PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 29 (1) 177-182 Jan. 2025

Evaluation of Protective Effects of Aqueous Extract of *S. Maydis* on Liver Cytoarchitecture of Wistar Rats Induced with Carbonated Herbal Alcoholic Beverages (*Orijin Bitters*)

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ABSTRACT: Alcoholic beverages are renowned as the most common types of alternative medicine, but the frequent over indulgence in the use of alcoholic beverages however, have been linked with a number of social and importantly health problems and death. Herbs, such as *Stigma maydis*, which have been used for centuries in treating various illnesses, play a major role in forming the basic platform of modern medicines. Consequently, this work evaluated the protective effects of aqueous extract of *Stigma maydis* (AESM) on the liver cytoarchitecture of carbonated herbal alcoholic beverage-induced Wistar rats using appropriate standard methods. A total of Thirty-six Wistar rats (180-190 g), were divided into groups (n=6). Group A: Control (untreated), Group B: Orijin bitters, Group C: Orijin bitters + AESM (200 mg/kg), Group D: Orijin bitters + AESM (600 mg/kg), Group E: AESM (200 mg/kg) and Group F: AESM (600 mg/kg). Oral administration lasted for 60 consecutive days. After sacrifice, the livers were harvested, fixed in 10% neutral buffered formalin, and processed for histological assessment using haematoxylin and eosin staining. The results showed administration of orijin bitters caused significant alterations to the cytoarchitecture of the liver, characterized by severe vascular ulceration, mild to severe vascular congestion, and infiltration of inflammatory cells. However, treatment with graded doses of AESM effectively attenuated these histopathological features, with higher doses demonstrating more pronounced protective effects. In conclusion, these findings suggest that AESM possesses hepatoprotective activity against alcoholic beverage-induced liver damage, and it could be a potential therapeutic agent for mitigating liver injury.

DOI: https://dx.doi.org/10.4314/jasem.v29i1.23

Cite this Article as: AGBORTAI, A; EZE, G. I; OLUKAYODE, S. B; INNIH, S. O. (2025). Evaluation of Protective Effects of Aqueous Extract of *S. Maydis* on Liver Cytoarchitecture of Wistar Rats Induced with Carbonated Herbal Alcoholic Beverages (*Orijin Bitters*). *J. Appl. Sci. Environ. Manage.* 29 (1) 177-182

Dates: Received: 22 October 2024; Revised: 20 November 2024; Accepted: 28 December 2024; Published: 31 January 2025

Keywords: *Stigma maydis*; alcoholic beverages; vascular congestion; inflammatory cells.

Alcoholic beverages contain significant amounts of ethanol, a psychoactive substance that acts as a depressant at low consumption levels and an intoxicant at high consumption levels (Elijah *et al.*, 2010). The global consumption patterns of these beverages exhibit considerable variation across different countries and even among ethnic groups within the same country, with a history dating back to prehistoric times. Notably, distinct regional preferences for specific types of alcoholic beverages exist. For example, beer is the preferred choice in several European and African countries, while wine is favored in most wine-producing countries (Elijah *et al.*, 2010). In contrast, spirits are more popular in Eastern Europe and Asia, whereas palm wine is the preferred beverage in many African countries and parts of Asia. Beyond their recreational use, alcoholic beverages have been reported to

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possess various nutritional, medicinal, religious, and social applications (Elijah *et al.,* 2010). Herbal bitters, a category of alcoholic beverages, are widely recognized as a common form of alternative medicine, with a history dating back to ancient times. According to reports, approximately 80% of the global population in both developed and developing countries rely on herbal bitters as a form of healthcare (Oyewo *et al.,* 2013). These polyherbal formulations typically comprise a mixture of plant parts from various species and families. Orijin bitters is a well-established herbal bitter in Nigeria, acclaimed for its purported therapeutic benefits due to the presence of bitter substances. In traditional medicine, Orijin bitters is alleged to treat or manage a range of health issues, including poor digestion, constipation, appetite loss, jaundice, anemia, poisoning, immunological disorders, diarrhea, malaria, inflammation, and more (Schulz, 2010). However, excessive consumption of alcoholic beverages, including herbal bitters, has been linked to various social and health problems, as well as increased mortality rates. The addictive consumption of alcohol beverages has been associated with numerous health issues, including metabolic syndrome, liver and kidney problems, cardiovascular disease, neurodegenerative disorders, and inflammatory diseases (Grant *et al*., 2004). For centuries, herbs have played a vital role in treating various illnesses, laying the foundation for modern medicine (Grant *et al*., 2004).

The therapeutic effects of traditional herbs can be attributed to the presence of natural antioxidants, particularly phenolic compounds (Liu *et al.,* 2011). These compounds neutralize reactive oxygen species (ROS), which can cause diseases related to oxidative stress, such as cancer, hypertension, and cognitive dysfunction. One herb with potential health benefits is *Stigma maydis*, derived from the stigmas of the female maize flower. This abundant, waste material from corn cultivation has been consumed for centuries as a therapeutic remedy for various illnesses. *S. maydis* is used in traditional medicine worldwide, including in China, Turkey, the United States, and France, to treat conditions such as cystitis, edema, kidney stones, prostate disorders, and urinary infections (Bastien, 1982; Caceres *et al.,* 1987; Dat *et al.,* 1992; Grases *et al.,* 1993; Yesilada *et al.,* 1995; Hu *et al.,* 2010). *S. maydis* has been reported to have anti-fatigue, anti-depressant, and kaliuretic properties, as well as excellent antioxidant capacity (Maksimovic and Kovačević, 2003; Ebrahimzadeh *et al.,* 2008; Hu *et al.,* 2010). It has also demonstrated protective effects against radiation and nephrotoxicity (Bai *et al.,* 2010; Sepehri *et al.,* 2011). However, one study found no antibacterial activity in *S. maydis* against certain bacterial species (Alam, 2011). In China, *S. maydis* is considered an important medicinal plant for treating prostate problems (Hasanudin *et al.,* 2012). Native Americans have used it to treat urinary tract infections, malaria, and heart problems (Hasanudin *et al.,* 2012). Although not scientifically proven, *S. maydis* tea is claimed to have various health benefits, including lowering blood pressure and reducing prostate inflammation. Stigma maydis is rich in phenolic compounds, particularly flavonoids (Liu *et al.,* 2011), and contains proteins, vitamins, carbohydrates, minerals, volatile oils, steroids, alkaloids, and saponins (Ebrahimzadeh *et al.,* 2008). Given its potential benefits, numerous studies have investigated the pharmacological activities of *S. maydis*. Hence, the objective of this paper is to evaluate the protective effects of the aqueous extract of *S. maydis* (AESM) on the liver cytoarchitecture of Wistar rats induced with carbonated herbal alcoholic beverages (orijin bitters).

MATERIALS AND METHODS

*Collection, Identification and Preparation of Plant Material***:** Fresh *S. maydis* was obtained from the bustling New Benin Market in Benin City, Edo State. To verify its identity, the sample was taken to the Department of Plant Biology and Biotechnology at the University of Benin, where it was authenticated and assigned the herbarium number UBH-1045. The fresh sample was then cleaned with tap water, air-dried, and pulverized. A portion of the powdered sample, weighing 200 g, was soaked in 1000 ml of water for 48 hours. The resulting crude aqueous extract was filtered, and the filtrate was freeze-dried using Kumar's method (2019) at the University of Benin's Natural Product Research Laboratory. The freeze-dried extract was stored in the refrigerator at -4°C until further use.

*Experimental Animals***:** Thirty-six adult Wistar rats, weighing 180-190g, were sourced from the Animal House, Department of Anatomy, University of Benin. The animals were provided with food and water *ad libitum* and housed under controlled laboratory conditions, which included a temperature range of 28 ± 2°C, relative humidity of 50 ± 5%, and a 12-hour light-dark cycle, to ensure their optimal comfort and well-being.

Drugs Preparation: A 10 g sample of AESM was dissolved in 100 ml of water to create a stock solution. This solution was then administered orally to Wistar rats at doses of 200 mg/kg and 600 mg/kg body weight, following the determination of the

median lethal dose (LD_{50}) as described by Lorke (1983).

Animal Groupings: Thirty-six adult Wistar rats were randomly divided into six experimental groups (n=6). Group A: Control (untreated), Group B: Orijin bitters only, Group C: Orijin bitters + AESM (200 mg/kg), Group D: Orijin bitters + AESM (600 mg/kg), Group E: AESM (200 mg/kg) only and Group F: AESM (600 mg/kg) only.

The oral administration of AESM continued for 60 consecutive days. Following the treatment period, all animals were sacrificed under chloroform anesthesia, 24 hours after the final administration. An abdomino-thoracic incision was made to expose the thoracic viscera, and the liver was carefully harvested and dissected. The liver tissue was immediately fixed in 10% formol saline to prepare it for histological examination.

*Histological analysis***:** Following fixation in formol saline, the liver tissues underwent routine histological processing. This involved dehydration in a graded ethanol series (70-100%), xylene clearing, and paraffin wax embedding. Sections were then cut, stained with hematoxylin and eosin (H&E) using the method of Drury and Wallington (1980), and examined under a light microscope to assess histological changes.

RESULTS AND DISCUSSION

The control group (Group A) showed normal liver cytoarchitectures, with radially arranged hepatocytes with large round nucleus, central vein and sinusoids (Plate 1). The liver of the group given orijin bitters (Group B) showed severe vascular ulceration, mild vascular congestion and moderate infiltrates of inflammatory cells (Plate 2).

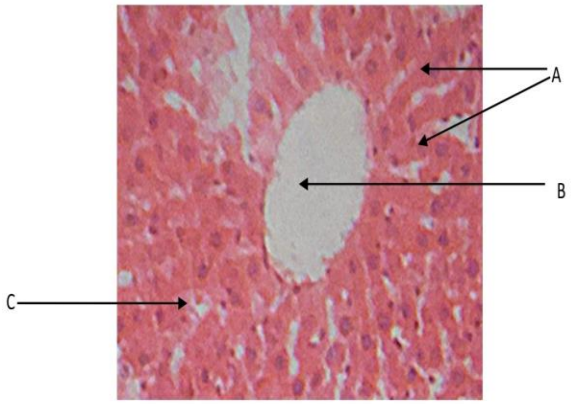

Plate 1. Photomicrograph of the liver of the control group (group A) showing normal histological features: radially arranged hepatocytes (A), central vein (B) and sinusoids (C). H and E 100x.

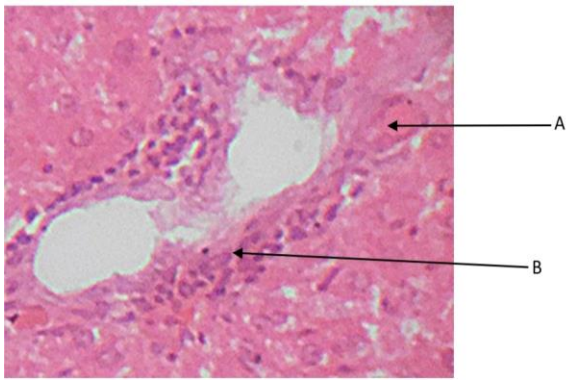

Plate 2. Photomicrograph of the liver of rat given orijin bitters only (Group B), showing: moderate infiltrates of inflammatory cells (A) and mild vascular congestion (B) H and E 100x.

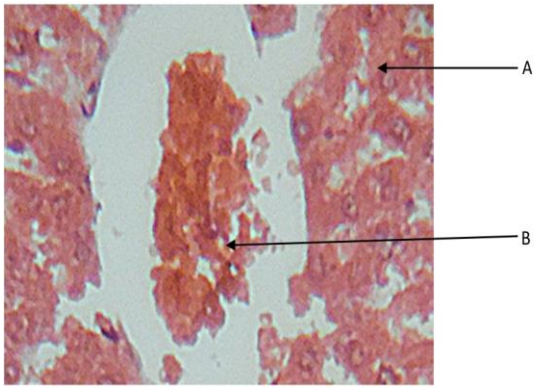

Plate 3. Photomicrograph of the liver of rat given orijin bitters and 200 mg/kg body weight of AESM (Group C), showing: normal hepatocytes (A) mild vascular congestion (B). H and E 100x.

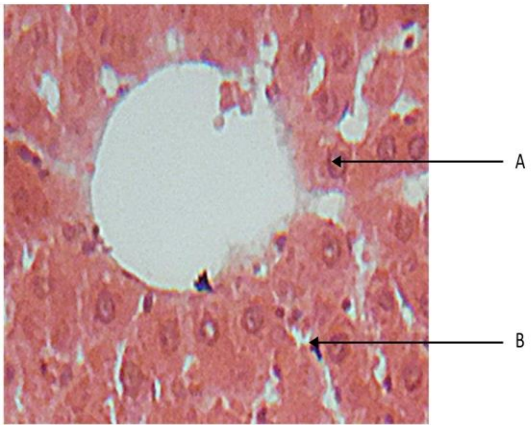

Plate 4. Photomicrograph of the liver of rat given orijin bitters and 600 mg/kg body weight of AESM (Group D), showing: normal hepatocytes (A) and moderate Kupffer cell activation (B) H and E 100x.

The liver of the group given orijin bitters and 200 mg/kg body weight of AESM (Group C) showed normal hepatocytes and mild vascular congestion (Plate 3). The liver of the group given orijin bitters and 600 mg/kg body weight of AESM (Group D) showed normal hepatocytes and moderate Kupffer cell activation (Plate 4).

The liver of the group given 200 mg/kg body weight of AESM only (Group E) showed normal hepatocyte architecture and mild Kupffer cell activation (Plate 5). The liver of the group given 600 mg/kg body weight of AESM only (Group F) showed normal hepatocyte architecture and mild Kupffer cell activation (Plate 6).

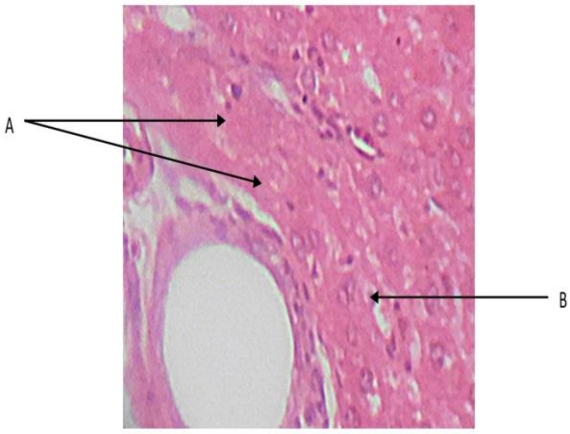

Plate 5. Photomicrograph of the liver of rat given 200 mg/kg body weight of AESM only (Group E) showing: normal hepatocyte architecture (A) and mild Kupffer cell activation (B). H and E 100x.

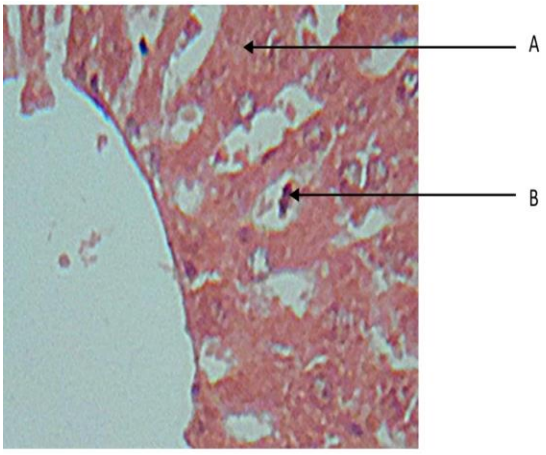

Plate 6. Photomicrograph of the liver of rat given 600 mg/kg body weight of AESM only (Group F), showing: normal hepatocyte architecture (A) and mild Kupffer cell activation (B). H and E 100x.

Alcohol use disorders pose a significant threat to public health, affecting approximately 10% of the population worldwide (Grant *et al.,* 2004). The economic burden of alcohol abuse is substantial, with far-reaching consequences for individuals, families, and communities (Office of National Drug Control Policy, 2004). Beyond the measurable economic costs, alcohol abuse also exacts a profound emotional toll, causing immeasurable suffering for individuals and those around them. Chronic alcohol consumption is closely linked to various liver diseases, including fatty liver, alcoholic hepatitis, and cirrhosis (Lim *et al.,* 2012). The breakdown of ethanol in the liver triggers a cascade of damaging effects, including the production of free radicals, acetaldehyde, and fatty acid ethyl esters, which ultimately harm liver cells (Lim *et al.,* 2012). In Nigeria, Orijin bitters have gained widespread acceptance for their purported therapeutic benefits, attributed to the herbal ingredients blended with flavors. The distinctive taste of Orijin bitters is characterized by a subtle fruitiness, bitterness, and medicinal undertones. Available in 20cl and 75cl sizes, Orijin bitters contain 30% alcohol by volume. The histological examination of liver tissues in this study revealed significant vascular congestion and inflammatory cell infiltration following treatment with Orijin bitters. These findings indicate a pronounced inflammatory response, characteristic of alcoholic hepatitis, which is marked by widespread liver tissue inflammation and destruction. The formation of scar tissue may also occur, replacing healthy liver tissue. Consistent with the findings of Oyewo *et al*. (2017), our results show that orijin bitters induce vascular congestion and inflammatory cell infiltration in the liver. However, co-administration of graded doses (200 mg/kg and 600 mg/kg body weight) of *S. maydis* with orijin bitters attenuated hepatitis, with the higher dose exhibiting a more potent effect. This finding is in line with Karami *et al*. (2013), who reported that *S. maydis* alleviates hepatitis in adult Wistar rats. The results suggest that *S. maydis* activates the liver's local immune system, with the higher dose proving optimal. This is consistent with Guo *et al.* (2009), who found that high doses of *S. maydis* extract activate Kupffer cells. The group treated with 200 mg/kg of AESM exhibited normal hepatocyte architecture and mild Kupffer cell activation, consistent with Kim *et al.* (2017) findings. These researchers demonstrated that *S. maydis* extract reduces the formation of reactive oxygen species and oxidative stress, thereby minimizing lipid peroxidation. This suggests that *S. maydis* possesses hepato-protective properties. Notably, even at a higher dose (600 mg/kg), the liver features remained normal, indicating that *S. maydis* can be well-tolerated without adverse effects.

Conclusion: This study provides evidence that AESM exerts a protective effect against carbonated herbal alcoholic-induced liver damage in adult Wistar rats. These findings suggest that AESM has potential as a hepatoprotective agent.

Declaration of Conflict of interest: The authors declare no conflict of interest.

Data availability: Data are available upon request from the first author.

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