Hepatotoxicity due to acetaminophen overdose in mice: assessing the protective potentials of ginsomin® supplementation.

Adejoke ONAOLAPO¹, O.J ONAOLAPO²*, Stella BAMIGBOLA³, O.O ADEGOKE⁴

1. Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
2. Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University Technology, Osogbo, Osun State, Nigeria.
3. Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University Technology, Osogbo, Osun State, Nigeria.

Corresponding author email: olakunleonaoalapo@yahoo.co.uk

Introduction

Ginsin is an over-the-counter nutritional supplement which possesses the natural energy-boosting properties of Panax ginseng and essential traditional vitamins and minerals. Panax ginseng has been used as a multipurpose medicine for centuries, especially in Asian countries (Yun, 2001; Park et al., 2005). Its root extract contains ginseng saponins referred to as ginsenosides, which play a major role in most of the physiological and pharmacological activities of ginseng (Yue et al., 2007; Park et al., 2012). Korean panax ginseng has been used as a general herbal tonic and is also known to have adaptogenic properties (Kim and Park, 2010). Ithas been reported to be useful in the treatment of various human diseases (e.g., rheumatoid arthritis, sexual dysfunction, ischemic injury and diabetes mellitus) (Kim and Kim, 2008; Shin et al., 2011), it promotes longevity, and assists the body in stress management (Lee et al., 2008) and homeostatic balance (Choi, 2008). It also has antioxidant properties due to its ability to scavenge free-radicals and to neutralize free ions induced by lipid peroxidation (Karadeniz et al., 2009). Other than the energising properties of ginseng, Ginsomin®, containing Ginseng® (containing Ginseng) was evaluated for possible hepatoprotective effect against acetaminophen-induced liver injury in this study. We hypothesised that Ginsomin pre-treatment may protect against biochemical and tissue changes associated with acetaminophen hepatotoxicity in mice. Forty adult mice (weighing 20-22 g) were assigned to five groups (n=8). Control group received vehicle (normal saline), one group received oral acetaminophen (800 mg/kg daily) for 3 days, while three groups were pre-treated with Ginsomin at 0.5, 1.0 and 1.5 mg/kg body weight respectively for 14 days before administration of acetaminophen (800 mg/kg daily) for 3 days. On day 18, animals were sacrificed, blood samples taken via an intracardiac puncture for estimation of biochemical parameters and sections of the right lobe of the liver processed for general histological study. Body weight decreased significantly in all groups of mice pre-treated with ginsomin compared to vehicle and increased compared to acetaminophen-only group.

Acetaminopen toxicity was evident by significant increase in serum aspartate aminotransferase, alanine aminotransferase and total protein, a decrease in plasma albumin and histologic evidence of liver injury. Pre-treatment with ginsomin resulted in a significant decrease in AST and ALT levels, total protein and varying degrees of protection from hepatic injury.

© 2016 PSCI Publisher All rights reserved.

Key words: Acetaminopen, Hepatotoxicity, Panax ginseng, Morphology, Mice
Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is a para-aminophenol derivative commonly used as analgesic and antipyretic (Garry and Kieran, 2005). It is generally considered safe when the recommended dose is taken but may cause severe hepatic damage when an overdose is administered both in humans and experimental animals (Sener et al., 2005) due to excessive production and/or decreased glutathione conjugation of N-acetyl-parabenzoquinone imines (NAPQI) which damages cell membranes (Kwan et al., 1995). More recent concerns are suspicions that repeated ingestion, even at clinically-prescribed doses may be associated with hepatic injury (Jalan et al., 2006). Acetaminophen toxicity has been attributed either to an acute overdose, repeated excessive dosing or use of a variety of medications containing acetaminophen (Gum and Cho, 2013b). Toxic effects are largely not from the drug itself but due to the formation of highly electrophilic and reactive intermediate metabolite, N-acetyl-para-benzoquinone imine (NAPQI), which at therapeutic doses preferentially conjugates with hepatic glutathione sulfide (GSH) (Gum and Cho, 2013b). However in overdose, large amounts of acetaminophen are metabolised by oxidation because of saturation of the glucuronide and sulphate conjugation pathway (Benjamin et al. 2002; Pajoumand et al., 2003), and the protective intracellular glutathione (GSH) stores are therefore depleted faster than they can be regenerated due to excessive production of NAPQI. NAPQI formed covalently binds to essential nucleophilic macromolecules such as cellular proteins and membrane macromolecules in the hepatocyte therefore initiating lipid peroxidation leading to cell death or injury and release of intracellular contents including a significant release of liver enzymes especially the transaminases in plasma (Yen et al., 2008). This may lead to hepatic necrosis, renal tubular necrosis, hypoglycaemic coma and death as a result of its toxicity (Gum and Cho, 2013a).

In many parts of the world, acetaminophen is cheap and easily gotten without a need for prescription. There exists a subset of the population (usually blue collar employees) who ingest acetaminophen daily, (to help in coping daily with physically-demanding work) many times without adhering to clinically prescribed doses. This group of people are at risk of acetaminophen toxicity.

It is common practice in many developing countries for people to rely on herbal or other natural products in the management of illnesses, including toxicities. A number of studies monitoring beneficial effects of natural or herbal products against paracetamol-induced toxicities have been carried out, with results that point towards hepatoprotection (Lee et al., 2001; Chattopadhyay, 2003; Onaolapo and Onaolapo, 2012; Fakurazi et al., 2012; Osamh, 2013). One of the main limitations to use of herbs (especially in third world countries where they are popular) is the lack of standardized formulations as well as the absence of detailed dosing regimens. The result is indiscriminate use and a tendency to develop hepatic and/or renal injury from herbal product overdose. The question here is, can there be a standardized herbal product that is useful in prevention of acetaminophen hepatotoxicity?

Herb-containing supplements like Ginsomin which until now has been marketed and sold mainly for its immune and mental-health boosting properties is believed to increase the body’s resistance to stress and fatigue. Ginsomin supplementation may offer protection against liver injury considering that the potential beneficial effects of Korean panax ginseng (which is the main constituent of ginsomin) in the management of several medical conditions is the subject of several studies (Kitt et al., 2000; Zhang et al., 2008; Lu et al., 2009; Ramesh et al., 2012; Osamh, 2013; Mohammed et al., 2015). Since Ginsomin is standardized and its dosing formula well-documented, incidences of overdose or indiscriminate use is much less. Also it is fortified with a lot of essential vitamins and minerals all of which may increase its antioxidant and immune modulatory property.

**Materials and Methods**

Ginsomin capsules (Mega LifeSciencePty.Ltd., Australia), Acetaminophen (paracetamol) tablets (Juhel Pharmaceuticals, Lagos, Nigeria). All the diagnostic kits for assaying hepatic function tests were obtained from Randot Laboratories. All other chemicals were of analytical grade. Ginsomin capsule containing 50 mg of ginseng extract and acetaminophen tablets (500 mg) were dissolved separately in measured volumes of normal saline and administered orally via a cannula.

**Animal care and management**

Forty (40) adult Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing between 20–22 g were used. Mice were housed in plastic cages measuring 16 x 12 x 10 inches (8 mice in each cage). Housing was a temperature-controlled (22.5°C ±2.5°C) quarters with 12 hours of light at the animal house of the College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria. Mice had free access to food and water. The experimental protocol was approved by the Ladoke Akintola University of Technology Animal Ethics Committee. All rules applying to animal safety and care were observed.

**Experimental method**

Animals were assigned randomly to five (5) groups of eight (8) mice each. They received vehicle (normal saline) or pre-treatment with one of three doses of ginsomin (0.5, 1.0 and 1.5 mg/kg body weight respectively) for 14 days before administration of acetaminophen (800 mg/kg) daily for 3 days. There was a negative control group that received acetaminophen without drug pre-treatment. Animals were weighed weekly using a Mettler weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland). At the end of the experimental period, animals were anaesthetised with diethyl-ether, sacrificed by cervical dislocation and samples taken via an intracardiac puncture for estimation of alanine and
aspartate transaminase (ALT, AST), total protein and plasma albumin levels. Sections of the right lobe of the liver were cut, processed for paraffin-embedding and stained with hematoxylin and eosin for general histological study.

**Biochemical analysis**

Blood samples were collected into lithium-heparin bottles. Plasma samples were then separated and stored at -20°C for biochemical analysis. For the assessment of the liver functions of the mice, plasma total protein was measured using direct Biuret method (Kingsley and Frankel, 1939) plasma albumin was estimated by using Bromocresol green method (Dormas et al., 1971) and also the activities of liver enzymes Aspartate and Alanine transaminase (AST, ALT) in plasma were determined using colorimetric method by measuring concentration of oxaloacetate hydrazone and the pyruvate hydrazones formed with 2,4-dinitrophenyl-hydrazine, respectively. The colour was measured at 546 nm according to Reitman and Frankel (1957).

**Liver Histology**

Liver was dissected out, washed in normal saline and sections of the right lobe sectioned and fixed in 10% formol-saline and processed for paraffin-embedding. Sections were taken at 5 microns, dehydrated in alcohol, cleared in xylene and stained with haematoxylin and eosin for general histological study. An Olympus BX50 digital light microscope was used to examine the slides and acquire photomicrographs.

**Statistical analysis**

All data were first tested for normality and variance homogeneity, prior to statistical analysis. Having been found to be normally distributed, and variances homogeneous, data was then analysed by one way analysis of variance (ANOVA) test to determine effects of the treatments using Chris Rorden’s ezANOVA statistical package, (version 0.98). Post-hoc tests with Tukey’s multiple comparison test (Tukey HSD) were used to determine the source of a significant effect. Results were expressed as Mean ± S.E.M. and p < 0.05 taken as accepted level of significant difference from control.

**Results**

**Effect of oral ginsomin on body weight**

Figure 1 represents the effect of ginsomin on body weight over a 14 day period. Body weight was measured as percentage change in weight defined as the difference between the final and initial body weights divided by initial weight multiplied by 100. There was a significant (F=161, p<0.001) decrease in weight gain at all doses of ginsomin compared to vehicle, and an increase in percentage weight gain compared to acetaminophen control.

![Figure 1. Effect of ginsomin on % weight change. Each bar represents Mean ±S.E.M, *p<0.05 vs.VEH, †p<0.05 vs.ACP, n=8; VEH. Vehicle, ACP: Acetaminophen](image)
Effect of ginsomin on aspartate transaminase

Figure 2 shows the effects of ginsomin on aspartate transaminase levels. There was a significant (F= 61.5, p<0.001) increase in serum aspartate transaminase levels at all doses of ginsomin compared to vehicle and a decrease compared to acetaminophen control.

![Figure 2](image1.png)

Figure 2. Effect of ginsomin on aspartate transaminase levels. Each bar represents Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. ACP, n=8; VEH. Vehicle, ACP: Acetaminophen.

Effect of ginsomin on alanine transaminase

Figure 3 shows the effects of ginsomin on alanine transaminase levels. There was a significant (F= 34.1, p<0.001) increase in serum alanine transaminase levels at 0.5 mg/kg of ginsomin compared to vehicle and a decrease at all doses of ginsomin compared to acetaminophen control. At 1.0 mg/kg of ginsomin, the increase seen compared to vehicle was only visual while at 1.5 mg/kg there was a visual decrease.

![Figure 3](image2.png)

Figure 3. Effect of ginsomin on alanine transaminase levels. Each bar represents Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. ACP, n=8; VEH. Vehicle, ACP: Acetaminophen.
Effect of ginsomin on total protein

Figure 4 represents the effects of ginsomin on total protein. There was a significant (F= 127, p<0.001) increase in total protein at all doses of ginsomin compared to vehicle, and a decrease at 0.5 and 1.0 mg/kg of ginsomin compared to acetaminophen control, at 1.5 mg/kg of ginsomin, the difference seen compared to acetaminophen control was only visual.

![Bar chart showing effect of ginsomin on total protein](image)

**Ginsomin (mg/kg, p.o.)**

<table>
<thead>
<tr>
<th></th>
<th>VEH</th>
<th>ACP</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein g/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 4. Effect of ginsomin on total protein. Each bar represents Mean ±S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. ACP, n=8; VEH. Vehicle, ACP: Acetaminophen.*

Effect of ginsomin on serum albumin

Figure 5 represents the effects of ginsomin on serum albumin. There was a significant (F=24.6, p<0.001) increase in serum albumin at all doses of ginsomin compared to vehicle and no significant difference compared to acetaminophen control.

![Bar chart showing effect of ginsomin on serum albumin](image)

**Ginsomin (mg/kg, p.o.)**

<table>
<thead>
<tr>
<th></th>
<th>VEH</th>
<th>ACP</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin g/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 5. Effect of ginsomin on serum albumin. Each bar represents Mean ±S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. ACP, n=8; VEH. Vehicle, ACP: Acetaminophen.*

Effect of ginsomin on liver morphology

Examination of haematoxylin and eosin stained liver sections of animals that were administered vehicle (figure 6a)
revealed sheets of radially-arranged hepatocytes around a terminal hepatic venule. There were small intervening sinusoidal spaces between cords of hepatocytes and hepatocytes had well-staining nuclei; these features are in keeping with normal histology. Liver slides from mice in the acetaminophen group (figure 6b) showed poorly-staining hepatocytes with loss of normal architecture, numerous vacuoles, dilatation of the central vein and numerous pale-staining irregularly-shaped hepatic nuclei; these features are in keeping with hepatic injury. Examination of the slides from animals in group that received ginsomin at 0.5 mg/kg (figure 6c), 1.0 mg/kg (figure 6d) and 1.5 mg/kg (figure 6e) showed varying degrees of protection from liver injury as evidenced by mild loss of normal liver architecture with some radially-arranged cords of hepatocytes with normal nuclei, a few hepatocytes with either pale or deeply staining nuclei. There was also dose-related reduction in central vein dilatation; these features are in keeping with probable protection from acetaminophen overdose.

![Figure 6.](image)

Figure 6. (a-c) Photomicrographs of liver of mice in all experimental groups. Hepatocyte: H, nuclei: N, intervening sinusoids: S, the central vein (C). ACP, n=8; VEH. Vehicle, ACP: Acetaminophen H&E x 100.

**Discussion**

Acetaminophen is a common, over-the-counter medication known to be hepatotoxic at doses above the therapeutic dose, as a result of excessive production of highly electrophilic intermediate metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which binds covalently to nucleophilic macromolecules such as cellular proteins and lipids in the hepatocytes, inducing lipid peroxidation (Muriel et al., 1992). This study set out to ascertain the possible hepatoprotective capability of oral ginsomin in acetaminophen overdose in mice.

The results of this study showed that acetaminophen at doses administered caused significant reduction in weight, an increase in the AST, ALT, total protein and decrease in plasma albumin levels associated with histological evidence of hepatotoxicity (disorganisation of the hepatic architecture with numerous vacuoles within the hepatocytes, dilated central vein and irregularly shaped nuclei). Pre-treatment with ginsomin however resulted in dose-related protection of the liver from the effects of acetaminophen-overdose.

In a number of toxicological studies, parameters such as body weight and relative organ weights are important criteria for evaluation of toxicity (Crissman et al., 2004). In this study, a reduction in body weight seen with acetaminophen overdose corroborates findings from an earlier study in rats (Onaolapo and Onaolapo, 2012) in which similar results were seen. Weight loss from acetaminophen overdose may be attributed to a reduction in food intake or poor food palatability. Acetaminophen overdose may also cause generation of free radicals from increase in oxidative stress and alterations in antioxidant levels which may put a burden on metabolism and cause weight loss. Pre-treatment with ginsomin which is known to have high antioxidant activity is seen to attenuate the effects of acetaminophen overdose on body weight.

Liver enzymes (AST and ALT) are cytoplasmic in origin and are found in the blood in significant quantities only after hepatic injury; however, ALT is considered the more reliable marker of hepatic injury of the two [24]. In this study, the decrease in the level of ALT and AST (compared to ACP group) may be attributable to the protective effect of ginsomin. A number of studies have shown ginseng, a major component of ginsomin is hepatoprotective and this function can be
attributed to its antioxidant activity that arises from constituents such as ginsenosides, phenolic acids and flavonoids (Karakus et al., 2011; Ramesh et al., 2012; Mohamed et al. 2015).

The liver plays a major role in metabolism and has a number of other functions in the body including plasma protein synthesis. With acetaminophen overdose, there is hepatic injury and a consequent decrease in plasma albumin; the liver cells may compensate for the decrease in albumin production by increasing the production of globulins and therefore increasing total protein. This study showed a significant decrease in plasma albumin and an increase in total protein at all doses of ginsomin compared to vehicle. Also, a significant decrease in total protein at doses of 0.5 and 1.0 mg/kg of ginsomin was seen compared to acetaminophen control. This result shows the ameliorative effect of ginsomin on total protein by decreasing its level in ginsomin pre-treatment groups. Liver histology showed that acetaminophen overdose was associated with hepatic injury in mice in the acetaminophen without pre-treatment group, compared to the vehicle. However, pre-treatment with ginsomin resulted in significant qualitative improvement in liver architecture compared to acetaminophen without pre-treatment, with the greatest improvement seen at 1.5 mg/kg.

In conclusion, this study demonstrated that ginsomin administration was protective against the ill effects of acetaminophen overdose on liver cells, suggesting that ginsomin capsules may be useful in protecting against development of liver injury in acetaminophen overdose.

References
