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**Carbonated Herbal Alcoholic Beverage-Induced Hepatotoxicity in Wistar rats; Role of Aqueous Extracts of *Stigma maydis***

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<https://orcid.org/0009-0001-0248-5561>. <https://dx.doi.org/10.4314/sokjmls.v10i1.12>**Abstract**

People consume beverages that contain alcohol for various reasons, ranging from therapeutic to the well acclaimed 'depression killer', without little or no concern about their adverse effects. *Stigma maydis* is a readily available herb of significant therapeutic importance. Consequently, this work sought to investigate the role of aqueous extracts of *S. maydis* in alcoholic beverage-induced hepatotoxicity by assessing the antioxidant activity and liver panel in Wistar rats using appropriate and standard methods. The results showed that the administration of alcoholic beverage was associated with a significant increase in the activities of serum alanine aminotransferases and serum aspartate aminotransferases of the Wistar rats, but there were no statistically significant differences in alkaline phosphatase, total bilirubin and conjugated bilirubin levels. It also caused significant reduction in glutathione peroxidase and superoxide dismutase activities, and significantly elevated level of malondialdehyde activity, but no statistically significant difference in catalase activity. Administration of aqueous extract of *S. maydis* attenuated these biochemical manifestations in the liver. In conclusion, the results from this work revealed the hepatotoxicity of alcoholic beverages and the attenuating potential of aqueous extract of *S. maydis*.

**Keywords:** *Stigma maydis*; alcoholic beverage; antioxidant; liver panel.

**Introduction**

Certain locally produced alcoholic beverages, including Alomo bitters, Action bitters, Origin

bitters, Pasa bitters, 1960 bitters, and Osomo bitters, have successfully penetrated the market. Initial reservations regarding their safety and ingredients (Chineke *et al.*, 2015; Igbokwe *et al.*, 2017) have largely given way to their popularity, driven in part by claims of their purported health benefits and aphrodisiac properties. The misuse of alcohol is a pervasive issue with far-reaching consequences, impacting social, economic, and health outcomes. It is a major factor in elevated rates of violent fatalities and a leading cause of hospital admissions globally (Elijah *et al.*, 2010). Alcohol consumption, whether acute or chronic, can cause irreversible damage to internal organs and bodily systems, often accompanied by weight loss, ultimately eroding overall health and quality of life. Alcoholic beverages contain substantial amounts of ethanol, a psychoactive compound that exhibits depressant effects at moderate consumption levels and intoxicating effects at higher consumption levels (Elijah *et al.*, 2010).

Herbal bitters have a long history of use as a form of alternative medicine, dating back centuries (Oyewo *et al.*, 2013). Orijin bitters, a popular herbal bitter in Nigeria, is believed to possess therapeutic properties due to its bitter constituents. According to traditional practices, Orijin bitters are used to treat or manage a range of health conditions, including digestive issues, constipation, appetite loss, jaundice, anaemia, poisoning, immunological disorders, diarrhoea, malaria, inflammation, and more (Schulz, 2010). Bitters are typically composed of water and alcohol, which act as a solvent for botanical extracts and a preservative. Advocates of bitters attribute several health benefits to their

consumption, including relief from hemorrhoids and enhanced sexual function, improved blood circulation and kidney function, blood pressure regulation, prevention of kidney stone formation, colon cleansing, and potential antitumor effects (McGovern, 2009). Alcoholic bitters consumption is increasing in Nigeria, fueled by perceptions that these beverages offer various health benefits, such as body purification, anti-malarial properties, enhanced sex energy, anti-diabetic effects, and cholesterol reduction. Nevertheless, excessive consumption of alcoholic beverages, including herbal bitters, is associated with a multitude of social and health problems, as well as higher mortality rates (Oyewo *et al.*, 2013)

*Stigma maydis*, obtained from the female maize flower's stigmas, is an abundant byproduct of corn cultivation. Traditionally, it has been utilized for centuries as a therapeutic agent for various health issues. Globally, *S. maydis* is employed in traditional medicine 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). Research has demonstrated that *S. maydis* exhibits anti-fatigue, anti-depressant, and kaliuretic properties, as well as notable antioxidant capacity (Maksimovic and Kovačević, 2003; Ebrahimzadeh *et al.*, 2008; Hu *et al.*, 2010). Furthermore, it has shown protective effects against radiation and nephrotoxicity (Bai *et al.*, 2010; Sepehri *et al.*, 2011). Chemical analysis reveals that *S. maydis* is rich in phenolic compounds, particularly flavonoids (Liu *et al.*, 2011), and also contains proteins, vitamins, carbohydrates, minerals, volatile oils, steroids, alkaloids, and saponins (Ebrahimzadeh *et al.*, 2008).

Given its potential therapeutic benefits, numerous studies have investigated the pharmacological activities of *S. maydis*. This study investigates the protective effects of the aqueous extract of *S. maydis* (AESM) against hepatotoxicity induced by Orijin bitters in Wistar rats, with a focus on antioxidant activity and liver function.

## Materials and Methods

### Sourcing and Extract Preparation:

*S. maydis* samples were sourced from the bustling New Benin Market in Benin City, Edo State, a major hub for various commodities. To verify their authenticity, the samples were submitted to the Department of Plant Biology and Biotechnology at the University of Benin. Here, they were thoroughly authenticated and assigned the herbarium number UBH-1045, confirming their identity. The fresh sample was subjected to a multi-step processing protocol. First, it was cleaned with tap water, then air-dried, and subsequently pulverized into a fine powder. Next, 200g of the powdered sample was steeped in 1000ml of water for 48 hours to yield a crude aqueous extract. Following filtration, the resulting filtrate was freeze-dried using Kumar's method (2019) at the Natural Product Research Laboratory, University of Benin. Finally, the freeze-dried extract was refrigerated at  $-4^{\circ}\text{C}$  for subsequent analysis.

### Experimental Rats:

Thirty-six Wistar rats, weighing between 180-190g, were acquired from the Animal House, Department of Anatomy, University of Benin. The animals' well-being was prioritized by providing them with free access to food and water, as well as housing them in a controlled laboratory environment. This environment maintained a precise temperature of  $28 \pm 2^{\circ}\text{C}$ , relative humidity of  $50 \pm 5\%$ , and a consistent 12-hour light-dark cycle, ensuring optimal conditions for their health and comfort.

### Preparation of Extract:

A stock solution was prepared by dissolving 10g of the aqueous extract of *S. maydis* (AESM) in 100ml of water. This solution was then orally administered to Wistar rats at two doses: 200 mg/kg and 600 mg/kg body weight. The doses were determined based on the previously established median lethal dose ( $\text{LD}_{50}$ ), using the method described by Lorke (1983).

### Grouping of Rats:

Thirty-six Wistar rats were randomly divided into six experimental groups, each comprising six animals ( $n=6$ ), and designated as follows:

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
- Group F: AESM (600 mg/kg) only

The animals underwent oral administrations of AESM for 60 consecutive days. Following the treatment period, all animals were humanely sacrificed under chloroform anesthesia, 24 hours after the final administration. A surgical abdomino-thoracic incision was made to expose the thoracic viscera, and the liver was carefully excised, dissected and homogenized. Thereafter blood samples were collected through cardiac puncture using 5mls syringes. The blood samples were turned into plain bottles and sera were obtained from it by allowing it to stand for 2 hours at room temperature, followed by centrifuging at 2000 rpm for biochemical parameters.

**Analysis of Liver Panel:**

Standardized commercial kits were utilized to evaluate various serum biochemical parameters. The assessment included the measurement of Serum alkaline phosphatase (ALP) levels evaluated by Englehardt's method (1970), serum total bilirubin (TB) and conjugated bilirubin (CB) levels were estimated using the Jendrassik and Grof method (1938), serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to Reitman and Frankel (1957).

**Antioxidant activities:**

The levels of oxidative stress markers and antioxidant enzymes in the serum were evaluated using established methods. Malondialdehyde (MDA) levels were determined according to Buege and Aust (1978). Glutathione peroxidase (GPx) activity was measured using the method described by Nyman (1959). Superoxide dismutase (SOD) activity was assessed using the method of Misra and Fridovich (1972). Catalase (CAT) activity was determined in the serum according to the method described by Cohen (1970).

**Statistical Analysis:**

The results obtained were presented as mean ± standard error of mean (SEM). Statistical significance was determined using a p-value threshold of 0.05, where values of p < 0.05 were considered statistically significant.

**Results**

The results of liver panel analysis show that the administration of Orijin bitters only (group B) led to significant elevations in both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared to the control group. However, no statistically significant differences in ALT and AST levels were observed in the other treatment groups compared to the control group. Additionally, no statistically significant differences were found in alkaline phosphatase (ALP), total bilirubin (TB), and conjugated bilirubin (CB) levels across all treatment groups when compared to the control group.

**Table 1: Result of Liver Panel in the experimental animals.**

Parameters (µ/L)	Group A	Group B	Group C	Group D	Group E	Group F
ALP	27.33 ± 2.60	47.00 ± 5.86*	32.33 ± 2.85	28.33 ± 7.97	40.67 ± 7.79	32.00 ± 7.00
AST	34.33 ± 3.53	38.67 ± 5.36	55.33 ± 9.77	33.33 ± 2.33	38.67 ± 7.31	47.67 ± 8.01
ALT	150.0 ± 18.19	131.3 ± 16.83	188.3 ± 13.98	165.3 ± 18.50	190.0 ± 19.29	182.3 ± 7.75
TB	0.76 ± 0.13	0.47 ± 0.13	0.78 ± 0.07	0.64 ± 0.07	1.05 ± 0.13	0.69 ± 0.14
CB	0.50 ± 0.16	0.31 ± 0.08	0.59 ± 0.08	0.29 ± 0.04	0.67 ± 0.05	0.40 ± 0.09

**Key:** \* = p < 0.05 indicating a statistically significant difference as group A (control) is compared with the other groups.

Orijin bitters administration alone was found to significantly alter liver oxidative stress parameters, characterized by decreased glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, and increased malondialdehyde (MDA) activity, relative to the control group. Conversely, these parameters remained unaffected in the other treatment groups. Notably, catalase (CAT) activity was unaltered across all treated groups, exhibiting no statistically significant difference compared to the control group.

**Table 2: Result of oxidative stress markers**

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
GPx (U/mg)	170.7 ±	142.1 ±	162.1 ±	173.7 ±	179.7 ±	173.3 ±
	15.89	13.13*	15.41	26.96	16.29	12.36
SOD (U/mg)	51.41 ±	34.79 ±	51.57 ±	56.76 ±	43.29 ±	43.24 ±
	1.54	4.46*	8.88	10.89	11.19	8.67
CAT (U/mg)	31.04 ±	29.22 ±	37.89 ±	43.79 ±	33.43 ±	31.51 ±
	4.48	4.64	6.77	3.05	3.15	7.16
MDA (µmol/ml)	4.58 ±	8.24 ±	4.77 ±	3.59 ±	4.43 ±	5.42 ±
	0.45	0.76*	0.60	0.35	1.03	1.52

**Key:** \*= $p < 0.05$  indicating a statistically significant difference as group A (control) is compared with the other groups

**Discussion**

Alcohol use disorders are substantial public health problems, affecting approximately 10 percent of the population, and resulting in the enormous economic costs to any nation (Grant *et al.*, 2004). Alcohol consumption is associated with various liver diseases and is most strongly related to fatty liver, alcoholic hepatitis and cirrhosis (Lim *et al.*, 2012), with the main biological mechanism likely involving the breakdown of ethanol through oxidative and non-oxidative pathways, resulting in the production of free radicals, acetaldehyde, and fatty acid ethyl ester which then damage liver cells (Igboh *et al.*, 2009). Although previous research by Okareh *et al.* (2018) identified phytochemicals such as tannins, saponins, and alkaloids in Orijin bitters, which may offer health benefits, the liver panel results from this study aligns with the findings of Oyewo *et al.* (2017). The latter study demonstrated alterations in liver enzymes following the administration of Orijin bitters alone and in combination with an atherogenic diet, suggesting potential liver disturbances.

Alcohol's effects are also mediated through the induction of oxidative stress, a well-established mechanism. This occurs when alcohol triggers

the excessive release of prooxidants, such as NAD phosphate oxidase and inducible nitric oxide synthase, from Kupffer cells. Concurrently, alcohol consumption leads to a depletion of antioxidants, including selenium, glutathione, and vitamin E (Carithers and McClain, 2016). As a result, oxidative stress causes cellular damage through protein, lipid, and DNA oxidation, as well as direct cell injury via DNA damage, lipid peroxidation, and TNF production, ultimately activating nuclear factor kappa B signaling pathways (Arteel *et al.*, 2003; Carithers and McClain, 2016). Contrary to expectations, the present study found that treatment with Orijin bitters increased glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, while malondialdehyde (MDA) levels were decreased. This outcome aligns with the findings of Oyewo (2017) but contradicts the results of Alabi *et al.* (2013), who reported that herbal bitters reduced lipid peroxidation and increased catalase activity. However, it is essential to note that Alabi *et al.* (2013) investigated different brands of bitters, whereas the present study specifically examined Orijin bitters.

Elevated liver enzyme levels are indicative of compromised cellular function and integrity, hepatocellular damage, necrosis, altered membrane permeability, and cholestasis (Rosalski and McIntyre, 1999; Begum, 2004; Adeogun *et al.*, 2013). Specifically, increased aspartate aminotransferase (AST) activity serves as a marker for liver damage and disease. Elevated alanine aminotransferase (ALT) levels are associated with liver degeneration, fat accumulation (Westerbacka *et al.*, 2004), and impaired hepatic insulin sensitivity (Vojarova *et al.*, 2002). Furthermore, increased alkaline phosphatase (ALP) levels indicate tubular degeneration and necrosis. The significant increase in AST and ALT levels in the group administered with Orijin bitters alone indicates potential liver toxicity. However, no significant change was observed in ALP levels. In contrast, co-administration of Orijin bitters with AESM resulted in decreased AST and ALT levels, suggesting that AESM may have a protective effect against the hepatotoxicity induced by the alcoholic content of Orijin bitters.

### Conclusion

This study demonstrates that concurrent treatment with graded doses of AESM significantly attenuates liver damage, with the high dose exhibiting the most potent effect. These findings contribute to the growing evidence that AESM possesses hepatoprotective properties, likely attributed to its bioactive components with antioxidant properties. As such, AESM may be considered a promising hepatoprotective agent, warranting further investigation.

### Declaration

The authors declare no conflict of interest.

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