Original Research Article

HEPATO-PROTECTIVE EFFECT OF COSTUS AFER (OKPETE) JUICE EXTRACT ON ALCOHOL-INDUCED LIVER TOXICITY OF ADULT ALBINO FEMALE WISTAR RATS.

ABSTRACT

Background: The oral intake of alcohol has become a widespread concern due to its high risk to body health. Therefore, our purpose in this study was to reveal the antioxidant efficacies of natural *Costus'safer* stem juice extract on hepatotoxicity induced by ethanol in adult female wistar rats.

Methods: We examined the impacts of *C. afer* in female albino wistar rats orally treated with *C. afer* (200 and 400 mg/kg) in combination with 1ml, 10% ethanol daily for 21 days. The rats were divided into four (4) groups; Group A served as the control group, which was given rat chow and water. Groups B, C and D were given 1ml of 10% ethanol. Afterwards, groups C and D were treated with 200 and 400 mg/kg using 1ml and 2ml of the juice extract of *Costusafer*, respectively. A liver function test and histological analysis were carried out.

Results: The results showed that treatment with *C. afer* after the oral consumption of ethanol caused elevation in serum liver function parameters (alanine transferases, aspartate transaminase, and alkaline phosphate), which is significant compared to the control group. There was also a slight restoration in histopathological changes in the liver, as revealed by decreased areas of inflammation.

INTRODUCTION

The utilization of herbal remedies for treating or curing illnesses can be traced back to ancient times, such as the Stone Age. The field of pharmacology has seen significant progress over time, leading to the development of numerous synthetic drugs. This underscores the value of ethnomedicinal plants in the quest for new drugs (Boison et al., 2019). *Costus afer*, a tall perennial herbaceous plant with an unbranched structure and creeping rhizome, is commonly found in damp or shaded areas of tropical West Africa, including Nigeria, Ghana, and Cameroon (Anaga et al., 2004). It belongs to the Zingiberaceae family and is often called ginger lily. In Nigeria, it is known by various names such as "Okpete" or "Okpoto" in Igboland, "Kakizawa" in Hausa, "tete-egun" in Yoruba, and "Mbritem" in Efik (Iwu, 2014).

The medicinal attributes of *Costus afer* encompass its ability to protect the kidneys and liver and its antioxidant properties (Ezejiofor et al., 2014; 2015). It exhibits antinociceptive characteristics

and has a hypolipidemic impact (Ijioma et al., 2014; Emeh et al., 2014). *Costus afer* has also demonstrated antimicrobial and anti-inflammatory effects (Akpan et al., 2011; Ezejiofor et al., 2017; Boison et al., 2019).

Chronic consumption of alcohol is linked to an increased risk of various chronic and acute diseases, notably alcoholic liver disease (ALD) (Tsukamoto, 2007), which can progress to hepatocellular carcinoma (Lieber, 2004; Breitkopf et al., 2009). Despite its severe implications, there is currently no effective ALD treatment.

The pathogenesis of ALD involves intricate biological and molecular mechanisms, with alcohol metabolism being a crucial and fundamental aspect (Mello et al., 2008; Albano, 2008). Alcohol metabolism encompasses several enzymatic systems or enzymes, such as the aldehyde dehydrogenase 2 (ALDH2) pathway, alcohol dehydrogenase (ADH), and cytochrome P4502E1 (CYP2E1) system. Chronic ethanol consumption has been shown to inhibit ALDH2 activity, leading to significantly elevated acetaldehyde levels in tissues and plasma (Mello et al., 2008). Acetaldehyde, a major toxic metabolite of alcohol, plays a pivotal role in mediating alcohol-induced mutagenic and fibrogenic effects in the liver. It accelerates the formation of adducts that impair vital enzymes and proteins, contributing to ALD (Setshedi et al., 2010). Therefore, the research aimed to investigate the hepatoprotective effects of *Costus afer* stem juice extract against alcohol-induced liver toxicity in adult female albino Wistar rats.

MATERIALS AND METHOD

Collection and identification of *Costus afer*

Costus afer was harvested daily from the university environment at Abia State University Uturu, Abia State, Nigeria. The Botanist of the Department of Botany at Abia State University Uturu, Abia State, Nigeria, identified and authenticated the plant material.

Preparation of the plant extract

Fresh stems of *Costus afer* were collected from Uturu environment in Abia State University, they were washed clean to remove dirts and sand particles. The stems were debarked and cut into sections, crushed with a crucible mortar and pestle; the ground stems were squeezed and filtered to obtain the fresh juice extract (Uchegbu *et al.*, 2016). The process was done daily to get freshly prepared extract for daily administration.

Animal care handling

Twenty (20) adult female Wistar rats weighing 100-120g were bought from the animal house of Abia State University Uturu, Abia State, Nigeria, for the study. The test animals were kept for fourteen days in iron cages under standard conditions following the procedures of Ezejiofor and Orisakwe (2015). The protocol for the experiment was approved by the Abia State University Faculty of Basic Medical Science Research Ethics Committee. The animals were given standard feed and water.

Experimental design

Weight-matched rats were divided into four groups of five rats each. Group A was maintained as the normal control and was given only feed and water, while group B was maintained as the negative control and given 1ml of 10% ethanol only for 14 days. Rats belonging to groups C and D received 1ml of 10% ethanol, 1ml and 2ml of extract at 200 and 400mg/kg respectively using oral gavage. Following the procedures recorded in the work of Alahmari *et al.* (2022). Based on 1g of stock solution, the volume given to the animals for 200 mg/kg and 400 mg/kg doses of *Costus after* is 1 ml, and 2 ml, respectively, for 7 days. The volume was calculated according to the average weight of the animals in each treatment group.

Phytochemical Analysis of *Costus afer* **Extract**

Phytochemical analysis of *Costus afer* extract was conducted u***sing Gas Chromatography-Mass Spectrometry (G.C.–M.S.)*** to determine the nature of the extract components, which might contribute to the protective impacts against ethanol toxicity according to the method described by Uchegbu *et al.*, (2016)

Sample Collection and Sacrifice

At the end of the treatment (7 days), the rats were sacrificed by cervical dislocation (blood was collected by ophthalmic puncture). The liver was quickly removed and fixed in 10% of formaldehyde. After centrifugation of the homogenates (3000 rpm, 15 min), the supernatants were used for biochemical assays related to alcohol-induced liver function damage, described below.

Biochemical Analysis

Evaluation of Liver Function

Heparinized blood was centrifuged at 3000 rpm for 15 min for biochemical analysis. We measured the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP). AST and ALT were measured, as described by Reitman and Frankel (Reitman and Frankel, 1957). ALP level was estimated by colourimetric endpoint method,

Histological Analysis

The twenty Wistar albino rats used for the study were sacrificed under cervical dislocation, and the liver was harvested and fixed in 10% formalin. After 72 hrs, the organs were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. Auto technicians carried out tissue processing, and the prepared 5 µ thick section was mounted on slides and stained with hematoxylin and eosin. Stained sections were morphologically evaluated, and the pictures of the slides were taken for comparison**.**

Statistical Analysis

Statistical analysis will be performed using the Graphpad Prism for Windows statistical package, version 6.0 (Graphpad Software Inc). Data expressed as means ± S.D. The effects of treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by Turkey's post-hoc test to correct for multiple comparison treatments. Statistical significance is set at the $p < 0.05$ level.

RESULT

Phytochemical Screening of Costus afer Juice Extract

Table 1 illustrates the phytochemical contents along with the respective traces in the *Costus afer* extract. The chromatographic analysis revealed the presence of 9 components in the extract. A trace amount of alkaloids, tannins, saponins, steroids, carbohydrates, cardiac glycosides, triterpenoids, and proteins is present *in the C. afer* juice extract. All compounds were present in trace amounts, except for a Flavonoid, which occurred in a rare amount.

Table 1:Phytochemical Analysis of *Costus Afer*

PHYTOCHEMICAL	JUICE EXTRACT
Alkaloid	+
Flavonoid	-
Tannins	+
Saponins	+
Steroid	+
Carbohydrate	+
Cardiac glycoside	+
Triterpenoid	+
Protein	+
Keys: -rare; + trace;	

Liver Function test

The hepatic function parameters we tested in this experiment were AST, ALT, and ALP (Table 2, Fig. 1). Group B, which received ethanol only, showed a decrease in the levels of liver function parameters tested; we recorded no significant differences in the values of AST, ALT, or ALP ($p > 0.05$) in comparison with group 1 (control). We found highly significant elevations in AST, ALT, and ASP levels ($p < 0.003$; $p < 0.01$; $p > 0.05$, respectively) in group C and D rats after cotreatment with *C. after* extract at low and high doses, respectively.

Table 2: Effect of *Costus afer* juice extract on the liver biochemical parameters following alcohol-induced toxicity in adult female albino Wistar rat

Liver Biomarkers (mg/dL)	Groups				
	A	B	C	D	p-value
AST	52.00±3.00	47.50±0.50	63.50±1.50a	74.50±2.50a	0.003
ALT	17.50±1.50	14.50±0.50	20.00±1.00	23.50±0.50a	0.011
ALP	48.00±1.00	42.50±1.50	53.00±1.00	52.50±3.50	0.062

Values are given as Mean ± S.E. for each group where S.E. is the Standard Error. 'a' indicates a significant difference ($p<0.05$) compared to Group A (Control).

Figure 1. Effects of *C. afer* juice extract (200 and 400 mg/kg) and 1ml of 10% ethanol intake on the liver function parameters in serum after 21 days of the experiment. Aspartate transaminase (AST), Alanine transaminase (ALT), and alkaline phosphate (ALP) levels are expressed as milligrams per deciliter (mg/dL). All values are expressed as mean ± standard error (S.E.) of $n = 5$. [a] $p < 0.05$ vs. control group.

Effect of Costus afer juice extract on the histoarchitecture of alcohol-induced liver toxicity in adult female albino Wistar rat Histological examination

Histopathological examination of the liver showed marked inflammation and cytoplasmic vacuolation in the ethanol group (b), indicating toxicity, which was slightly restored dose-dependently in the treated group, as seen in Figure 2.

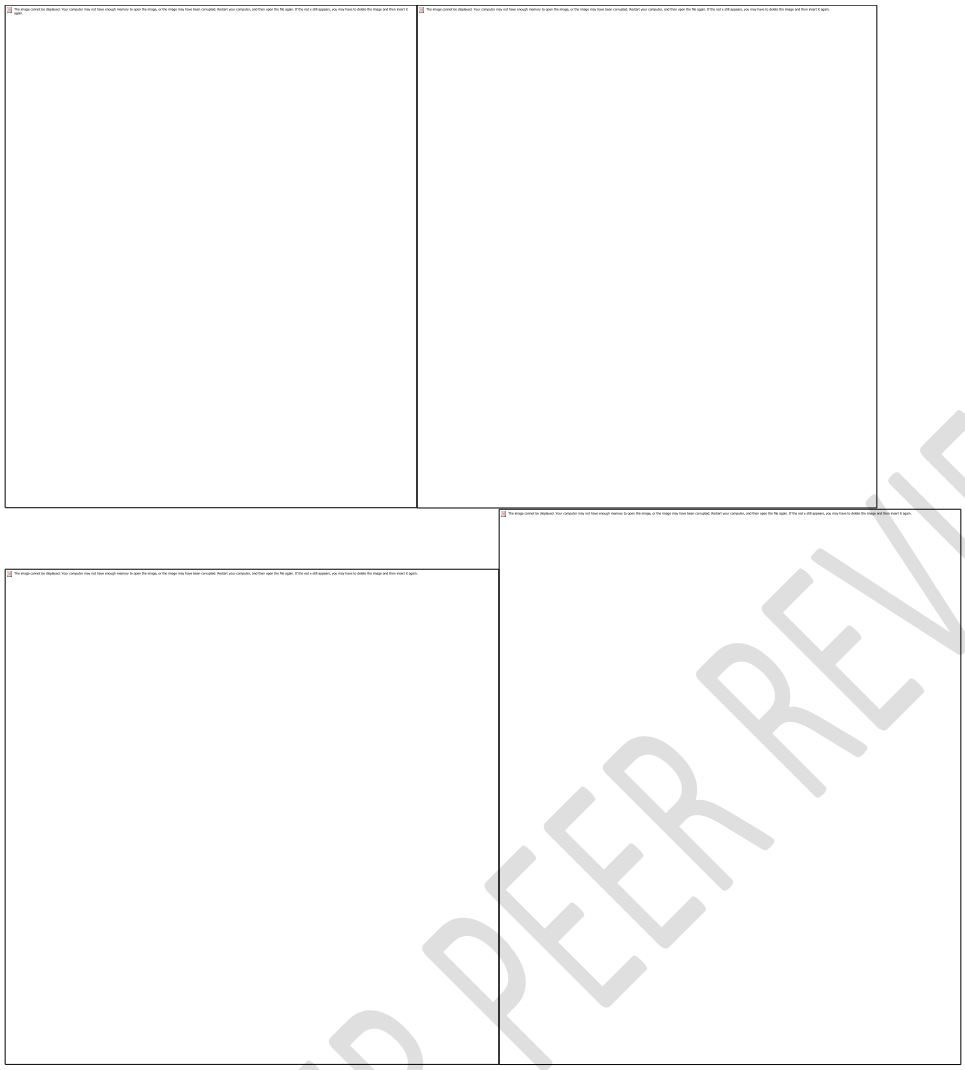

Figure 2. Photomicrograph of the liver of alcohol-induced hepatotoxic rats treated with *Costus afer* juice extract. (a) Normal control. (b) Toxic control. (c) ethanol ± 200mg/kg *Costus afer*. (d) ethanol ± 200mg/kg *Costus afer* (X400).

DISCUSSION.

In this study, we investigated the protective effects of *Costus afer* (*C. afer*) juice extract against alcohol-induced hepatotoxicity in rats, considering the widespread health risks associated with oral alcohol consumption. Our focus was on evaluating improvements in liver function and structure using hepatic function markers and histopathological examinations, as the precise role of this natural extract in mitigating alcohol-induced liver damage still needs to be completed.

Our results revealed that ethanol administration at a concentration of 10% significantly decreased hepatic function parameters, indicating hepatotoxicity. Histopathological changes in hepatic tissue supported this observation. These findings align with previous studies conducted on rat

models (Hyun et al., 2021). Ethanol-induced oxidative stress in hepatocytes plays a crucial role in hepatotoxicity and liver dysfunction, as indicated by previous research (Patel et al., 2021). The reduced levels of serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are indicative of increased permeability, damage, and necrosis of hepatocytes, a common consequence of ethanol consumption (Hyun et al., 2021).

Ethanol metabolism generates toxic byproducts that damage liver cells. Enzymes such as ALT and AST are released into the bloodstream, initially increasing serum levels and decreasing them with chronic administration. Moreover, elevated levels of hepatic tumour markers may serve as indicators of liver injury and mechanisms of cellular damage (Hussein and Khalifa, 2014). Therefore, measuring these markers during the 21-day experiment provided early evidence of cellular damage onset.

Histopathological examination of the liver revealed significant inflammation and cytoplasmic vacuolation in the group treated with ethanol alone (Group B), indicating toxicity, which is consistent with findings from previous studies by Arshed et al. (2012) and Hyun et al. (2021). Conversely, the control group (Group A) exhibited normal liver histology without inflammation or tissue distortion, with clearly visible central veins, hepatocytes, and portal tracts. Treatment with *Costus afer* extract showed mild restoration of liver hepatocytes and reduced inflammation. However, it did not fully reverse the hepatic injury caused by ethanol during the treatment period.

To assess its efficacy against ethanol toxicity, we administered ethanol + 400 mg/kg of *C. afer* extract to the animals (group d). Our data indicated that treatment with *C. afer* extract in combination with ethanol (group d) resulted in increased levels of ALT, ALP, and AST, with a significance level of $P < 0.05$ compared to the control group. These findings confirm that *C. afer* could mitigate the hepatotoxic effects induced by ethanol consumption. Similar results have been observed in multiple studies investigating the protective effects of *C. afer* extract against various toxic agents (Ezejiofor et al., 2014; 2017; Anyanwu et al., 2021; Amadi and Anyasor, 2023). Amadi and Anyasor (2023) demonstrated that *C. afer* extract can alleviate liver function biomarker disorders in a concentration-dependent manner, attributed to its natural bioactive components that may attenuate hepatic damage caused by free radicals. Furthermore, Anyanwu et al. (2021) reported that *C. afer* can inhibit hepatocarcinogenesis proliferation in a dose-dependent manner.

The beneficial effects of *C. afer* observed in our study may be linked to its antioxidant activities. These activities play a crucial role in hepatoprotection by scavenging free radicals, restoring antioxidant defense activity, and maintaining cell membrane integrity and stability, as evidenced in previous research (Ezejiofor et al., 2015). These mechanisms contribute to improving the structure and function of the liver.

CONCLUSION

In summary, administration of 10% ethanol at a dose of 1ml/kg for 21 days led to (i) a notable reduction in liver function parameters, which are key indicators of liver damage and toxicity, and (ii) histopathological changes in rat hepatic tissues, indicating mechanisms of cellular injury. Concurrent treatment with Costus afer alongside ethanol appeared to offer protective effects on the liver, evidenced by increased liver function biomarkers and improved liver histology, attributed to its phytochemical properties and ROS-scavenging activities. However, this combined treatment did not completely restore the liver's histoarchitecture. Our study indicates that the stem juice extract of *C. afer* may prevent or alleviate the risks associated with exposure to various toxic agents.

References :

Akpan, M.M., Odeomen, C.S. and Nwachukwu, C.N., (2011). Antimicrobial assessment of ethanolic extract of Costus afer leaves. *Asian Journal of Plant Science & Research*.

Alahmari, A.S., El-Mekkawy, H.I., Al-Doaiss, A.A. and Alduwish, M.A., (2022). Effect of natural Commiphora myrrha extract against hepatotoxicity induced by alcohol intake in rat model. *Toxics*, *10*(12), p.729.

Albano, E., (2008). Oxidative mechanisms in the pathogenesis of alcoholic liver disease. *Molecular aspects of medicine*, *29*(1-2), pp.9-16.

Amadi, B.N. and Anyasor, G.N., (2023). Evaluation of Costus afer Ker Gawl. Rhizome fractions for hepatoprotective function and characterization of its bioactive compounds. *Drug Discovery*, *17*, p.e36dd1960.

Anaga, A.O., Njoku, C.J., Ekejiuba, E.S., Esiaka, M.N. and Asuzu, I.U., (2004). Investigations of the methanolic leaf extract of Costus afer. Ker for pharmacological activities in vitro and in vivo. *Phytomedicine*, *11*(2-3), pp.242-248.

Anyanwu, B.O., Ezejiofor, A.N., Nwaogazie, I.L. and Orisakwe, O.E., (2021). Hepatoprotective Effect of Costus afer on Trace Metal Mixture Treated Rats Mediated by Regulation of Oxidative Stress Markers, Inflammatory Cytokines and Bio-Metal Chelation.

Arshed Iqbal, D., Ramesh Chandra, S. and Suresh Kumar, B., (2012). Hepatoprotection: a hallmark of Citrullus colocynthis L. against paracetamol induced hepatotoxicity in Swiss albino rats. *American Journal of Plant Sciences*, *2012*.

Boison, D., Adinortey, C.A., Babanyinah, G.K., Quasie, O., Agbeko, R., Wiabo-Asabil, G.K. and Adinortey, M.B., (2019). Costus afer: a systematic review of evidence-based data in support of its medicinal relevance. *Scientifica*, *2019*.

Breitkopf, K., Nagy, L.E., Beier, J.I., Mueller, S., Weng, H. and Dooley, S., (2009). Current experimental perspectives on the clinical progression of alcoholic liver disease. *Alcoholism: Clinical and Experimental Research*, *33*(10), pp.1647-1655.

Emeh, C.C., Abbey, B.W. and Essien, E.B., (2014). Hypolipidemic activity of aqueous extract of Costus afer stems in diet induced hyperlipidemic rats.

Ezejiofor, A.N. and Orisakwe, O.E., (2015). Assessment of the hepatoprotective and antioxidant effect of aqueous leaf extract of costus afer "ker gawl" on cyclosporine a induced hepatotoxicity. *Toxicol Int*, *22*, pp.83-91.

Ezejiofor, A.N., Orish, C.N. and Orisakwe, O.E., (2014). Costus afer ker gawl leaves against gentamicin-induced nephrotoxicity in rats. *Iranian Journal of Kidney Diseases*, *8*(4), p.310.

Ezejiofor, A.N., Udowelle, N.A. and Orisakwe, O.E., (2017). Nephroprotective and antioxidant effect of aqueous leaf extract of Costus Afer Ker gawl on cyclosporin-a (Csa) induced nephrotoxicity. *Clinical Phytoscience*, *2*, pp.1-7.

Hussein, R.H. and Khalifa, F.K., (2014). The protective role of ellagitannins flavonoids pretreatment against N-nitrosodiethylamine induced-hepatocellular carcinoma. *Saudi Journal of Biological Sciences*, *21*(6), pp.589-596.

Hyun, J., Han, J., Lee, C., Yoon, M. and Jung, Y., (2021). Pathophysiological aspects of alcohol metabolism in the liver. *International journal of molecular sciences*, *22*(11), p.5717.

Ijioma, S.N., Nwosu, C.O., Emelike, C.U., Okafor, A.I. and Nwankwo, A.A., (2014). Antinociceptive property of Costus afer Ker stem juice and ethanol leaf extract in albino rats. *Comprehensive Journal of Medical Sciences*, *2*(2), pp.14-19.

Iwu MM. (2014). *Handbook of African medicinal plants.* CRC press. p. 161–2.

Lieber, C.S., (2004). Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol*, *34*(1), pp.9-19.

Mello, T., Ceni, E., Surrenti, C. and Galli, A., (2008). Alcohol induced hepatic fibrosis: role of acetaldehyde. *Molecular aspects of medicine*, *29*(1-2), pp.17-21.

Patel, F., Parwani, K., Patel, D. and Mandal, P., (2021). Metformin and probiotics interplay in amelioration of ethanol-induced oxidative stress and inflammatory response in an in vitro and in vivo model of hepatic injury. *Mediators of inflammation*, *2021*, pp.1-31.

Reitman, S. and Frankel, S., (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), pp.56-63.

Setshedi, M., Wands, J.R. and de la Monte, S.M., (2010). Acetaldehyde adducts in alcoholic liver disease. *Oxidative medicine and cellular longevity*, *3*, pp.178-185.

Tsukamoto, H., (2007). Conceptual importance of identifying alcoholic liver disease as a lifestyle disease. *Journal of gastroenterology*, *42*, pp.603-609.

Uchegbu, R., Akalazu, J., Ibe, C., Ahuchaogu, A. and Amadikwa, C., (2016). Chemical composition of the stem extract of Costus afer (Bush Cane) and its antimicrobial activity. *British Journal of Pharmaceutical Research*, *10*(5), pp.1-9.