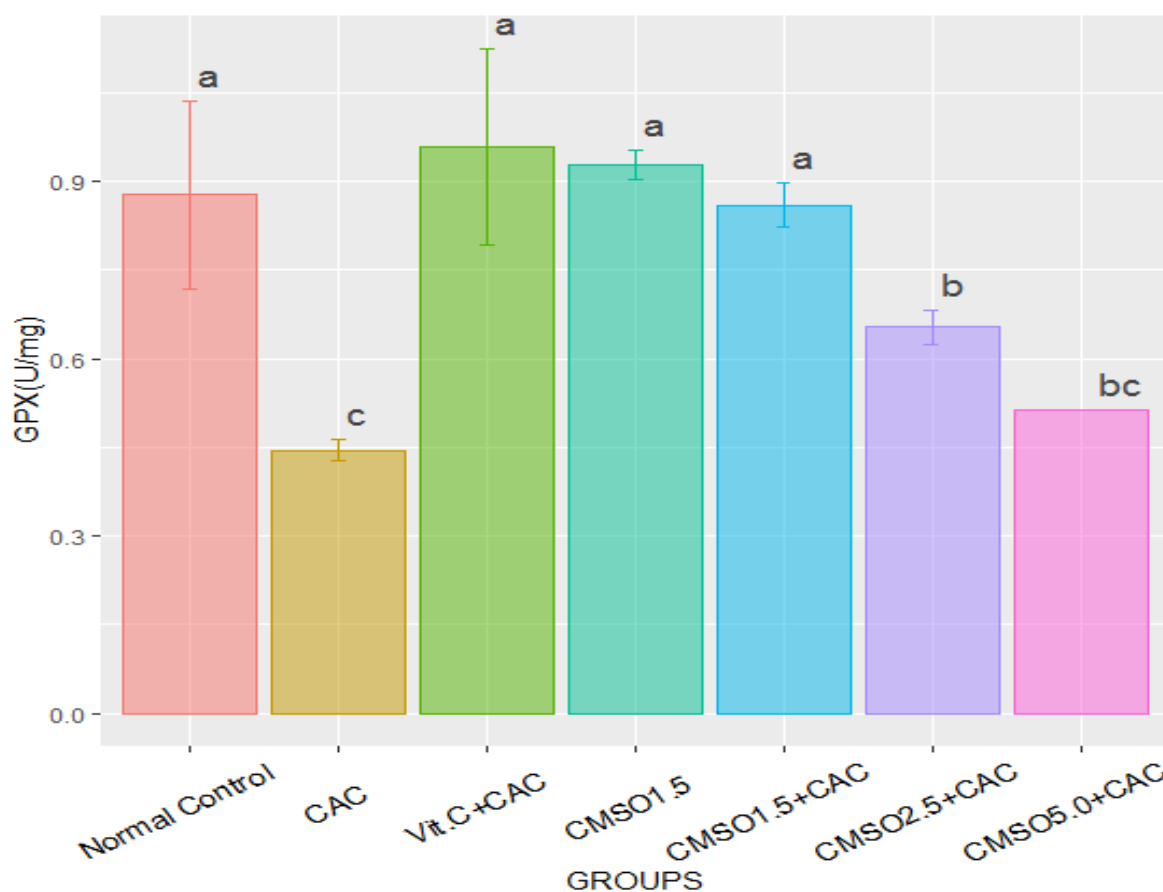


Figure 3: Effects of CMSO treatment on Glutathione peroxidase activity in the spleen of albino rats intoxicated with calcium carbide. Values are expressed as mean \pm standard deviation ($n = 4$). Bars bearing different alphabetical letters indicate statistically significant differences at $P < 0.05$. Bars with two alphabetical labels are significantly different from any bars lacking both letters. CAC (Calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

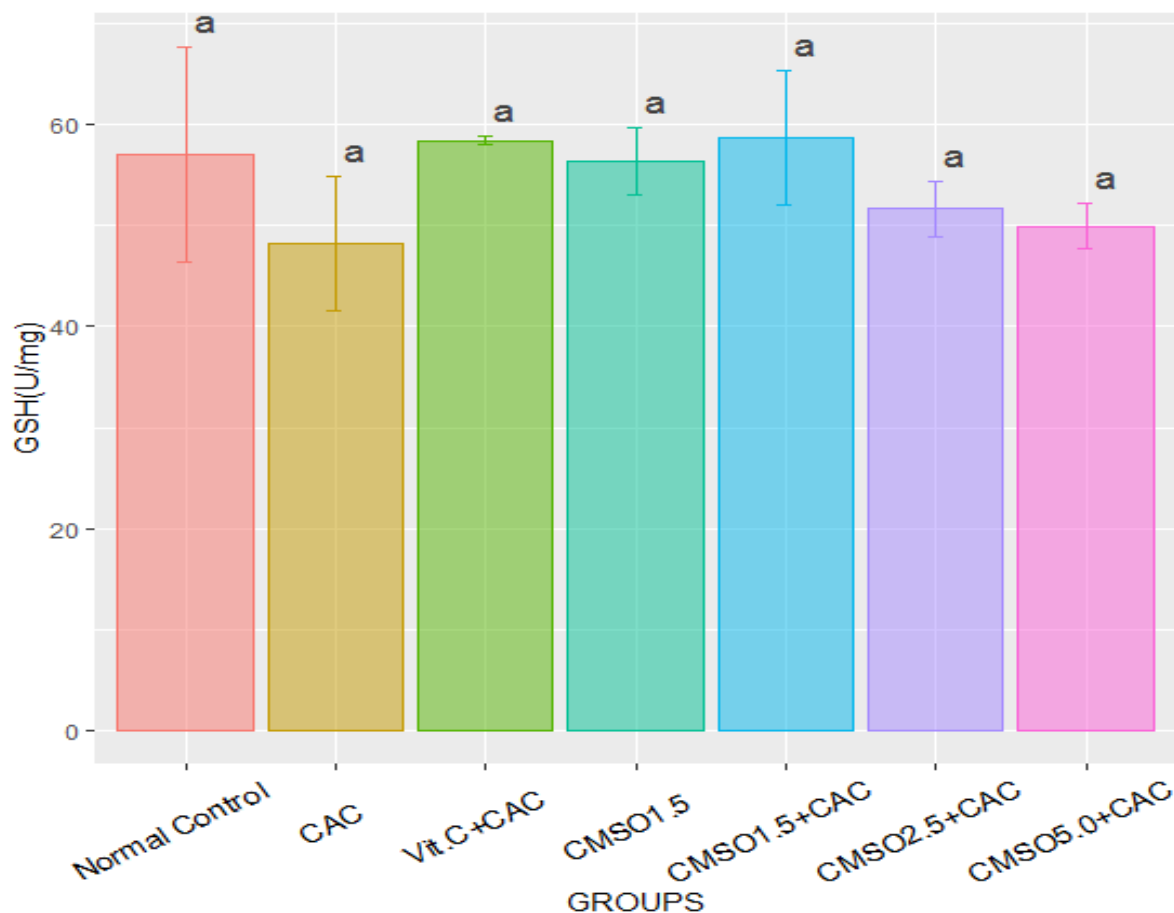


Figure 4: Effects of CMSO treatment on Reduced Glutathione activity in the spleen of albino rats intoxicated with calcium carbide. Values are expressed as mean \pm standard deviation ($n = 4$). Bars bearing different alphabetical letters indicate statistically significant differences at $P < 0.05$. Bars with two alphabetical labels are significantly different from any bars lacking both letters. CAC (Calcium carbide), CMSO (*Cucumeropsis mannii* seed oil).

Discussion

Antioxidants are vital for protecting the body from dangers of oxidative stress, which usually arises when reactive oxygen species (ROS) surpass the system's capacity to counteract them. This imbalance can lead to cellular injury, inflammation, and various health problems, particularly when aggravated by environmental toxins such as calcium carbide, regularly used in agriculture as a ripening agent. Calcium carbide generates lethal metabolites that can harm several organs (Ogunsuyi *et al.*, 2021). Calcium carbide exposure results in the production of reactive oxygen species (ROS), that contribute to oxidative stress (Adeyemi and Oyeyemi, 2020).

The spleen, a vastly vascular organ saddled with the responsibility of filtering blood and immune surveillance, is particularly susceptible to oxidative damage. ROS can attack cellular mechanisms for instance, proteins, DNA and lipids, resulting in cell dysfunction and eventually, cell death. This oxidative damage can weaken the spleen's ability to accomplish its vital functions, such as blood filtration and immune response orchestration (Galaris and Pantopoulos, 2019).

Cucumeropsis mannii seed oil (CMSO), derived from the egusi melon, is a natural source of antioxidants, abundant in tocopherols, phenolic acids and phytosterols, demonstrating outstanding antioxidant and anti-inflammatory properties (Adebayo *et al.*, 2020). The spleen, essential for blood filtration and immune function, is especially susceptible to oxidative stress as a result of calcium carbide exposure. This results in considerable increase in ROS production, leading to inflammation and potential cellular injury, thereby impairing the spleen's capability to filter pathogens and produce immune cells, thereby, elevating the risk of infections (Ibrahim *et al.*, 2019).

Superoxide dismutase (SOD) is an essential antioxidant enzyme saddled with the responsibility of deactivating reactive oxygen species (ROS) and averting oxidative injury to cells and tissues.

This study revealed that the activity of SOD reduced considerably in the untreated group when compared to animals in normal control, reflecting oxidative distress due to calcium carbide exposure, signifying a considerable impairment in the spleen's ability to combat oxidative stress. This outcome is consistent with the study of Adebayo and Onyekachi (2022), that established calcium carbide exposure results in increased reactive oxygen species (ROS) production, undermining the antioxidant defense mechanisms

within various tissues in the body. Moreso, the decline in SOD activity aligns with findings from Okoye *et al.* (2023), who also established that reduced SOD activities can adversely impair the spleen's role in immune response. The consequences of lessened SOD activity are profound, as the spleen majorly plays an essential role in the body's immune system by producing lymphocytes and filtering blood.

Furthermore, increased SOD activity after treatment with CMSO is an indication that CMSO can enhance the spleen's ability to neutralize superoxide radicals, thus decreasing their harmful effects on cellular structures. This observation is in conformity with recent research that emphasizes the antioxidant properties of CMSO and its potential to strengthen cellular function under conditions of oxidative stress (Onodugo *et al.*, 2024). The increase in SOD activity due to CMSO suggests a protective effect, improving the spleen's ability to address oxidative stress and sustain vital immune functions (Adeleye *et al.*, 2021). The collective effects of tocopherols, phenolics, and fatty acids help counter the oxidative stress caused by agents like calcium carbide, allowing SOD activity to reemerge and protect spleen cells from oxidative injury. These bioactive compounds in CMSO work synergistically to reduce ROS levels, thereby, creating an environment that encourages increased SOD activity (Igwenyi *et al.*, 2022).

As an enzyme that decomposes hydrogen peroxide into water and oxygen, catalase (CAT) plays a vital role in protecting cells from oxidative harm due to reactive oxygen species (ROS) buildup (Garcia and Evans, 2020). Administration of CMSO demonstrated an outstanding restoration of CAT activity in the spleen, highlighting CMSO's ability in defending cells against oxidative stress. This improvement supports existing knowledge that natural antioxidants, like those present in *Cucumeropsis mannii*, can bolster CAT activity and protect tissues from oxidative injury, contributing to overall organ resilience under cellular distress.

Glutathione peroxidase (GPx) and reduced glutathione (GSH) are vital for the splenic defense against oxidative stress. As an important antioxidant enzyme, GPx catalyzes the conversion of hydrogen peroxide and lipid peroxides into inoffensive byproducts, thereby, protecting cellular structures from injury or damage. GSH, on the other hand, not only assists as a key cofactor for GPx but also acts self-sufficiently to counteract reactive oxygen species (ROS), thereby maintaining the balance desirable for

cellular health and wellbeing (Chen *et al.*, 2021). Simultaneously, GPx and GSH form a strong defense mechanism that becomes impaired under oxidative stress conditions, as evident in the calcium carbide-exposed group. The reduction in GPx activity and GSH levels in the calcium carbide-exposed group indicates that prolonged ROS exposure overpowers the spleen's innate antioxidant defenses, therefore, compromising its immune function. CMSO treatment effectively restored both GPx activity and GSH levels, demonstrating that its bioactive compounds may play a substantial role in detoxifying ROS and stabilizing antioxidant systems within the spleen (Agu *et al.*, 2022). This restorative effect aligns with the research of Wu *et al.* (2022) signifying that natural antioxidants can enhance and preserve GPx activity and GSH levels, even under intense oxidative stress.

This protective ability likely stems from the antioxidant properties embedded in CMSO, which decreases oxidative stress and cellular injury within splenic tissues. These findings align with recent research demonstrating that natural antioxidants has the capability to preserve tissue structures and thereby alleviating oxidative stress-related challenges (Obi *et al.*, 2021).

Conclusion

CMSO possess antioxidant properties which aids in mitigating oxidative stress induced by calcium carbide in the spleen of rats. This study reveals that CMSO, particularly at a lower dose, effectively enhances the activities of key antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and maintains reduced glutathione (GSH) levels. This study proposes that CMSO is a potent natural anti-oxidant with potential therapeutic applications in oxidative stress-related conditions. These findings support the further exploration and development of CMSO as a natural intervention to combat oxidative damage and improve cellular health.

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