

Living and Making Sustainable Choices That Nourish Our Body

**A Celebratory Publication
In Honour of**

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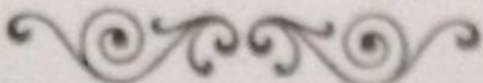
Published by
The Department of Biochemistry
University of Ilorin

ISBN: 978-978-8556-57-2

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Printed by:
UNILORIN PRESS
University of Ilorin, Ilorin
Email: unilorinpress@gmail.com

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CHAPTER SIXTEEN

BCH WL Festschrift HOB 006

CUCUMIS SATIVUS FRUIT HOMOGENATE'S HEPATOPROTECTIVE EFFECTS ON AUGMENTIN-INDUCED LIVER TOXICITY IN WISTAR RATS

Abubakar, F.A, Oloyede, H.O.B and Bamidale B. A

Abstract

Augmentin, a commonly prescribed antibiotic, poses a risk of liver toxicity, highlighting a significant health concern. This study aimed to explore the potential hepatoprotective effects of cucumber (*Cucumis sativus*) fruit homogenate against Augmentin-induced liver toxicity in Wistar rats. Thirty-six rats were randomly assigned to six groups: Control, Non-induced (receiving *C. sativus* homogenate), Augmentin-induced (receiving distilled water), Augmentin-induced + silymarin treated, Augmentin-induced + low dose of *C. sativus* homogenate treated, and Augmentin-induced + high dose of *C. sativus* homogenate treated. Evaluation encompassed phytochemical analysis of the homogenate, liver function parameters, and histological changes. Results revealed the presence of alkaloids, steroids, glycosides, terpenoids, and saponins in the homogenate. Augmentin administration induced hepatic injury, evidenced by elevated serum liver enzymes and altered histology. However, supplementation with *C. sativus* fruit homogenate mitigated these effects, leading to decreased liver enzyme levels and improved histological architecture. These findings suggest the potential hepatoprotective properties of *C. sativus* fruit homogenate against Augmentin-induced liver toxicity, possibly attributed to its phytochemical constituents. Further investigations are needed to delineate the underlying mechanisms and clinical implications of these observed effects.

Keywords: *Cucumis sativus*, fruit homogenate, Augmentin, hepatoprotective, phytochemical, histological

Introduction

The liver orchestrates vital biochemical and physiological functions, including growth, energy regulation, nutrient distribution, homeostasis maintenance, xenobiotic detoxification, and immune defense mechanisms, rendering it highly vulnerable to damage from hepatotoxic agents. Liver diseases, stemming from various factors such as genetic predispositions, viral infections, chemical or drug-induced injuries, obesity, diabetes, and autoimmune disorders, pose grave risks, leading to

irreversible harm to the liver or bile ducts, and potentially culminating in malignancies like liver cancer (Blachier *et al.*, 2013).

Augmentin, a widely prescribed antibiotic composed of amoxicillin and clavulanate, effectively tackles bacterial infections like otitis media, sinusitis, bronchitis, skin infections, and pneumonia (Pujagic, 2022). Although acute liver injury induced by amoxicillin-clavulanate typically presents as jaundice, lasting up to 24 weeks, fatalities or chronic damage are

infrequent, except in cases involving multiple exposures or comorbidities such as cirrhosis (Garcia-Cortes *et al.*, 2020).

Furthermore, medicinal plants offer significant therapeutic potential owing to their non-toxic nature and diverse healing properties, including liver ailment management. *Cucumissativus* L., commonly known as cucumber, has been esteemed as a significant vegetable crop in traditional Indian medicine for centuries. These low-calorie, high-water-content vegetables contain a plethora of phytochemicals, including carbohydrates, resins, saponins, terpenoids, tannins, cardiac glycosides, phytosterols, phlorotannins, alkaloids, flavonoids, glycosides, and steroid terpenes (Sari *et al.*, 2021). Additionally, cucumber plants exhibit a range of pharmacological activities, such as cytotoxic, antacid, carminative, hepatoprotective, hypoglycemic, and hypolipidemic effects (Khan *et al.*, 2022).

Given these findings and the observed properties of *Cucumissativus*, the hypothesis of its hepatoprotective potential, possibly through antioxidant and anti-inflammatory mechanisms, warrants investigation. Therefore, the present study aims to assess the hepatoprotective activity of *Cucumissativus* fruit homogenate using an Augmentin-induced liver damage rat model.

Material and Method

Chemicals:

Chemicals used in the study included Augmentin, manufactured by Glaxo Wellcome (Mayenne, France), and Silybon-70, manufactured by Micro Labs Ltd (Silymarin; Sipcot, Hosur - 635 126, India). Additionally, all other chemicals and reagents utilized were of analytical grade.

Collection of Plant Material.

The fruit of *Cucumissativus* was procured from a local market. Subsequently, the plant specimen will undergo authentication at the Department of Plant Biology Herbarium, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (UILH/001/1790/2024) has been deposited.

Preparation of fruit homogenate

The cucumber fruit underwent a thorough rinse in clean water before being homogenized with an electric blender (Mazeda Mill, MT 4100, Japan). Subsequently, it was preserved in an airtight container and refrigerated at 4°C until needed.

Hepatoprotective Assay.

The *in vivo* hepatoprotective activity of *Cucumissativus* fruit homogenate was assessed using the Augmentin-induced hepatotoxicity test in rats. The animals ($n=6$) were randomly divided into six

experimental groups and administered test solutions as follows:

Group I: Naive control, receiving distilled water.

Group II: Augmentin-induced at 80 mg/kg bw+ distilled water (negative control).

Group III: Augmentin-induced+ 100 mg/kg of Sylimarin (positive control).

Group IV: Augmentin-induced + 2ml/kg bw of *Cucumis sativus* fruit homogenate.

Group V: Augmentin-induced, + 4ml/kg bw of *Cucumis sativus* fruit homogenate.

Group VI: Non- induced rat receiving 4ml/kg bw of *Cucumis sativus* fruit homogenate

The animals were fasted for 48 hours before the experiment under standard laboratory conditions, with access to distilled water ad libitum. After fasting, each group received the respective dose of the test solution orally once daily for 12 consecutive days. On the 12th day, oral administration of Augmentin was performed 3 hours after the last homogenate administration, except for Group I and VI, which received only distilled water and *Cucumis sativus* fruit homogenate respectively.

After 48 hours of inducing hepatic injury, the animals were lightly anesthetized with diethyl ether, and blood was collected via cardiac puncture into sterilized centrifuge tubes. The blood was then centrifuged at 3000 rpm for 10 minutes to obtain serum for biochemical parameter analysis. Subsequently, the animals were

euthanized via cervical dislocation, and liver tissues were excised for histopathological examination.

Liver Enzymes Assessment

The collected serum was analyzed using standard methods for assessing liver enzymes. Levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were determined utilizing the Hitachi 902 Automatic Chemical Analyzer.

Non-enzymatic liver parameter

Serum albumin concentration was assessed following the method outlined by Doumas *et al.* (1971), while serum bilirubin concentration was determined using the method described by Jendrassik and Grof (1938). Serum globulin levels were measured according to the method outlined by Tietz (1995), and total protein was determined utilizing the biuret method as described by Gornall *et al.* (1949).

Histopathology

The liver tissue was excised and preserved in 10% formalin, then subjected to dehydration through a gradual ethanol series (50-100%), followed by clearance in xylene, and embedding in paraffin wax. Subsequently, sections of 5-6 mm thickness were prepared using a rotary microtome (Leica RM 2125 RTS, Singapore) and stained with hematoxylin and eosin dye for microscopic examination.

histopathological changes in the liver. The liver sections the severity of hepatic injury according to the method described by El-Beshbishy *et al.* (2010) with minor modifications.

Statistical Analysis

The data were presented as the mean $\pm$ standard error of mean (SEM) from six determinations. One-way Analysis of Variance (ANOVA) followed by Duncan's test was used for statistical analysis. A p-value of less than 0.05 was considered statistically significant (Mahajan, 1997). Graph Pad Prism 5 and SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) were used for data analysis.

Results

Table 1 presents the phytochemical constituents found in the *Cucumissativus* homogenate, including saponins, terpenoids, coumarins, glycosides, steroids, and alkaloids, with concentrations of 0.047, 0.14, 0.15, 0.63, 5.26, and 46.78 mg/100g, respectively.

were further evaluated and scored for

Table 2 illustrates the impact of serum concentrations of selected liver function indices in rats orally administered *Cucumissativus* homogenate at doses of 2.0 and 4.0 mg/kg body weight for 12 days. The serum protein concentration of the induced but treated groups showed no significant difference ($p>0.05$) compared to the control group. However, it exhibited a significant decrease ($p<0.05$) when compared to the untreated group, yet demonstrated a favorable comparison with the Silymarin-treated group. A similar trend was observed in the concentrations of serum albumin, globulin, and bilirubin when compared to their respective controls.

Figure 1 illustrates the impact of serum activities of transaminases in rats orally administered *Cucumis sativus* homogenate at doses of 2.0 and 4.0 mg/kg body weight for 12 days. The levels of aspartate transaminase (AST) and alanine transaminase (ALT) in the homogenate-treated group showed no significant difference ($p>0.05$) compared to the control and Silymarin-treated group, but exhibited a significant decrease ($p<0.05$) compared to the untreated group.

Table 1. Phytoconstituents present in *Cucumis sativus* homogenate

Secondary metabolites	Concentration (mg/100g of homogenate)
Alkaloids	46.78 ± 0.62
Steroids	5.26 ± 0.65
Glycosides	0.63 ± 0.62
Carbohydrates	0.15 ± 0.65
Terpenoids	0.14 ± 0.66
Saponins	0.067 ± 0.66

Values expressed as mean ± SEM of three replicates

Table 2. Effect of *Cucumis sativus* homogenate on selected liver function indices in rats

Parameters	Total Protein (mg/dL)	Albumin (mg/dL)	Globulin (mg/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Control	22.16 ± 0.53 ^a	15.61 ± 0.48 ^a	6.46 ± 0.12 ^a	31.26 ± 0.58 ^a	21.34 ± 0.67 ^a
Extracted	23.46 ± 0.55 ^a	20.17 ± 0.20 ^a	9.61 ± 0.15 ^a	43.51 ± 0.55 ^a	29.28 ± 0.68 ^a
C	22.56 ± 0.71 ^a	15.14 ± 0.55 ^a	6.58 ± 0.17 ^a	34.65 ± 0.61 ^a	21.71 ± 0.66 ^a
D	22.85 ± 0.48 ^a	15.26 ± 0.66 ^a	7.62 ± 0.20 ^a	34.21 ± 0.20 ^a	22.36 ± 0.68 ^a
E	21.51 ± 0.66 ^a	15.16 ± 0.46 ^a	6.47 ± 0.10 ^a	32.32 ± 0.52 ^a	20.87 ± 0.38 ^a
F	21.33 ± 0.64 ^a	15.09 ± 0.70 ^a	6.45 ± 0.20 ^a	31.66 ± 0.47 ^a	20.68 ± 0.58 ^a

Values are means ± SEM of five replicates. Values in the same column with the same superscript are not significantly different ($p > 0.05$). C=Induced+100 mg/kg Syllmarin; D=Induced+2 mL/kg homogenate; E=Induced+4 mL/kg homogenate; F=4 mL/kg homogenate.

Conversely, for lactate dehydrogenase (LDH), the group treated with 2.0 mg/kg homogenate showed no significant difference ($p>0.05$) compared to the control, while at 4.0 mg/kg homogenate, both the induced and non-induced groups showed a decrease compared to the control. However, all treated groups showed a significant decrease compared to the untreated group.

Figure 2 shows the effect of serum activities of transaminases in rats orally administered *Cucumis sativus* homogenate at doses of 2.0 and 4.0 mg/kg body weight for 12 days. The activities of acid phosphatase (ACP), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in the homogenate-treated group showed no significant difference ($p>0.05$) compared to the control and Silymarin-treated group, but exhibited a significant decrease ($p<0.05$) compared to the untreated group.

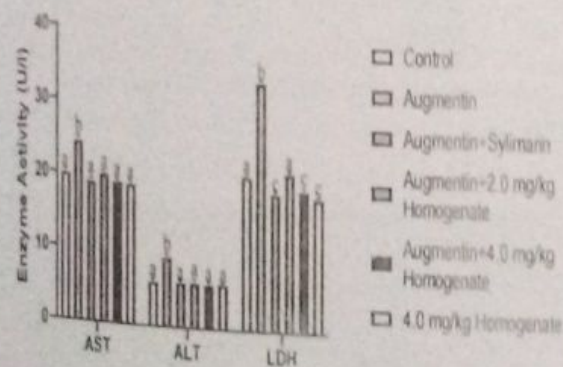


Figure 1. Effect of *Cucumis sativus* homogenate on selected liver enzyme activities in rats treated for 12 days

Values are means $\pm$ S.E.M of five replicates. Values in the same column with different superscripts are

significantly different ($p<0.05$).
 AST=Aspartate transaminase;
 ALT=Alanine transaminase;
 LDH=Lactate dehydrogenase.

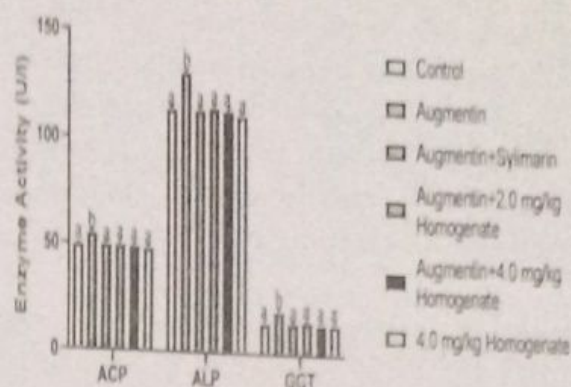


Figure 2. Effect of *Cucumis sativus* homogenate on selected liver enzyme activities in rats treated for 12 days

Values are means $\pm$ S.E.M of five replicates. Values in the same column with different superscripts are significantly different ($p<0.05$).
 ACP=Acid phosphatase; ALP=Alkaline phosphatase;
 GGT=Gamma-glutamyltransferase.

Plates 2-6 display photomicrographs of rat liver cross-sections, illustrating the histomorphology of each experimental group. The slides provide clear definition of liver structure, highlighting its histological components including parenchyma, stroma, and sinusoids. The parenchyma, represented by hepatocytes (indicated by black arrows), is surrounded by connective tissue and vessels forming the stroma, while the sinusoids (indicated by yellow arrows) are capillaries traversing between hepatocytes, observed at high-power magnification (x100).

In the control group, a central vein is evident, characterized by medium-sized nuclei and well-separated hepatocytes along the sinusoids. Conversely, the untreated rats displayed a central vein with conspicuous large-sized nuclei and similarly well-separated hepatocytes. The treated groups exhibited central veins with smaller-sized nuclei, resembling the control, and hepatocytes

well-separated by sinusoids.

Overall, liver histology in both the control and treated groups demonstrated well-conserved hepatocyte cords, non-vacuolated cytoplasm, and clearly delineated sinusoids without infiltration by inflammatory cells or fatty degenerative changes, indicative of normal hepatic integrity.



Plate 1: Photomicrograph of the liver of rat administered 0.5 mL distilled water showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes. Mild atrophy of central vein is

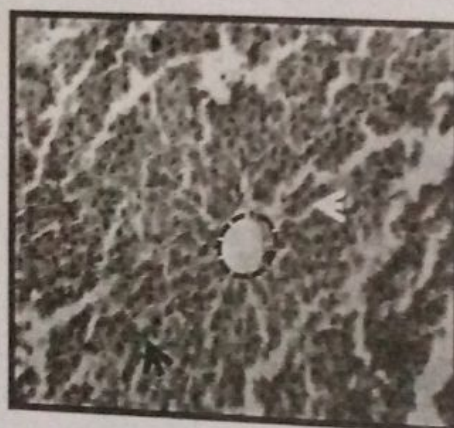


Plate 2: Photomicrograph of the liver of rat induced with 80 mg/kg Augmentin showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes. Mild atrophy of central vein and dilation of the sinusoidal space



Plate 3: Photomicrograph of the liver of rat induced with 80 mg/kg Augmentin but treated with 100 mg/kg Sylimar showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes. Mild atrophy of central vein is apparent.

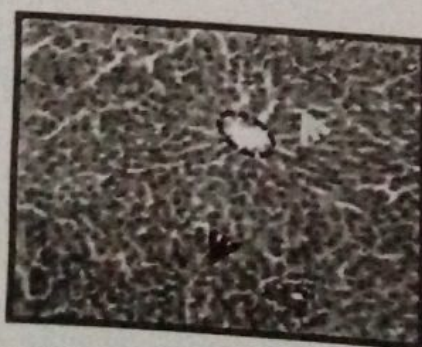


Plate 4: Photomicrograph of the liver of rat induced with 80 mg/kg Augmentin but treated with 2.0 mg/kg *Cucumissativus* homogenate showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes.

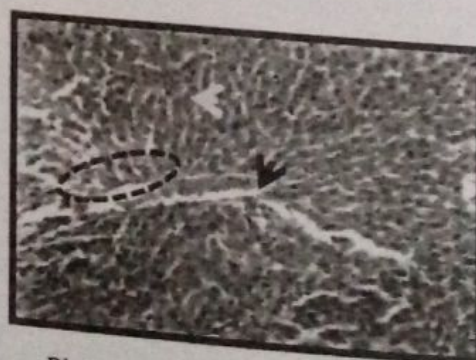


Plate 5: Photomicrograph of the liver of rat induced with 80 mg/kg Augmentin but treated with 4.0 mg/kg *Cucumissativus* homogenate showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes.

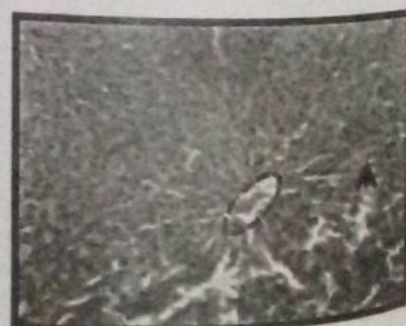


Plate 6: Photomicrograph of the liver of rat administered 4.0 mg/kg *Cucumissativus* homogenate showing typically-sized halo-spaced central vein surrounded by densely distributed hepatocytes.

Discussion

Damage to hepatocyte and bile duct cells triggers bile acid accumulation within the liver, exacerbating liver damage (Agatemor *et al.*, 2018; Li *et al.*, 2022). However, the consumption of fruits and vegetables in daily diets has been strongly linked to a reduced risk of cardiovascular and chronic diseases, including liver ailments (Wallace *et al.*, 2020). Raw materials sourced from plants' seeds, roots, stems, leaves, and fruits serve as the foundation for modern drug development (Nwokonkwo and Okeke, 2014).

This study delves into the potential impact of *Cucumissativus* homogenate on liver health in rats afflicted with Augmentin-induced liver damage. *Cucumissativus*, a vegetable rich in bioactive compounds such as cucurbitacins, vitexin, and orientin, holds promise as prophylactic and curative agents for managing various non-communicable diseases, including liver disease, diabetes mellitus, obesity, cancer, and cardiovascular ailments (Ezeodili *et al.*, 2017; Li *et al.*, 2022; Noor *et al.*, 2022; Varela *et al.*, 2022; Dai *et al.*, 2023; Rodriguez *et al.*, 2023). The presence of saponins, terpenoids, coumarins, glycosides, steroids, and alkaloids in *Cucumissativus* may contribute to liver protection, consistent with previous findings (Agatemor *et al.*, 2018; Uthpala *et al.*, 2020).

Of note, alkaloids, present in significant quantities in *Cucumissativus*

homogenate, have been associated with antibacterial, analgesic, and central nervous system stimulant properties (Abubakar *et al.*, 2015; Manske *et al.*, 2007; Ahmad *et al.*, 2013). Additionally, alkaloids exhibit antioxidant activity, attributed to their redox properties, which can act as reducing agents and singlet oxygen quenchers (Agatemor *et al.*, 2018b). Polyphenolics and phenols found in *Cucumissativus* possess antioxidant, anti-inflammatory, and anticancer activities, potentially mitigating conditions such as Augmentin-induced hepatotoxicity (Jing *et al.*, 2010; Anoop and Bindu, 2015; Karker *et al.*, 2016).

Moreover, the presence of saponins in cucumis may aid in controlling cholesterol levels and lessening the metabolic burden on the liver during metabolism, enhancing overall liver health (James *et al.*, 2010). The study's significant findings, especially regarding alkaloids and terpenoids in *Cucumissativus*, suggest the homogenate's potential as an effective antibacterial agent.

Liver cell destruction leads to the leakage of tissue content into the bloodstream, influencing serum protein, albumin, globulin, and bilirubin concentrations. Alterations in these biomarkers may indicate liver damage, impaired protein synthesis, or other underlying conditions (Udem *et al.*, 2010; Agarwal, 2021; Levitt and Levitt, 2016; Shimizu, 2008, 2016; Nwikwe and Balogun, 2021). Remarkably, after 12 days of oral administration of the homogenate, serum biomarker

concentrations aligned favorably with the control, suggesting preserved liver synthetic capability and functionality, akin to Silymarin, thereby reversing Augmentin-induced effects.

Aminotransferases, including AST, ALT, and ALP, are key indicators of liver damage, with elevated levels in the blood often signifying hepatocellular injury (Honda *et al.*, 2010; Rosenthal and Glew, 2011; Averbukh *et al.*, 2022). Similarly, ACP and GGT activities may indicate hepatobiliary diseases (Thapa and Walia, 2007; Agrawal *et al.*, 2016). The observed decrease in enzyme activities in treated groups compared to the untreated group suggests the homogenate's protective effect against liver damage induced by Augmentin.

LDH, an enzyme found in various tissues and organs, may indicate liver or muscular damage when elevated in blood (Chaudhary and Chauhan, 2015; Hussain *et al.*, 2023). Although LDH activities increased in treated groups, this may not solely indicate liver diseases but could result from muscular tissue damage.

Histological findings corroborate the biochemical assessments, demonstrating the homogenate's potential to mitigate liver damage induced by Augmentin. The observed mild lesions and preservation of liver architecture suggest the homogenate's favorable effect on liver structure and function.

Conclusion,

Cucumis sativus homogenate exhibits hepatoprotective properties against Augmentin-induced liver damage in rats, potentially attributed to its rich phytochemical composition. Further research is warranted to elucidate the mechanisms underlying.

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