Protective and curative effects of *Panax ginseng* aqueous crude extract on biochemical changes of BALB/c mice exposed to Aflatoxins

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**Abstract**

The current study was designed to investigate the protective and curative effects of *Panax ginseng* aqueous crude extract (PACE) against the changing of liver enzymes and some renal functions, induced by aflatoxins exposure *in vivo*. Aflatoxins (AFs) increased significantly (*P* ≤ 0.05) the levels of Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Blood Urea, Serum Uric Acid and Serum Creatinine in mice orally administrated with 9 mg/kg b. w crude extraction of AFs. Whereas the levels of Bilirubin showed different values in all groups ranging from 0.29 mg/dl to 0.45 mg/dl which were in normal range (0.17-1 mg/dl). The mice were pretreated with 150, 100, and 50 mg/kg b. w doses of PACE orally for ten consecutive days, led to significant reductions in levels of the parameters which were studied (100 mg/kg b. w was near to the normal values in a significant manner, whereas 50 mg/kg b. w was appeared non-significant result (*p* ≥ 0.05) with uric acid). Furthermore, post-treated mice with same doses of PACE orally for twenty one consecutive days revealed a significant result at 150 mg/kg b. w more than 100 mg/kg b. w, however the third concentration gave non-significant results (*p* ≥ 0.05).

**Keywords:** *Panax ginseng*, Aflatoxins, pretreated, post-treated.

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Introduction

Herbal medicine also known as phyotherapy has been used for thousands years in the treatment or prevention of many disorders. *Panax ginseng* is one of the most popular phyotherapeutic agents of Asian and Chinese medicine for patients with liver diseases [1,2]. Authors 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 [3].

Ginseng species has a wide range of pharmacological and therapeutical actions. The investigations of pharmacological acts have suggested that the basic action of ginseng extract is to increase nonspecific resistance of the organism or to normalize the physiology of the organism and stimulate various metabolic reactions in the liver cells [4]. After ethanol treatment, recovery of liver damage was appeared after using ginseng [5]. Also, it has been found that ginseng protects the human body from toxic substances [6] and disease [7] by several different mechanisms. It was reported that *P. ginseng* 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 [8]. The pharmacological properties of ginseng are mainly attributed to ginseng saponins (ginsenosides) which are the major and bioactive constituents [9]. The most of the previous data demonstrated the protective effect of ginseng extract on different pathological effects of liver, spleen, kidney and brain [10].

Mycotoxins are secondary metabolites with low molecular weight produced by fungi in various foods and feeds [11]. They are considered as risky to the consumers of contaminated foods and feeds [12]. Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus* [13]. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B1, B2, G1, and G2 [14]. Aflatoxin B1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species [13]. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize [13,14].

Materials and Methods

Source of Plant materials and Fungal Isolates

Dried roots of *Panax ginseng* (Korean ginseng) were purchased from Iraqi local market in Irbil-North Iraq. The dried roots were powdered by using electrical blender and then kept in polyethylene bags until use. Five pure cultures of *Aspergillus flavus* isolates were obtained from the department of Biotechnology-College of science - University of Baghdad.

Aflatoxins production, extraction and analysis

Selective isolates of *A. flavus* (cultured initially on PDA medium) were grown in 250 ml Erlenmeyer flask (80 flasks) containing 25g of sterile rice medium which was wetted with sterile water 5:1 (w/v). Then a discs of 5 mm diameter of fungi culture was punctured in the rice media to prepare the inoculums for *A. flavus*. Then the flasks were incubated for 21 days at 28°C [15].

After incubation time, the moldy rice was soaked overnight with 75 ml of chloroform in dark place. Then the soaked medium was homogenized with electric homogenizer for 15 min. The extracted solution was filtered through gauze then was filtered through a Whatman No.1 filter paper. The residue was washed with 50 ml of chloroform, and then was filtered. Chloroform fractions were pooled and evaporated to dryness at 50°C. The extract stored at 4°C until use [16].

Detection and quantification of aflatoxin using High Performance Liquid Chromatography (HPLC)

It was prepared by diluting in absolute methanol, 1mg /ml for AFB2 and AFG2, 10mg/ml for AFB1 and AFG1. Then 100 µl aliquot of each aflatoxin standard solution was combined in a 2-ml vial and mixed well. This mixture was further diluted in series to 100,000 folds in water: methanol (7:3v/v) and stored at -70 °C in deep freeze until using to detect the types of aflatoxins in rice culture extract [17]. The HPLC conditions were:

- Analytical column:C18 (30 cm x4.6 mm) at 30°C.
- Injection volume : 20 µl
Detection: EX :365 EM : 455
The mobile phase: CAN (Acetonitrile): H$_2$O (40:60)
Flow rate: 1 ml/min.

**Laboratory animals and experimental design**

Seventy eight of Swiss albino mice (male) were purchased from the National Centre for Drug Control and Research /Baghdad were used, their ages were ranged (8-12) weeks and weighting (25-30) gm. The mice were acclimatized for two weeks before treatment. They housed in plastic cages containing hard wood chips for bedding, in controlled animal house at 25± 2Cº, 4/10 hour’s light / dark cycle. The animals were given water and fed with suitable quantity of complete diet.

The animals were divided into twelve groups and orally administered with 0.2ml of either plant or aflatoxins crude extract as shown below:

- Control groups (consisted of six groups) each group contained (5) mice.
  - **Group 1**: Animals without any treatments.
  - The following groups were treated: 0.2ml by oral gavage with:
    - **Group 2**: Crude extract of aflatoxins (9 mg /kg b. w), two times in a week
    - **Group 3**: PACE (150mg / kg b. w) for ten days.
    - **Group 4**: PACE (100mg / kg b. w) for ten days.
    - **Group 5**: PACE (50mg / kg b. w) for ten days.
    - **Group 6**: Methanol 10% two times in a week.

The pre and post treatment groups with PACE and crude extract of AFs were divided into six groups, each group contained (8) mice («1» refers to pretreated groups (which treated with PACE for ten days then were treated with crude extract of AFs (9mg/kg b. w)). Whereas «2» refers to post treated groups (which treated with crude extract of AFs then they were treated with PACE. The letters a, b and c refer to concentration of *P. ginseng* extract (150, 100 and 50mg/kg b. w) respectively.

**Blood Collection**

The blood samples were collected at the end of the experiment from all groups. Mice were fasted for twelve hours before they were anesthetized with ethyl ether inhalation. About 0.5-1ml of blood was collected directly from heart through cardiac puncture using 1ml disposable insulin syringes. Blood was collected in a sterile eppendorf tube and left for about 30 minutes to clot at room temperature, then centrifuged at 3000 rpm for 15 minutes. Serum was transferred to 0.5 ml eppendorf tube using micropipette, and then kept in a deep freezer for biochemical analysis.

**Biochemical analysis**

The biochemical analytics: liver function (Alkaline phosphatase ALP, Aspartate Aminotransferase AST, Alanine Aminotransferase ALT and serum Bilirubin) and renal function (Blood Urea, Serum Creatinine and Serum Uric Acid) were assayed in Laboratory analyzes pathological by using kits provided from Bio Lab Company.

**Results and Discussion**

**Screening and quantification the types of aflatoxins in rice culture extract by High Performance Liquid Chromatography (HPLC)**

The HPLC analysis for aflatoxins in rice culture extract revealed that, the solvent system composed of ACN: H$_2$O at ratio (40:60v/v) was very satisfactory, because it gives one peak at retention time 6.2 minutes for AF B2 and 8.5 for AFB1 in comparison with standard aflatoxins, and the concentration of each was 0.06264 ppm and 2.50503 ppm respectively Figure-1 and 2.
Liver functions

The parameters measured included Alkaline Phosphatase ALP, Aspartate Aminotransferase AST, Alanine Aminotransferase ALT and Bilirubin. The outcome indicated that all the three enzymes were significantly higher (P ≤ 0.05) in mice treated with aflatoxins (9 mg /kg b.w) (Oral gavage) comparing with control group (without any treatment), whereas Bilirubin appeared different values in all groups ranged between 0.29 mg/dl for control group- to 0.45 mg/dl for aflatoxins (9 mg /kg b.w) treated group- which were in normal range (0.17-1 mg/dl [18]). On the other hand, the values of the previous enzymes in animals pre and post treated with various concentrations of PACE were lower than those treated with aflatoxins only. The pretreatment of ginseng extract reduced of aflatoxins effects.
compared with post-treated groups as shown in Table-1. That is may attributed to protection effects properties of ginseng extract and its stimulation for the immune system to prepare the body to resist any pathogens.

The results showed disparate decrease in enzymes and bilirubin levels either in post or pre-treatment groups comparing with AFs (9mg/kg b. w) treated group. While the results showed an elevation in enzymes and Bilirubin levels (especially in the pre-treated groups) comparing with the control group. These results were in agreement with many previous studies applied on aflatoxins [19,20]. Alkaline phosphatase ALP was significantly higher (P≤ 0.05) in animals treated with aflatoxins (9 mg /kg b.w) (Oral gavage), which increased to 230 IU/L comparing with control animals (without any treatment) 119 IU/L, whereas the values of ALP in animals pretreated with plant extract (150,100, 50 mg/kg b.w) were significantly decrease (P≤ 0.05) to 140, 130 and 148IU/L respectively. Furthermore the values of ALP in animals post-treated with plant extract (150, 100, 50 mg/kg b.w) were 150, 184 and 210 IU/L respectively. The first and second values were significantly lower (P≤ 0.05) than AFs (9mg/kg b.w) treated group, whereas the third value was non-significant (p ≥ 0.05).

Alanine Aminotransferase ALT and Aspartate Aminotransferase AST were increased significantly in animals gavaged with AFs (9 mg /kg b.w), they were 55.6 IU/L and 88.6 IU/L respectively comparing with control group which were 20.7 IU/L and 42.1IU/L respectively. However, the values of ALT in animals pretreated with plant extract (150, 100 and 50 mg/kg b.w) were 33.4, 27.4 and 39.4 IU/L respectively, whereas the values of AST were 62.6, 52.3 and 74.4 IU/L respectively. The assessment of ALT and AST were significantly lower (P≤ 0.05) than assessment of AFs (9 mg/kg b.w) treated group. While the values of ALT and AST in animals post-treated with plant extract (150, 100, 50 mg/kg b.w) were 37.4, 46.3 and 49.6 IU/L for ALT; 50.7, 75.5 and 80.3 IU/L for AST respectively. The first and second values of both enzymes were significantly lower (P≤ 0.05) than value of AFs (9 mg/kg b.w) treated group, whereas the third value was non-significant (p ≥ 0.05). Most Bilirubin is chemically attached to another molecule in the liver before it is released in the bile. This conjugated (attached) bilirubin is called direct bilirubin whereas unconjugated bilirubin is called indirect bilirubin [21]. So total serum bilirubin equals to direct bilirubin plus indirect bilirubin. The results showed no significant alteration value in Bilirubin levels.

The increase of liver enzymes strongly in serum depends on the degree of damage and alteration in organ tissues. The more elevation in enzymes levels is due to release them to the serum and that clearly found in animal with aflatoxicosis [22]. High serum levels of ALP, AST and ALT are usually sensitive indicators of hepatic necrosis and liver damage in animals and humans that was mentioned in several reports such as [23, 24]. Many studies on the mechanisms of aflatoxins induced liver injury have demonstrated that in animals fed diets contaminated with toxicants, the serum levels of these enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum [25-27]. In addition, the metabolites produced during biotransformation of aflatoxins may justify the hepatotoxicity and the increase of serum activities of liver enzymes. They may cause cellular damage by covalent binding to cellular components such as enzymes, nucleic acids and proteins or by another mechanism. Damage of cellular components may play an important role in death of liver cells [28,29] hence, ALP, AST and ALT may be released to serum, so the levels of these enzymes would increase.
Ginseng, Panax ginseng, is considered to be the most important components contributing to the multiple medicinal properties of ginseng [30]. The full pharmaceutical activity which is due to a range of ginsenosides, were demonstrated in mice treated with aflatoxins (9 mg/kg b. w). In the pre and post treatment groups may be attributed to its ability in increasing the antioxidant status as well as lowering the oxidative damage of nucleic acids in the body [31].

Renal functions

Blood Urea, Serum Uric Acid and Serum Creatinine are numbers of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys, which can help to determine the cause and extent of kidney dysfunction.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALP (IU/L)</td>
</tr>
<tr>
<td>Negative Control</td>
<td>119±8</td>
</tr>
<tr>
<td>Methanol 10%</td>
<td>128±10</td>
</tr>
<tr>
<td>Positive Control</td>
<td>230±14</td>
</tr>
<tr>
<td>Ginseng</td>
<td>119±12</td>
</tr>
<tr>
<td>Ginseng (100mg/kg)</td>
<td>121±15</td>
</tr>
<tr>
<td>Ginseng (50mg/kg)</td>
<td>119±15</td>
</tr>
<tr>
<td>Ginseng (150mg/kg)+AFs(9mg/kg)</td>
<td>140±14</td>
</tr>
<tr>
<td>Ginseng (100mg/kg)+AFs(9mg/kg)</td>
<td>130±15</td>
</tr>
<tr>
<td>Ginseng (50mg/kg)+AFs(9mg/kg)</td>
<td>148±16</td>
</tr>
<tr>
<td>AFs(9mg/kg)+Ginseng (150mg/kg)</td>
<td>150±16</td>
</tr>
<tr>
<td>AFs(9mg/kg)+Ginseng (100mg/kg)</td>
<td>184±19</td>
</tr>
<tr>
<td>AFs(9mg/kg)+Ginseng (50mg/kg)</td>
<td>210±17</td>
</tr>
</tbody>
</table>

Each value expressed as Mean ± Standard Error (S.E) of three replicates, A = Negative Control (mice without treatment), B = Positive Control (mice with aflatoxins (9mg/kg) treatment) NS = P >0.05, a = P< 0.05, b = P< 0.01, c = P< 0.001.

In the same regards, researchers reported that ginseng has a potent protective action against CCl₄ induced toxicity [33]. Therefore, it is suggested that the protective effect of ginseng is attributed to its free radical scavenging activity [34]. Rudakewich et al., reported that ginseng roots and ginsenosides are effective in stimulating learning, memory and physical capabilities, in supporting radioprotection and provide resistance to infection [35]. Generally, the present results indicated that ginseng has protective effects against liver injury induced by aflatoxins and it plays a role in increasing the antioxidant status as well as lowering the oxidative damage of nucleic acids in the body [32].
treatment). The values of blood urea, uric acid and serum creatinine were 68.5, 4.42 and 0.88 mg/dl respectively for AFs (9 mg/kg b. w) treated group, whereas the values were 30.2, 2.11 and 0.30 mg/dl respectively for control group. On the other hand, the assessments of blood urea (mg/dl) in animals pretreated with plant extract (150, 100, 50 mg/kg b. w) were 45.3, 35.2 and 55.3 mg/dl respectively, whilst 3.55, 2.74 and 3.81 mg/dl respectively for uric acid, however the serum creatinine results were 0.54, 0.38 and 0.76 mg/dl respectively. Depending on the previous results, the values were significantly lower (P≤ 0.05) than those of AFs (9 mg/kg b. w) treated group, except of uric acid (50mg/kg b. w pretreatment group) was non-significant. While the values of blood urea, uric acid and serum creatinine in animals post-treated with plant extract (150, 100 and 50 mg/kg b. w) were 38.7, 55.3 and 60.1 mg/dl for urea, 3.05, 3.87 and 4.1 mg/dl for uric acid while 0.45, 0.70 and 0.81 mg/dl for serum creatinine, respectively. The values of first and second concentrations of post-treated with plant extract were significantly lower (P≤ 0.05) than the value of AFs (9 mg/kg b. w) treated group, whereas the third concentration gave a non-significant (p ≥ 0.05) results. The pretreated groups did not appear high rise in blood urea, uric acid and serum creatinine levels when compared with post-treated and AFs (9 mg/kg b.w) group.

Based on results, elevation of values of blood urea, uric acid and serum creatinine seem to be reasons of kidney damage and renal dysfunction obtained under the effect of mycotoxicosis, that was cleared in AFs group which was produced toxic changes as evident by all the parameters used. Moreover, these findings were consistent with reports indicated that AFs have stressful effects on the renal tissues [36].

Pre and post treatment with ginseng maybe related to the ameliorative effects of aflatoxins. It is possible that the modulation of the toxic response by AFs might have been marked by the alterations induced by pre and post treatment of ginseng doses at the different concentrations. This finding is keeping in line with the report of previous investigator, [37] who observed that the lower toxicities of AFs are mainly as a result of a faster rate of clearance via urine and feces at pre and post treatment with ginseng extract.

Treated intoxicated mice with ginseng extract showed significant improvement in kidney function as indicated by the marked decrease in blood urea, uric acid and creatinine levels. These results were in conformity with those reported by [38] who demonstrated that white ginseng and its active component, saponin, could significantly reduce the blood urea nitrogen, uric acid and creatinine levels in the blood of nephrectomized rats. Other researchers mentioned that rats administered with an enriched mixture of ginsenosides Rk3 and Rh4 before and after cisplatin injection showed decreased blood levels of creatinine [39]. It was hypothesized that relationship between the structure of ginsenosides and their effect on antioxidant system in chemical induced hemolysis was considered important in human because protopanaxatriol or protopanaxdiol act as pro-oxidant or anti-oxidant agent, and ginsenosides could decrease the severity of renal injury induced by cisplatin. Additionally other scholars asserted the nephroprotective effect of Korean ginseng saponin against cisplatin-nephrotoxicity. They suggested that Korean ginseng saponin reduced cisplatin induced cytosolic free ions (Ca2+) overload and formation of DNA interstrand cross-link and DNA–protein cross-link. They demonstrated that ginsenoside could decreased the severity of renal injury induced by cisplatin. These authors suggested that decreased level of urea in serum in rats given ginseng reflected the protective action of ginsenoside against the renal dysfunction [32,40].
Table 2- Effect of Panax ginseng crude aqueous extract on renal functions (Urea, Uric acid and Creatinine) in BALB/c mice treated with aflatoxins

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>30.2± 5.5</td>
<td>2.11± 0.05</td>
<td>0.30± 0.07</td>
</tr>
<tr>
<td>Methanol 10%</td>
<td>33.3± 6.5</td>
<td>2.23± 0.07</td>
<td>0.34± 0.08</td>
</tr>
<tr>
<td>Positive AFs(9mg/kg)</td>
<td>68.5± 4.2</td>
<td>4.42± 1.0</td>
<td>0.88± 0.06</td>
</tr>
<tr>
<td>Ginseng(150mg/kg)</td>
<td>37.4± 5.3</td>
<td>1.98± 0.08</td>
<td>0.31± 0.04</td>
</tr>
<tr>
<td>Ginseng(100mg/kg)</td>
<td>33.7±6.4</td>
<td>2.02±0.07</td>
<td>0.33±0.06</td>
</tr>
<tr>
<td>Ginseng (50mg/kg)</td>
<td>32.7±5.5</td>
<td>2.11±0.08</td>
<td>0.35±0.05</td>
</tr>
<tr>
<td>Ginseng(150mg/kg)+AFs(9mg/kg)</td>
<td>45.3±3.3</td>
<td>3.55±1.13</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>Ginseng(100mg/kg)+ AFs(9mg/kg)</td>
<td>35.2±3.1</td>
<td>2.74±0.96</td>
<td>0.38±0.05</td>
</tr>
<tr>
<td>Ginseng(50mg/kg)+ AFs(9mg/kg)</td>
<td>55.3±2.4</td>
<td>3.81±1.11</td>
<td>0.76±0.03</td>
</tr>
<tr>
<td>AFs(9mg/kg)+Ginseng(150mg/kg)</td>
<td>38.7±1.3</td>
<td>3.05±0.32</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>AFs(9mg/kg)+Ginseng(100mg/kg)</td>
<td>55.3±3.9</td>
<td>3.87±1.12</td>
<td>0.70±0.04</td>
</tr>
<tr>
<td>AFs(9mg/kg)+ Ginseng (50mg/kg)</td>
<td>60.1±3.3</td>
<td>4.1±1.21</td>
<td>0.81±0.07</td>
</tr>
</tbody>
</table>

Each value expressed as Mean ± Standard Error (S.E) of three replicates, A = Negative Control (mice without treatment). B = Positive Control (mice with aflatoxins (9mg/kg) treatment) NS = P >0.05, a = P< 0.05, b = P< 0.01, c = P< 0.001

References


