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Protective Effects of *Cyperus esculentus* against Arsenic Trioxide-induced Gastrotoxicity in Wistar Rats

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ABSTRACT

The ingestion of metal particles or fumes poses a significant threat to human health, with prolonged exposure potentially causing a variety of gastrointestinal disorders and other adverse health effects. This study investigated the protective effects of ethanol tuber extract of *Cyperus esculentus* (ETECE) against arsenic trioxide (ATO)-induced gastrotoxicity in Wistar rats. A total of 49 adult Wistar rats (n=7) were randomly assigned to seven groups: Groups A (control), B (10 mg/kg body weight (bw) of ATO only), C (200 mg/kg bw of ETECE + ATO), D (400 mg/kg bw of ETECE + ATO), E and F (200 and 400 mg/kg bw of ETECE respectively) and G (ATO + 100 mg/kg bw of Vitamin C). Following 60 days of administration, the animals were humanely euthanized. Blood and stomach tissue were collected for oxidative stress and histopathological analyses respectively. Arsenic trioxide caused significant decreases in glutathione, catalase and superoxide dismutase levels, increased malondialdehyde levels ($p < 0.05$), and widespread gastric mucosal necrosis and significant infiltration of inflammatory cells. Treatment with ETECE significantly improved the oxidative stress markers, while treatment with 400 mg/kg bw of ETECE substantially alleviated the detrimental histological effects. The findings of this study suggest that ETECE, at a dose of 400 mg/kg, exerts a protective effect against ATO-induced gastrotoxicity in adult Wistar rats, highlighting its potential as a gastroprotective agent at this dose.

Keywords: *Cyperus esculentus*; arsenic trioxide; inflammatory cells; mucosa necrosis; antioxidant.

INTRODUCTION

Arsenic trioxide (ATO) is a highly toxic substance that poses significant health risks upon ingestion. Exposure to arsenic trioxide has been linked to severe gastrointestinal symptoms, including abdominal pain, nausea, vomiting, and diarrhea[1]. Due to its toxicity, the commercial applications of arsenic trioxide are limited. Prolonged ingestion of arsenic trioxide, even in medical settings as an anticancer treatment, can lead to devastating health consequences, including skin cancer, reproductive problems and digestive issues[2]. Prolonged exposure to arsenic trioxide, particularly through the consumption of contaminated drinking water, can lead to severe and long-lasting health complications[3].

The stomach is one of the primary digestive organs affected by arsenic trioxide's toxic effects. These adverse effects can compromise the stomach's essential functions, including digestion and pathogen defense. As a result, arsenic trioxide-induced gastrotoxicity can lead to broader systemic health issues, underscoring the importance of mitigating exposure to this toxic substance[4]. The severe implications of arsenic trioxide exposure have sparked a growing interest in discovering effective treatments to counteract its harmful effects. One promising area of research involves the utilization of medicinal plants, which have been employed for some centuries in traditional medicine to treat a myriad of ailments. Throughout history, plants have played a vital role in traditional medicine, owing to their remarkable therapeutic properties. These properties are often attributed to bioactive compounds that possess antioxidant, anti-inflammatory, and detoxifying effects[5]. Medicinal plants offer a natural, accessible and potentially safer alternative to synthetic drugs, which can have detrimental side effects. By harnessing the therapeutic potential of medicinal plants, researchers may uncover novel treatments to protect the devastating effects of arsenic trioxide exposure.

Cyperus esculentus, a member of the Cyperaceae family, is a cultivated crop renowned for its numerous therapeutic effects[6]. This nutrient-rich tuber serves as an excellent energy source, boasting significant amounts of starch, fat, protein, sugar, and essential dietary minerals, making it a valuable and nutritious food resource[7]. In Nigeria, *C. esculentus* is a widely recognized plant with diverse local names across ethnic groups, including "Aya" in Hausa, "Imumu" in Yoruba, and "Ofio" in Igbo[8]. Nigerians utilize this versatile plant in various forms, consuming it fresh, dried, roasted, or as a key ingredient in the traditional beverage "Kunu"[9]. *C. esculentus* is primarily cultivated in Nigeria's middle belt and northern regions, where three distinct varieties - black, brown, and yellow - are grown. While all three varieties are cultivated, the yellow and brown varieties are more commonly available in markets. *C. esculentus* boasts an impressive nutritional profile, rich in antioxidants such as vitamin E, vitamin C and quercetin, essential minerals like zinc, potassium and phosphorus[10]. This nutrient-rich tuber offers a promising natural resource for promoting health and well-being.

Beyond its nutritional value, *C esculentus* has been traditionally revered for its potential to enhance male fertility and sexual wellness. Research has demonstrated its ability to augment libido, improve sexual performance and restore sexual function in individuals with pre-existing sexual abnormalities, positioning it as a valuable natural remedy for promoting reproductive health[11]. *C. esculentus* has also been traditionally utilized in treating urinary tract and bacterial infections, reducing the risk of colon cancer when consumed[12]. In recent years, researchers have shown increasing interest in exploring the potential of medicinal plants with antioxidant properties, such as *C. esculentus*, to counteract metal toxicity[13]. This study aimed to investigate the protective potential of ethanol tuber extract of *C. esculentus* (ETECE) against arsenic trioxide-induced gastrotoxicity in Wistar rats.

MATERIALS AND METHODS

Collection and extraction of plant materials

Ethical approval for this study was obtained from the Research Ethics Committee of College of Medicine, University of Benin, with approval number: CMS/REC/2024/743. *C. esculentus* tubers were obtained from the New Benin Market in Benin-City, Edo State, Nigeria. To ensure authenticity, a sample was submitted to the University of Benin's Department of Plant Biology and Biotechnology for verification. The sample was positively identified and assigned the herbarium number UBH-C419. Following authentication, the tubers underwent thorough washing with tap water air-drying to remove excess moisture and pulverization into a fine powder. A 150 g portion of the powdered tubers was then soaked in 1000 ml of 50 % ethanol for 72 hours. The crude ethanol extract was filtered using a Buchner funnel and Whatman No.1 filter paper to obtain a clear filtrate. The filtrate was subsequently freeze-dried using described method[14] at the University of Benin Natural Product Research Laboratory. The freeze-dried extract was stored in a refrigerator at 4°C pending further analysis.

Animal model and experimental design

A total of 49 adult Wistar rats, weighing between 190-210 g, were used for this study. These rats were bred in the anatomy animal house at the School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The animals were provided with unlimited access to food and water and a controlled laboratory environment designed to ensure their optimal comfort and well-being. The laboratory conditions were carefully maintained within a narrow range of temperature: 28 ± 2 °C, relative humidity: 50 ± 5 % and 12-hour light-dark cycle. After a 2-week acclimatization period, the rats were randomly assigned to seven groups (n=7 per group) and received predetermined dosages of ethanol tubal extract of *C. esculentus* and ATO via oral gavage. The dosages were determined based on the LD_{50} values obtained using the described method[15]. The treatment groups were Group A (Control): received 1 ml of distilled water, Group B: received 10 mg/kg bw of ATO only, Group C: received 200 mg/kg bw of ETECE and ATO, Group D: received 400 mg/kg bw of ETECE and ATO, Group E: received 200 mg/kg bw of ETECE only, Group F: received 400 mg/kg bw of ETECE only and Group G: received ATO and 100 mg/kg bw of Vitamin C.

Humane euthanasia and tissue collection

Upon completion of the 60-day treatment regimen, the rats were humanely euthanized using ketamine anesthesia. Anesthesia was induced by briefly exposing the rats to cotton wool soaked in approximately 30ml of ketamine within an enclosed container. Once anesthetized, each rat was positioned on the dissection table, and a thoraco-abdominal incision was made to access the abdominal cavity. The stomach was carefully excised and immediately preserved in 10% neutral buffered formalin solution within a universal container, in preparation for subsequent histopathological analysis and blood samples were collected from each rat via cardiac puncture.

Assessment of oxidative stress markers and antioxidant enzymes

The following biochemical assays were conducted to evaluate oxidative stress and antioxidant status: lipid peroxidation was assessed using earlier method described[16], superoxide dismutase (SOD) activity was measured according to the method described[17], catalase (CAT) activity was evaluated using the described method by Cohen[8] and glutathione peroxidase (GPx) activity was assessed accordingly[19].

Histological processing and evaluation

The formalin-fixed stomach tissue underwent routine histological processing, which included: dehydration in a graded ethanol series (70-100%), clearing with xylene and embedding in paraffin wax. Thin sections were then cut from the embedded tissue and stained with hematoxylin and eosin (H&E) according to the protocol described by Drury[20]. The stained sections were examined under a light microscope to assess any histological alterations.

RESULTS

Effect of treatment on oxidative stress

The result shows that rats treated with arsenic trioxide only showed significant decreases ($p<0.05$) in superoxide dismutase, catalase and glutathione peroxidase levels and a corresponding significant increase ($p<0.05$) in malondialdehyde concentration. In contrast, rats pretreated with ETECE showed significant increases ($p<0.05$) in SOD, CAT, and GPx levels and a corresponding significant decrease ($p<0.05$) in MDA (Figures 1-4).

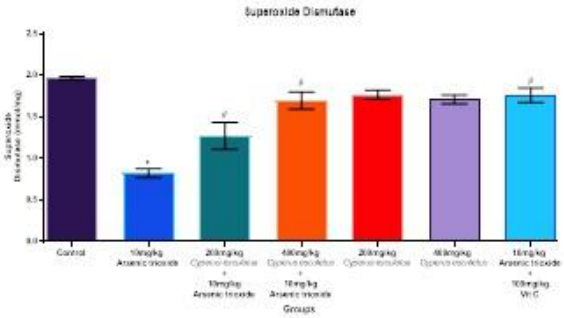

Figure 1. Superoxide dismutase Level after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with Control group; $^{\#}p<0.05$ compared with Arsenic trioxide alone group

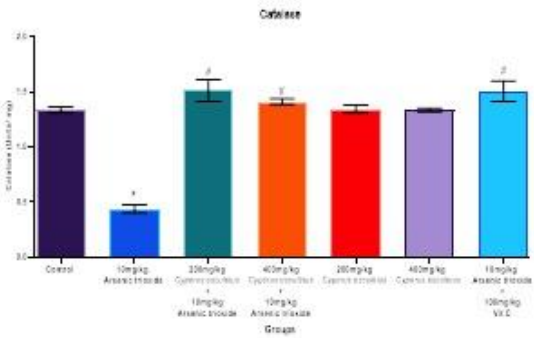

Figure 2. Catalase Level after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with Control group; $^{\#}p<0.05$ compared with Arsenic trioxide alone group

Effect of treatment on gastric histology

The control group (Group A) showed normal stomach cytoarchitectures of pitting mucosal

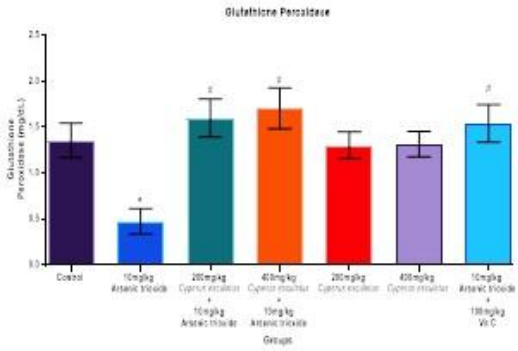

Figure 3. Glutathione peroxidase Level after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with Control group; $^{\#}p<0.05$ compared with Arsenic trioxide alone group

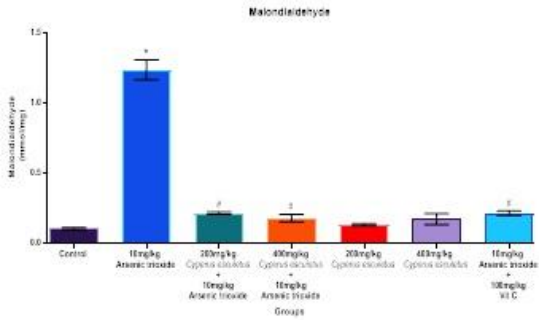

Figure 4. Malondialdehyde Level after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with Control group; $^{\#}p<0.05$ compared with Arsenic trioxide alone group.

membrane lining the mucosa, mucosal glands, submucosa and muscularis propria. The stomach of the group given arsenic trioxide only (Group B) showed extensive mucosal necrosis and heavy mucosal infiltrates of inflammatory cells. The stomach of the group given 200 mg/kg bw of ethanol tube extract of *C. esculentus* and arsenic trioxide (Group C) showed marked mucosal necrosis and heavy mural infiltrates of inflammatory cells. The stomach the group given 400 mg/kg bw of ethanol tube extract of *C. esculentus* and arsenic trioxide (Group D) showed normal architecture: gastric, submucosa and muscularis propria. The stomach of the group given 200 mg/kg bw of ethanol tube extract of *C. esculentus* only (Group E) showed normal architecture of gastric glands, submucosa and muscularis propria. The stomach of the group given 400 mg/kg bw of ethanol tube extract of *C. esculentus* only (Group F) showed normal architecture of gastric glands, mural infiltrates of inflammatory cells and submucosa. The stomach of the group given 100 mg/kg bw of vitamin C and arsenic trioxide (Group G) showed marked mural necrosis and heavy infiltrates of inflammatory cells.

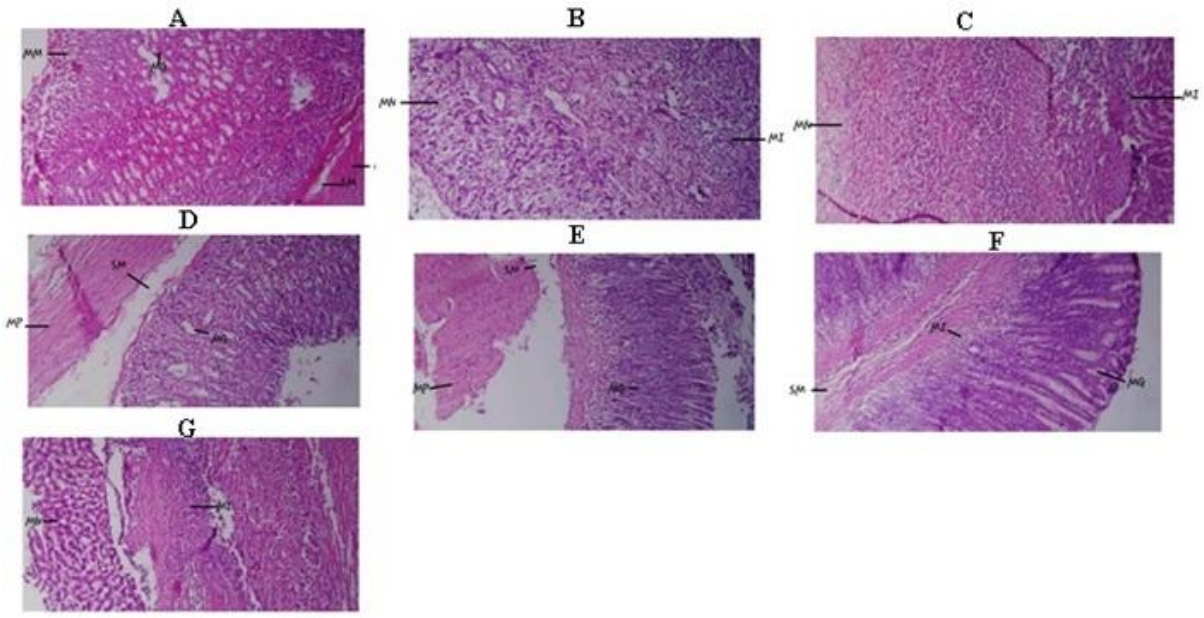

Figure 5. Photomicrographs of the stomach of Groups A-G, H and E; 100x. MM: pitting mucosa membrane, MG: mucosa gland, SM: submucosa, MP: muscularis propria, MN: mucosa necrosis, MI: mucosa infiltrates

DISCUSSION

Extensive evidence has demonstrated that arsenic trioxide exposure leads to organ toxicity in both humans and animals[21, 22]. The primary mechanism underlying arsenic trioxide's toxic effects involves the induction of oxidative stress, which disrupts cellular redox balance and generates reactive oxygen species. This results in damage to cellular macromolecules, including proteins, lipids, and DNA[23]. Furthermore, the toxic effects of arsenic trioxide are also mediated by its binding to sulfhydryl groups in proteins, leading to impaired enzyme function, mitochondrial dysfunction, and compromised energy production. This ultimately triggers cell death pathways, including apoptosis and necrosis[24]. These findings are consistent with previous studies, which have shown that arsenic trioxide toxicity primarily impairs gastric tissues through oxidative stress and inflammatory pathways[25].

Lipid peroxidation is a critical factor in the toxicity of various agents. Consistent with this notion, our study demonstrates that arsenic trioxide exposure leads to increased malondialdehyde levels, a biomarker of lipid peroxidation[25]. This finding suggests that lipid peroxidation plays a significant role in arsenic trioxide-induced toxicity. To counteract the deleterious effects of reactive oxygen species and free radicals, cells employ robust antioxidant defense mechanisms, including enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase[26]. However, our study reveals that arsenic trioxide exposure not only induces lipid peroxidation but also corresponds with a significant decrease in the activities of these cellular enzymatic antioxidants. Pretreatment with ethanol tubal extract of *C. esculentus* effectively protected against the dysregulation of antioxidant enzyme activity and induction of lipid peroxidation caused by arsenic trioxide exposure. This protective effect may be attributed to the extract's free radical scavenging and antioxidant properties, as reported by previous work[21]. The findings of this study suggest that *C. esculentus* extract may be a valuable adjunct in mitigating the toxic effects of arsenic trioxide.

Administration of arsenic trioxide resulted in significant histopathological changes in the stomach, characterized by extensive mucosal necrosis and heavy mucosal infiltrates of inflammatory cells. These findings are consistent with established reports of arsenic trioxide's potential gastrotoxic effects[4]. The gastric mucosal lesions caused by arsenic trioxide are likely attributed to oxidative stress, which disrupts the delicate balance between reactive oxygen species production and antioxidant defenses. Pretreatment with a higher dose of ethanol tubal extract of *C. esculentus* revealed notable improvements in the stomach cytoarchitecture of the rats, aligning with previous studies on the efficacy of herbal remedies in mitigating gastric lesions[27]. However, treatment with a higher dose of ethanol tubal extract of *C. esculentus*

alone showed mural infiltrates of inflammatory cells, suggesting a potential mechanism of action.

In contrast, sections of the stomach from rats treated with a lower dose of ethanol tubal extract of *C. esculentus* plus arsenic trioxide and vitamin C plus arsenic trioxide showed severe mucosal necrosis and heavy mural infiltrates of inflammatory cells. These findings indicate that a lower dose of ethanol tubal extract of *C. esculentus* and vitamin C, a known antioxidant, failed to prevent arsenic trioxide-induced gastrotoxicity. In contrast, a higher dose of ethanol tubal extract of *C. esculentus* provided remarkable protection to the stomach, suggesting that the efficacy of ethanol tubal extract of *C. esculentus* is dose-dependent, as reported in previous studies[28].

CONCLUSION

This study reveals that the ethanol tubal extract of *C. esculentus* exerts a dose-dependent protective effect against arsenic trioxide-induced gastrotoxicity, with the higher dose demonstrating enhanced protective efficacy.

Conflict of interest: The authors have no conflict of interest to declare.

Authors' contributions: SOI: Project design, data analysis and manuscript revision. SBO: Bench work, laboratory analysis, compilation of results and preparation of manuscript.

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