ABSTRACT:
Ginseng has a wide range of pharmacological and therapeutical actions. The present study was designed to investigate the protective effect of Panax ginseng (Pg) against the liver damage induced by paracetamol (acetaminophen; AA) in mice. Fifteen mice were divided into three groups; each contains five mice. Group "1": normal control; Group "2": mice treated via the intraperitoneal route with paracetamol (500 mg/kg) for five consecutive days; Group "3": the remaining mice were also injected intraperitoneally with ginseng (200 mg/kg) for five successive days; then injected with only one dose of paracetamol (500 mg/kg). Acetaminophen increased significantly the levels of alanine aminotransferase "ALT" and aspartate aminotransferase "AST" while decreased the level of serum albumin in significant value. Administration of Panax ginseng (Pg) in the third group reduced significantly the levels of "ALT" and "AST" that were induced with AA administration near to the control values and modulated the low level of albumin that was induced with AA near to the normal values in a significant manner. Liver histopathological studies showed that AA administration induced many pathological effects that can be ameliorated or prevented by applying pretreatment with Pg. These results recommend that Pg has a protective effect against AA-induced liver injury in mice.

KEY WORDS:
Panax ginseng (Pg), acetaminophen (AA), paracetamol, ALT, AST, albumin, liver

INTRODUCTION:
Paracetamol which is also known as acetaminophen (AA) is widely used as analgesic and antipyretic agent for relieving the moderate pain. The drug is safe and easily tolerated in therapeutic doses (Proudfoot and Wright, 1970). However, AA taken in overdose or long-term use at the therapeutic level may induce the saturation of conjugation pathway leading to the depletion of glutathione and to the increased formation of toxic reactive metabolites. Free toxic metabolites are then covalently bound to the macromolecules of cells leading to cell necrosis (Jollow et al., 1973; Mitchell et al., 1973; Pumford et al., 1997). N-acetyl-p-benzoquinone imine (NAPQI) - the toxic metabolite of acetaminophen - is responsible for inducing liver damage by binding to the liver cells’ protein leading to centrilobular necrosis (Dahlin et al., 1984). There is considerable species variation with respect to susceptibility to AA-induced hepatic toxicity; the mouse is among the more susceptible species and is frequently used in many studies (Davis et al., 1974).

The liver is the largest internal organ in the human body and some animals’ bodies. It plays a key role in over 200 functions and in many biological activities, such as metabolism, digestion, elimination, and detoxification of xenobiotics, environmental pollutants and chemo-therapeutic agents (Maheswari, 2008). Lee et al. (2001) reported that herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, and there is relatively little knowledge with respect to their modes of action. Natural compounds that reduce enzymes related to bioactivation of chemicals could be considered as good candidates for protection against chemical-induced toxicities. Herbal medicine, also known as phytotherapy, has been used for thousands of years in the treatment or prevention of many disorders. Panax ginseng is one of the most popular phytotherapeutic agents of Asian and Chinese medicine for patients with liver diseases (Duke, 1989; Chung et al., 2011). The hepatoprotective effects of Pg on cirrhosis and liver injury have
been shown in previous studies using animal models (Park et al., 2010). The ginseng has wide range of pharmacological and therapeutical actions. Pharmacological investigations have suggested that the basic action of ginseng extract is to increase non-specific resistance of the organism or to normalize the physiology of the organism and stimulate various metabolic reactions in the liver cells (Gum et al., 2007). The recovery of liver damage by ginseng has been reported by Lin et al. (2003) after ethanol treatment. It has been found that ginseng protects the human body from toxic substances (Lee et al., 1984) and disease (Yang et al., 1993) by several different mechanisms. It was concluded that *Pg* and its constituents have been shown to exhibit both anti-stress and antioxidant activities and to exert various benefits relating to stress and the immune system (Simsek et al., 2007).

The pharmacological properties of ginseng are mainly attributed to ginseng saponins, commonly called ginsenosides, the major and bioactive constituents (Ernst, 2010). Most of the previous data demonstrated the protective effect of *Pg* on different pathological effects of liver, brain and kidney (Ibrahim, 2009). On the other hand there are not enough data available in relation to the hepatoprotective effect of *Panax ginseng* against AA intoxication. Therefore, the present study was designed to investigate the protective effect of *Pg* against AA-induced hepatotoxicity in mice.

**MATERIAL AND METHODS:**

**Animals:**

Fifteen male Swiss albino mice, each weighing 25-30 g, were obtained from the National Institute of Cancer, Cairo University. The mice were transferred to the study laboratory for one week before experimentation to ensure their adaptation. The mice were divided into three groups; each one contains five mice and placed into a separate cage. The mice were provided with standard mice feed and tap water *ad libitum*.

**Chemicals:**

Acetaminophen was purchased from Bristol-Myers Squibb Loc, Fontana del Ceraso, Anagni , Italy as a product called Perfalgan infusion "paracetamol" (one ml contains 10 mg paracetamol).

*Panax ginseng* was purchased in a refill containing (5g *Pg* root) from Imtenan Health Shop, Nasr City, Egypt.

Alanine aminotransferase and aspartate aminotransferase Kits were purchased from Human Society for Biochemicals and Diagnosis, Max-Plank, Germany. While, albumin reagents was purchased from Vitro Scient, Hannover, Germany.

**Experimental design:**

The mice were divided into three groups, each contained five animals. The first group served as healthy control. The second group contained mice injected *via* the intraperitoneal route with 500 mg/kg AA (Yapar et al., 2007) for five consecutive days. The third group of mice was injected *via* the intraperitoneal route with 200 mg/kg *Pg* for five consecutive days and with 500 mg/kg AA for only one dose (Uzkeser et al., 2012).

**Samples collection and processing:**

Twenty four hours after the last administration of the treatments, the mice from which blood samples were collected by cardiac puncture into dry tubes; were killed by cervical dislocation, and their livers were excised. Blood samples were centrifuged at 3000 rpm for 10 minutes for the sera separation.

**Biochemical studies:**

The activities of ALT and AST were measured using kinetic method according to recommendations of the expert panel of the IFCC (International Federation of Clinical Chemistry) (Schumann and Klauke, 2003). Albumin level was quantified according to the procedure of Doumas et al. (1971).

**Histopathological studies:**

Small pieces of liver were excised, fixed in 10% buffered formalin and processed by embedding in paraffin, then the paraffin blocks were cut into 5-7 µm thick sections. Staining with eosin and haematoxylin was done. The histopathological changes were evaluated using light microscope (Olympus, Japan). The slides were photographed using digital camera.

**Statistical analysis**

All values were expressed as means ± SD. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey’s post-hoc test. Differences with a value of *P*<0.05 were considered as statistically significant.

**RESULTS:**

**Biochemical Results:**

No death was recorded among of mice during experimentation of all the three groups. The effects of AA and *Pg* on the studied serum biochemical parameters are presented in tables 1&2 and figures 1-3. The comparison between the recorded values of the different studied groups showed that AA induced liver injury and this is observed in elevated values of ALT and AST in comparison to the control values. AA induced highly significant increase in liver ALT values "221.6 ± 14.41" versus "168 ± 7.02" (*P*< 0.01). On the other hand, the AA has a very highly significant effect on the liver AST values (*P*< 0.001) because of the recorded values of AST was 369.6 ± 22.8 versus 192.2 ± 4.97.
Table 1. Serum ALT, AST activities and albumin level of the control and the treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (µ/l)</th>
<th>AST (µ/l)</th>
<th>Albumin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>168 ± 7.02</td>
<td>192.2 ± 4.97</td>
<td>2.396 ± 0.018</td>
</tr>
<tr>
<td>AA</td>
<td>221.6 ± 14.41**</td>
<td>369.6 ± 22.8***</td>
<td>2.178 ± 0.039***</td>
</tr>
<tr>
<td>Pg &amp; AA</td>
<td>130 ± 20.26*</td>
<td>209.2 ± 32.6</td>
<td>2.362 ± 0.041</td>
</tr>
</tbody>
</table>

*: significant (P < 0.05); **: highly significant (P < 0.01); ***: very highly significant (P < 0.001).

Table 2. Serum ALT, AST activities and albumin level of acetaminophen AA group compared with Pg + AA group

<table>
<thead>
<tr>
<th>Groups</th>
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</tr>
</tbody>
</table>

***: very highly significant (P < 0.001).

On the other hand, P. ginseng pretreatment leads to modulation in the values of ALT and AST. ALT and AST levels decreased significantly as compared with the "AA" group (P < 0.001). These data are represented in table 2. In relation to albumin, AA induced reduction in their values as compared to control. Thus, AA induced very highly significant decrease in liver albumin values (P < 0.001). On the other hand, P. ginseng pretreatment leads to modulation of albumin values near to the normal values. These data are shown in tables 1&2 and represented in figure 3.

Fig. 1. Serum ALT activity in control, AA, and Pg + AA groups

Fig. 2. Serum AST activity in control, AA, Pg + AA groups

Histological Results:

The histopathological assessment of the liver was performed for all the animal groups. Examination of liver of the healthy control group revealed that the liver was formed of hepatocytes arranged in cords. The cords were radiating from a central vein and separated by blood sinusoids (Fig. 4).

Fig. 4. Photomicrograph of a transverse section of normal liver of a control mouse showing normal hepatocytes (H) with normal tissue architecture; normal blood sinusoids (S) and normal central vein (CV), H&E X 10

On the other hand, AA induced many histopathological effects in the liver as shown in figures 5 and 6; the central veins appear dilated with severe congestion, Kupffer cells appear abundant, the hepatocytes became vaculated with appearance of focal areas of necrosis. The administration of Pg in Pg & AA group showed moderate improvement as compared with acetaminophen group; most of the central veins appeared normal and the other one appeared congested but not dilated, the hepatocytes appeared normal without any vaculation, the necrotic areas disappeared and Kupffer cells became normal as shown in (Fig. 7).
DISCUSSION:

Acetaminophen is one of the commonest drugs used for the treatment of minor to moderate pain in humans. In case of the intake of high doses of AA; at first, it is converted into non-reactive metabolites in the liver by sulphation and glucuronidation reactions. These metabolites are later converted into a reactive metabolite known to be toxic for the liver; namely N-acetyl-p-benzo-quinoneimine; by the liver cytochrome P450 enzyme system. The resulting metabolite covalently binds to oxidized lipids and sulphhydryl groups in the liver tissue which leads to severe damage of cell membranes (Küpeli et al., 2006; Naziroğlu et al., 2009; Demirbas et al., 2011). Several studies on animals and humans have demonstrated that acetaminophen overdose causes liver damage due to enhanced production and/or decreased glutathione conjugation of N-acetyl benzoquinoneimine (NABQI) which damages the cell membrane (Kaira et al., 2012).

The determinations of liver enzyme levels such as AST and ALT are commonly used as a hepatic marker in assessment of AA-induced liver damage (Yanpallewar et al., 2003; Yen et al., 2008). AST is mainly found in the mitochondria of hepatocytes and the cytoplasm of liver, heart, skeletal muscles and red blood cells. However, the ALT enzyme is found predominantly in the cytoplasm of hepatocytes and thus, it is more specific to different liver injuries (Kuntz & Kuntz, 2006). In the present study, due to induction of liver toxicity by AA, the plasma levels of AST and ALT elevated significantly, while the level of albumin decreased significantly compared with reference normal values. Administration of acetaminophen (500 mg/kg) to mice produced marked hepatic damage as reflected by the histopathological findings (extensive necrosis, vaculation and congestion of the central veins) and the results of liver function biochemical tests (the acute elevation of serum ALT and AST and the acute reduction of Albumin). These findings were consistent with the findings of Kamanaka et al. (2003); Janbaz et al. (2004); Oz et al. (2004); and Abdel Salam et al. (2005) who reported that acetaminophen administration causes severe centrilobular necrosis resulted in marked reduction of albumin level and marked increase in serum ALT activity. On the other hand, the pretreatment with Panax ginseng, modulated the values of AST, ALT, and albumin significantly towards the normal values. These results are in agreement with some of the previous studies used Pg and some hepatotoxic agents such as acetaminophen, aflatoxin, carbon tetrachloride and cadmium (Abdel-Wahhab et al., 2010; Cayir et al., 2011; Karakus et al., 2011; Uzkeser et al., 2012).

Ginseng has antioxidant activity as it contains ginsenosides, phenolic acids, flavonoids and saponins. These properties of the ginseng are thought to provide many beneficial preventative effects against organ damage (He, 2012; Ramesh et al., 2012). AA induced severe acute hepatocellular necrosis; these results are consistent with Corcoran et al. (1985) and Douidar et al. (1985). Panax ginseng pretreatment exhibited significantly less damage to the hepatic cells. With
regarded to ginseng, the results of the present study showed significant improvement in liver function tests (significant decreases in the serum levels of ALT, AST, and significant increase in albumin). In relation to the histopathological findings (significant reduction of necrosis, vacuolation and central vein congestion) in comparison to acetaminophen group, and this was in agreement with Park et al. (2005) who mentioned that ginseng has been used as a valuable tonic and treatment of various diseases and coincided with Shim et al. (2010) and Ramesh et al. (2012) who reported that ginseng enhanced the antioxidant defense mechanism and increased self-antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GSH). This finding indicates that Pg has the ability to protect the structural integrity of hepatocytes from the damage induced by AA. Finally, our results suggest that Pg effectively protects against the AA-induced liver damage in mice.

REFERENCES:


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