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Research Article

Effect of *Cucurbita maxima* Leaf Extract on Acetaminophen-induced Toxicity in Liver Function and Histoarchitecture in Adult Wistar Rats

Nwanama Emmanuel Kenechulwu[1]*, Ibenyenwa John Chinaza[2] and Mbang Joshua Egbe[3]

[1]*Department of Anatomy, Faculty of Basic Medical Sciences, Edo State University, Edo State, Nigeria*

[2]*Department of Public Health, Peace Land College of Health Sciences and Technology, Enugu, Enugu State, Nigeria*

[3]*Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Cross Rivers State, Nigeria*

***Corresponding Author:** Nwanama Emmanuel Kenechulwu, Department of Anatomy, Faculty of Basic Medical Sciences, Edo State University, Edo State, Nigeria.

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Abstract

Context: *Cucurbita maxima* (Pumpkin) leaf is rich in carotenoid and flavonoid that are believed to perform protective effects. In this study, the effects of *Cucurbita maxima* extract are investigated in the treatment of Wistar rat liver injury induced by acetaminophen (paracetamol).

Materials and Methods: Twenty adult male Wistar rats weighing between 180 and 200 g were divided into five groups (n = 4). Normal control group; received 1ml of normal saline, negative control; paracetamol (500mg/kg) for fourteen days, experimental groups; paracetamol (500mg/kg/day) and *Cucurbita maxima* extract (25, 50 and 75mg/kg/day) 7 days respectively. At the end of the experiment, toxicity was measured histopathological by light microscopy and biochemically by checking levels of liver functions.

Results: The negative control group had increased level of alanine transaminase and aspartate aminotransferase and hepatocellular inflammation. When compared to the negative control, the *Cucurbita maxima* treatment decreased the serum levels of both aspartate aminotransferase and alanine transaminase. Additionally, hepatic tissues from our study showed that *Cucurbita maxima* treatment reduced liver damage caused by oxidative stress, which in turn activated autophagy.

Conclusions: Our research demonstrates that *Cucurbita maxima* has the potential to be a medicinal agent by protecting liver tissue from acetaminophen-induced toxicity.

Keywords: *Cucurbita maxima*; Hepatic-Disorder; Histoarchitecture; Paracetamol

Introduction

Acetaminophen (APAP) is currently one of the most frequently prescribed antipyretics, analgesic, and anti-inflammatory drugs in the globe [1]. When administered in excess, APAP can cause severe drug-induced liver damage, cell death, acute liver failure, and even high mortality in both animals and people [2]. At therapeutic doses, APAP is typically regarded as safe, with an adult daily maximum of 4g [3].

However, hepatotoxicity brought on by APAP overdose is a major contributor to acute liver failure globally [4]. In therapeutic doses, 90% of APAP undergoes glucuronidation metabolism and is excreted in the urine as sulphation [2]. The minor amounts that are left are changed into N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P450 enzymes (CYP2E1, CYP2A6), which quickly conjugates with cellular GSH and is eliminated through bile or urine

[5]. Any additional APAP is transformed into the extremely reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) when there is an APAP overdose because the anti-toxic phase II enzymes become saturated [6]. Since glutathione (GSH) is depleted as a result of excessive NAPQI accumulation, APAP protein adducts are formed when NAPQI binds covalently to intracellular proteins, which leads to mitochondrial dysfunction, reactive oxidative stress (ROS), and ultimately hepatocyte necrosis and apoptosis [7]. The processes underlying APAP-induced liver injury (ALI) are well established, however new medications to counteract the damaging effectiveness are still required.

Oxidative stress, mitochondrial dysfunction, inflammation, endoplasmic reticulum stress, and autophagy are all factors that are associated with liver injury [8-11]. According to Gaskell., *et al*. [12], the oxidative stress that results from mitochondrial damage causes mitochondrial permeability transition pore opening, mitochondrial membrane potential loss, and even necrotic cell death, which is indicated by the release of HMGB1 from the nuclei.

In recent years, since a significant portion of the global population today relies on organically derived medications for the treatment and management of ailments, the usage of plant products as a key component of complementary medicine has received more attention [13].

Pumpkin (*Cucurbita maxima*.) is a rich source of bioactive compounds and it belongs to the Cucurbitaceae family and is an annual plant [14]. According to Dotto and Chacha[15], the pumpkin is the vegetable with the widest variety of traits, including size, shape, and color.

Phytochemicals, unsaturated fatty acids, essential amino acids, vitamins, and minerals are all present in pumpkin seeds [14-16]. Carotenoids are thought to be present in pumpkin. So far, most studies on the chemical composition of pumpkin have focused on the content of these compounds [17]. Pumpkin's antimicrobial, antidiabetic, anti-hyperlipidemic, anti-carcinogenic, anti-hypertensive, anti-inflammatory, anti-depressant, antioxidant, and anthelmintic properties have been proven by results of animal and *in vitro* experimental research [18]. Since scavenging, anti-inflammatory, and free radical formation are strategically controlled, liver damage management. Thus, it is hypothesized that *Cucurbita maxima* has a hepatoprotective effect. Therefore, the aim of this study is to determine the liver functions and morphology of rats following acetaminophen-induced liver damage, as well as the hepatocurative effect of an ethanolic leaves extract of *C. maxima* against that damage.

Materials and Methods

Plants material and preparation

The fresh *Cucurbita maxima* (Pumpkin) leaves used in this research were collected from a farm in Emene, Enugu East Local Government, Enugu State, Nigeria and identified at the Department of Plant Sciences, Enugu state university of Science and Technology, Enugu State, Nigeria. The leaves were air dried under shade and blended to a coarse powder form; about 700g was macerated with water. Then, Whatman No. 1 filter paper was used to filter the solutions. The extract was evaporated to dryness using Rotary Evaporator [19].

Ethical approval

As described in the Guidelines for the care and use of laboratory animals, all animals were handled in accordance with the faculty's ethical standards for animal research.

Animal management and grouping

For this experiment, twenty adult male Wistar rats weighing an average of 150g were used. Throughout the experiment, they were kept in netted iron cages with a temperature of 25°C, a humidity of 60–70%, and 12-hour cycles of light and darkness. They were randomly selected and divided into (5) groups, groups 1-5, with four (4) rats in each group. And then kept for fourteen days for laboratory adaptation, while having free access to feeds (growers mesh) and water. Body weight of animals were obtained using Sansa electronic weighing scale before and after laboratory adaptation, and also at weekly intervals throughout the period of the experiment.

Hepatotoxicity induction

Hepatotoxicity was induced orally in groups 2-5, using 500 mg/kg of paracetamol daily for 7 days.

Administration of extract

For 14 days, Group 1 received 1ml of oral normal saline every day. In group 2, toxicity was induced using 500 mg/kg of oral paracetamol every day for 14 days. Toxicity induction was performed on Groups 3–5 using oral paracetamol 500 mg/kg/day for

7 days. Afterwards, they were given *Cucurbita maxima* gavage leaf extract at doses of 25 mg/kg, 50 mg/kg, and 75 mg/kg for a period of 7 days.

Chart 1: Administration of Extract.

Group	Day 1 – 7	Day 8 – 14
1 (Normal control)	Normal saline 1ml daily	Normal saline 1ml daily
2 (Negative control)	Paracetamol 500mg/kg daily	Paracetamol 500mg/kg daily
3 (Low dose extract)	Paracetamol 500mg/kg daily	Extract 25mg/kg daily
4 (Medium dose extract)	Paracetamol 500mg/kg daily	Extract 50mg/kg daily
5 (High dose extract)	Paracetamol 500mg/kg daily	Extract 75mg/kg daily

Sample collection

On day 15, the experimental animals were anaesthetized by chloroform inhalation. And blood samples were collected via ophthalmic vessels and immediately transferred into a plain bottle. And subsequently, the animals were sacrificed, and the liver excised and immediately transferred for histological analysis.

Sample analysis

Hematoxylin and Eosin stains were used to create the histological slides of the liver specimens following to standard histological procedures. Light microscopy analysis was performed on the slides. Spectrophotometric analysis was used to quantify the levels of blood alanine aminotransferases (ALT) and aspartate amino transferases (AST) in the test specimens.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 23 was used to conduct this study, and a P-value of 0.05 or lower was considered significant.

Results

Effect of ethanol leaf extract of *Cucurbita maxima* on Liver Histoarchitecture in paracetamol-induced toxicity

Figure 1 displays photomicrographs of the histological liver structures. While the histology of the control group (a) demonstrates no change, that of the APAP group (b–d) reveals visible lesions (black arrows), while that of group 5 (e) did not demonstrate any visible lesions in comparison to the control.

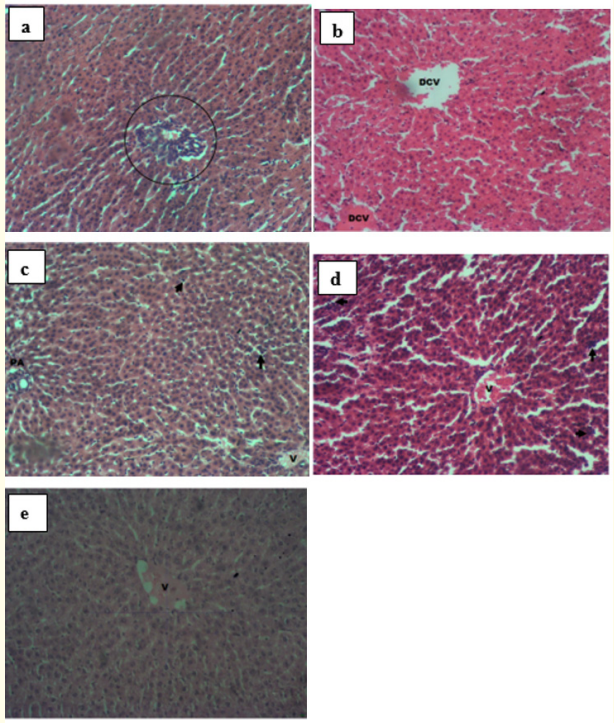

Figure 1: Photomicrographs of acetaminophen-induced toxicity in rat liver and treated with aqueous extract of Cucurbita maxima. (a) Control, (b) APAP only (500 mg/kg), (c) APAP + C. maxima (25 mg/kg), (d) APAP +C. maxima (50 mg/kg), (e) APAP + C. maxima (75 mg/kg). APAP: acetaminophen; H & E 100x V: central vein.

Evaluation of Effect of ethanolic leaf extract of *Cucurbita maxima* on Liver Function enzymes

Acetaminophen (500mg/kg) administration to rats significantly increased the levels of the AST and ALT enzymes when compared to the control (p 0:05). These enzyme levels were restored in the *C. maxima* leaf extract-treated groups to levels that were comparable to the control. Comparing AST and ALT to the corresponding control values revealed statistically significant differences. It was discovered that the reductions were dose-dependent (Table 1).

Group	AST	ALT
1 (Normal control)	29.50 ± 2.1	28.00 ± 1.4
2 (Negative control)	35.50 ± 3.5	44.50 ± 4.9
3 (Low dose extract)	28.00 ± 2.8	27.00 ± 1.4[c]
4 (Medium dose extract)	25.50 ± 2.1	26.50 ± 2.1
5 (High dose extract)	23.50 ± 3.5[e]	24.50 ± 2.1
P- Value	0.004	0.002

Table 1: Effect of *Cucurbita maxima* ethanolic leaf extracts on liver function enzymes in the liver of rats treated with APAP.
AST - Aspartate aminotransferase. ALT - Alanine aminotransferase.
Values expressed as mean ± SD, n = 5. [c]p =.05 vs group 5, [e]p =.05 vs control.

Discussion

Paracetamol is an antipyretic and analgesic agent. The liver can become toxic when paracetamol is used in excessive doses. Usually, this toxicity is accompanied by inflammation and oxidative stress [20,21].

In line with Hussain., *et al.* [22] research, acetaminophen induction considerably raises the level of serum enzymes like AST and ALT. While treatment with *Cucurbita maxima* significantly reduced both these biochemical markers and the cellular alterations in the liver. According to research study reports, APAP causes liver damage by increasing free radical generation and decreasing the liver's antioxidant status [2]. While the use of *Cucurbita maxima* as a treatment enhances the liver's antioxidant enzyme status and protects against liver damage via its scavenging properties.

The most common method for determining the severity of liver damage is by analyzing the transaminase enzyme levels in the serum. Serum ALT and AST values are specifically employed as a biomarker of hepatic necrosis [23]. The ALT and AST enzymes carry out the reductive transfer of amino acids from alanine or aspartate, respectively, to alpha ketoglutarate to create pyruvate or oxaloacetate. Damaged hepatocytes discharge their contents, including ALT and AST, into the extracellular space [23]. As a result, the increased level of these enzymes in the acetaminophen-treated negative control group is regarded as a sign of liver injury [11]. ALT is present in the heart, brain, skeletal muscle, and liver, with the liver having the highest concentration of all the organs [23]. Due to its presence in other organs, AST is considered to have a lower specificity for liver injury.

The reactive species (NAPQI) generated after paracetamol overdose affects the hepatic cells by lipid peroxidation, which also compromises cellular permeability and increases serum ALT and AST levels [8,23]. When compared to the healthy control group in this study, the APAP-treated group's ALT and AST levels significantly increased (Table 1). An agent's protective effects are indicated by a considerable decrease in serum ALT and AST. Treatment with *Cucurbita maxima* considerably lowers the biomarkers in a dose-dependent way. This outcome was directly related to *Cucurbita maxima*'s ability to prevent cells from the necrotic damage caused by APAP by lowering the release of the transaminase enzyme and stabilizing the cell membrane [24].

Histopathological findings show that the liver has hepatocellular inflammation and distended central veins (Figure 1b). Interestingly, administration of *Cucurbita maxima* demonstrated the potential to attenuate liver injury in a dose-dependent manner (figure 1d and e). Moreover, it also corrected the histopathological alterations as seen in the negative group (figure 1b) when compared with the *C. maxima* treated groups. The hepato-regeneration progress in the *C. maxima* 50 mg/kg group (figure 1d) was remarkably similar to that in the 75 mg/kg-treated group (figure 1e), which demonstrated an increase in the number of viable cells and a decrease in the serum levels of hepatic enzymes. The therapeutic properties of *C. maxima* against paracetamol-induced hepatotoxicity have been demonstrated to have a potential mechanism [9,11].

The post-treated *C maxima* group attenuated the alterations induced on by the damage that APAP produces, including centrilobular destruction and invading T cells. According to the findings, APAP intake led to liver cells damage, necrosis, when compared to the control section (Figure 1) [22]. However, the substantial antioxidant properties of *C maximum* in the work help to restore anatomical change. Previous research has demonstrated that natural derivatives with strong antioxidant activities can prevent liver cell damage [25,26]. According to previous studies, natural derivatives, which have potent antioxidant effects, have been shown to protect against cellular damage in the liver [25,26].

Conclusion

The studies show that *C. maxima* has a therapeutic effect by reducing inflammatory response, apoptosis, and serum indicators. We therefore concluded that *C. maxima*'s antioxidant, anti-inflam-

matory, and anti-apoptotic properties may be the cause of all of its beneficial benefits. These findings also suggest that C maxima may be an alternate remedy for liver damage. *Cucurbita maxima* should be ingested, in low to high quantity, by people constantly exposed to acetaminophen. It should also be considered as a diet supplement.

Conflict of interest

None.

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Consent for Publication

Not applicable.

Ethical Approval

The departmental research ethics committee granted ethical approval.

Availability of Data and Material

This article has all the data that were created or evaluated during this investigation.

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