Fresh Garlic Extract Protects The Liver Against Acetaminophen-Induced Toxicity

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Citation

Abstract
Acetaminophen toxicity is a major course of acute liver failure. Many plants have been reported to show hepatoprotective activities. This study was to determine the protective potential of fresh garlic extract on acetaminophen toxicity and to demonstrate its dose dependence. Sixty Swiss mice were divided into six groups of ten. Group I served as negative control and were treated with physiological saline. Group II served as the positive control and received 250 mg/kg body weight of acetaminophen only. Groups III, IV, and V were pretreated with daily administration of 250 mg/kg, 500 mg/kg, and 750 mg/kg of garlic respectively, for five days followed by 250 mg/kg of acetaminophen. Group VI were pretreated with 25 mg/kg silymarin prior to 250 mg/kg acetaminophen. Blood samples were collected after six hours of acetaminophen and used for biochemical studies, while liver was excised from each mouse and used to prepare hematoxylin / eosin sections. The alterations in AST, ALT, alkaline phosphatase, and serum albumin were significantly prevented by prior administration of garlic extract. Also the histological changes induced by acetaminophen overdose were prevented by garlic. We conclude that fresh garlic extract protected the liver against toxic doses of acetaminophen and suggest that its use could protect against hepatitis.

INTRODUCTION
Hepatitis is a major public health problem in the world today and acetaminophen overdose contributes significantly to cases of drug induced hepatitis. Acetaminophen is quite safe and well tolerated in therapeutic doses. However, at toxic doses, acetaminophen produces acute liver failure characterized by centrilobular necrosis in both man and experimental animals. This has been attributed to the metabolic activation of acetaminophen to a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) in the liver by cytochrome p450 isoenzymes especially CYP2E1. NAPQI reportedly depletes liver glutathione thereby inducing oxidative stress. It also binds to vital cellular and mitochondrial proteins leading to cellular necrosis, and it could activate cells of the immune system leading to the release of pro-inflammatory cytokines.

Many natural products have been reported to possess antioxidant and hepatoprotective effects in animal models of hepatotoxicity and quite a good number are employed in folk medicine for the treatment of liver diseases. Currently, many plant extracts have been studied, and some have received approval or are undergoing clinical trial for use in liver related conditions.

Garlic is widely consumed in many cultures as spice and as condiment in many dishes. Several cultures use garlic for medicinal purposes. A plethora of publications are also available on the pharmacological properties of garlic and their beneficial health effects. Although many studies have focused on the antioxidant and anti-lipid peroxidative properties of garlic extracts, many of these studies employed aged garlic extract (AGE) that is known to have a different phytochemical composition from fresh garlic, and just a few reports investigated the actions of garlic oil. To our knowledge, no study has investigated the effect of fresh extract of local Ugandan cultivars of garlic on acetaminophen hepatotoxicity. In other to provide crucial information that is needed for the possible development of a prophylactic strategy for drug induced hepatitis, this study was designed to determine the usefulness of ethanolic extract of fresh garlic bulbs in preventing acetaminophen induced hepatotoxicity in mice. Specifically, we investigated its usefulness in preventing acetaminophen induced changes in serum biochemistry and liver histology, and dose dependence of these effects.

MATERIALS AND METHODS
A. PLANT COLLECTION, IDENTIFICATION, AND
EXTRACTION

Local cultivars of garlic (Allium sativum L.) were purchased from a local market in Ishaka Town in Western Uganda, and identified by a qualified taxonomist, Professor Samson Bitawha of the Rukararwe Partnership Workshop for Rural Development, Bushenyi. A voucher specimen number KIU/PHM/0026 was deposited at the Herbarium of the Pharmacy Department of the Kampala International University, Western Campus.

Cold extraction of the garlic was carried out at room temperature (18-22 °C) as follows:

Fresh garlic bulbs were ground to a fine paste using a mechanical grinder.

50 g of the paste was put in a 250 ml conical flask and covered with about 100 ml of 80 % ethanol, stoppered with cotton wool, and allowed to stand in the dark at room temperature for 48 hours.

The ethanolic extract was filtered off into pre-weighed evaporating dishes, while the residue in the flask was washed with a further 100 ml of 80 % ethanol and added to the extracts in the evaporating dishes.

The filtrates were then evaporated to a syrupy residue using a rotary extractor at 40 °C.

The dishes were then weighed again on a triple beam balance and the percentage yield was calculated as follows:

Weight of extract = weight of evaporating dish after evaporation – weight of dish before addition of extract;

Percentage yield = total weight of extract ÷ weight of paste used (50 g) × 100.

The extracts were pooled together into an air-tight container and stored refrigerated (−4 °C) until required for use.

For use, a portion of the extract was weighed and dissolved in normal saline solution. Fresh preparations were made on each day of the experiment. The resulting solutions were injected intraperitonially into the mice.

B. LABORATORY ANIMALS

Swiss mice 6-8 weeks old weighing 18-32 g were purchased from the Pharmacology Department of the Mbarara University of Science and Technology in Uganda. They were maintained and habituated in plastic cages in the animal house of the School of Health Sciences, Kampala International University, Western Campus for one week before use. The mice had free access to water and were fed standard rodent pellets (purchased from a local commercial supplier) ad libitum. Habituation conditions were 12 hr dark/light cycles, and average environmental temperature of 20 °C.

C. ACUTE TOXICITY TEST AND DETERMINATION OF LD

The LD₉₀ of each of the extracts was determined in the mice by the procedure described by Bernas et al. (2004), and the confidence interval of the LD₉₀ was estimated by the Litchfield – Wilcoxon method (1949).

D. EXPERIMENTAL DESIGN

Sixty Swiss mice were grouped randomly into 6 groups of 10 each and administered with the drugs/extract as follows:

Group I (10 mice): given physiological saline i.p. only.

Group II (10 mice): given acetaminophen 250 mg/kg i.p. single dose only.

Group III (10 mice): given garlic extract 250 mg/kg for 5 days before a single i.p dose of acetaminophen 250 mg/kg.

Group IV (10 mice): given garlic extract 500 mg/kg for 5 days before a single i.p dose of acetaminophen 250 mg/kg.

Group V (10 mice): given Garlic extract 750 mg/kg for 5 days before a single i.p dose of acetaminophen 250 mg/kg.

Group VI (10 mice): given Sylimarin 25 mg/kg for 5 days before a single i.p dose of acetaminophen 250 mg/kg.

The extract was administered as a single once daily dose, while acetaminophen was administered after 12 hours fast after the last dose of garlic extract.

The study received approval from the ethics committee of the Kampala International University, Western Campus, Bushenyi Uganda, as well as the Postgraduate Committee of the College of Medicine, Abia State University, Uturu, Nigeria.

E. COLLECTION OF SAMPLES

a) Blood Samples: Blood samples were collected by cardiac puncture into sterile plastic tubes under ether anaesthesia exactly six hours after acetaminophen administration, and used for biochemical studies. The blood samples were allowed to clot and retract at room temperature, and the sera were separated into plastic vials and stored in the freezer.
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(-20 ° C) until required for use. For the biochemical assays, the sera were allowed to thaw at room temperature and mixed by repeated inversion of the vials.

b) Liver Samples: After sacrifice, livers were obtained from the mice and after physical examination they were fixed in 10 % formal saline (1 part of tissue to 10 parts of fixative). Tissue samples were processed by wax impregnation and embedding, and 5 uM sections cut using a rotary microtome. Staining was by Haematoxylin & Eosin method.

F. BIOCHEMICAL ASSAYS

Assays for serum aspartate transaminase (AST) and alanine transaminase (ALT) was carried out with Randox kits (Randox Laboratories UK) and based on the method of Reitman and Frankel. Serum Alkaline Phosphatase was determined by the method of Armstrong and King. Serum albumin was determined by reaction with BCG using Randox kits. Manufacturer’s procedures in the kits were diligently followed.

G. STATISTICAL ANALYSIS

Results were expressed as mean ± SE. Comparison of means was by the student’s t-test and the Mann-Whitney U test. A p value < 0.05 is considered significant.

RESULTS

Extraction of the fresh garlic bulbs yielded 22 % extract. The LD50 of the extract was determined to be 1524 +/- 83 mg/Kg body weight. Administration of acetaminophen to the untreated mice (group 2) resulted in a marked escalation of serum transaminases and alkaline phosphatase levels and a concomitant decrease in serum albumin values. However, treatment of the mice with the fresh garlic extracts for five days produced significant dose dependent prevention of these biochemical changes that is similar to that produced by silymarin, as shown in table 1 and figure 1.

Histological examination also showed characteristic pathological changes in the group 2 mice that received only acetaminophen. These include centrilobular necrosis, steatosis, leukocyte infiltration, portal triaditis, and evidence of edema as shown in figure 2 slides. Pretreatment of the mice in groups 3 – 5 demonstrated dose dependent prevention of these changes. Mice in groups 3 and 4 showed mild triaditis and mild venous congestion, whereas those in group 5 and 6 showed no remarkable changes as shown in figures 4-6.

Figure 1: Biochemical values from the mice in the six groups

Table 1: Biochemical values from the mice in the six groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALK PHOS (U/L)</th>
<th>S. ALB (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CONTR.</td>
<td>5 mg/Kg</td>
<td>25 mg/Kg</td>
<td>250 mg/Kg</td>
<td>500 mg/Kg</td>
</tr>
<tr>
<td>II</td>
<td>CONTR.</td>
<td>5 mg/Kg</td>
<td>25 mg/Kg</td>
<td>250 mg/Kg</td>
<td>500 mg/Kg</td>
</tr>
<tr>
<td>III</td>
<td>12.1±2.3</td>
<td>12.4±2.8</td>
<td>28.2±3.4</td>
<td>12.4±2.8</td>
<td>28.2±3.4</td>
</tr>
<tr>
<td>IV</td>
<td>30.3±2.4</td>
<td>30.4±2.8</td>
<td>44.2±3.6</td>
<td>30.3±2.4</td>
<td>44.2±3.6</td>
</tr>
<tr>
<td>V</td>
<td>125 mg/Kg</td>
<td>65.4±2.2</td>
<td>22.5±4.6</td>
<td>22.5±4.6</td>
<td>22.5±4.6</td>
</tr>
<tr>
<td>VI</td>
<td>250 mg/Kg</td>
<td>56.1±2.9</td>
<td>26.6±2.5</td>
<td>26.6±2.5</td>
<td>26.6±2.5</td>
</tr>
</tbody>
</table>

Figure 2: Serum AST, ALT, Alkaline Phosphatase, and albumin from the mice in the six study groups
DISCUSSION

This study has shown that pretreatment of mice with fresh ethanolic extract of garlic prevented the escalation of serum liver enzymes such as AST, ALT, and alkaline phosphatase, and the decrease in serum albumin that are usually associated with acetaminophen hepatotoxicity. Similarly, the characteristic histological changes associated with acetaminophen toxicity such as centrizonal necrosis, steatosis, and sinusoidal enlargement, were significantly prevented by the garlic extract in a dose-dependent manner. This adds to several reports on the pharmacological usefulness of several plant extracts as liver protective agents.

There is currently a dart of articles on the
hepatoprotective action of garlic, with those available reporting on aged garlic extract and garlic oil, but here we report that fresh ethanolic extract of garlic produced dose-dependent hepatoprotection in acetaminophen toxicity. The medical implication of this finding could be that consumption of raw garlic might be a useful prophylactic strategy against toxic hepatitis.

Although the mechanism by which garlic exerts its hepatoprotective effect was not investigated in this study, several reported pharmacological properties of garlic could contribute to this. It is now a well recognized finding that garlic possesses antioxidant and anti-lipid peroxidative effects. Oxidative stress and lipid peroxidation are key processes in the toxicity of acetaminophen. Therefore prevention of these processes could be central event through which garlic exerts its salutary effects in acetaminophen toxicity. Garlic contains several organosulfur compounds such as allicin, diallyl sulfide, and diallyl disulfide which are valuable precursors for glutathione biosynthesis. It is possible that garlic prevents glutathione depletion induced by acetaminophen overdose by upregulating its biosynthesis in the liver. Garlic contains high levels of selenium and other constituents that could actively scavenge free radicals. Selenium also upregulates glutathione peroxidase activity in the liver thereby contributing also to the antioxidant defense of the organ.

The constituents of garlic are reported to inhibit phase I metabolism through specific inhibition of cytochrome p450 enzymes, while they enhance the activities of phase II enzymes that are involved in the conjugation and excretion of drugs and their phase I metabolites. Limiting the production of NAPQI production by the above mechanisms would minimize the toxic effects of acetaminophen overdose.

NAPQI produces cellular necrosis through the formation of lethal protein adducts with important structural and functional proteins. Prevention of adduct formation could also be one way through which garlic exerts its effect. More so, garlic is known to inhibit the secretion of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin-1, and interleukin -6; these cytokines play significant roles in the toxicity of acetaminophen. Finally, many steroidal saponins and sapogenins are present in garlic, and could play vital roles as anti-inflammatory agents, in the induction of protein synthesis, and in tissue regeneration and repair.

CONCLUSION

This study has shown that fresh extract from garlic exerts significant protection of the liver against acetaminophen toxicity. Garlic contains many secondary metabolites, trace elements, amino acids, and proteins which could act in a variety of ways to protect the hepatocytes from toxic assault. Consumption of fresh garlic could therefore be a reasonable antidote to the development of hepatitis.

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