MMP-1 and MMP-7 Expression is Influenced by Ginsenosides in Mice Exposed to Aflatoxin B1: *in vivo* Study

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ABSTRACT

Panax ginseng (PG), one of the most widely used herbal medicines, has demonstrated various beneficial effects such as anti-inflammatory, antioxidant, and anticancer impacts. Naturally occurring ginsenosides in the ginseng plant inhibit cell proliferation and significantly reduce liver damage induced by certain chemicals. Aflatoxin B1 (AFB1) is a primary mycotoxin due to its hepatotoxic, immunotoxic, and oncogenic effects in animal models and humans. In this study, we examined the effects of assorted doses of PG aqueous crude extract on the expression of matrix metalloproteinase 1 and 7 (MMP-1 and MMP-7) in the kidney, spleen, and liver of experimental AFB1-exposed mice, using immunohistochemistry (IHC). Mice were orally administered 6 mg/kg body weight (bw) of refined AFB1 (isolated and extracted from Aspergillus flavus, conc. 0.05 ppm) twice weekly for two weeks. We then compared the effects of three different doses (50, 100, and 150 mg/kg bw) of crude ginseng. We estimated the expression of MMP-1 and 7 in organs using IHC. We used the 6 mg/kg of purified AFB1, representing a 60% concentration, as a control group. IHC analysis showed that MMP (1 and 7) expression in the spleen, liver, and kidney of mice decreased after treatment with ginseng crude extract. MMP-1 expression was reduced in the liver by approximately 2.6 times, while the effectiveness in the MMP-1 reduction reached 9 and 8 times, respectively, in the spleen and kidney when treated with a higher dose of PG compared to the control. MMP-7 expression was reduced in the liver by approximately 13 times, while the reduction effectiveness fell to 2.3 and 5.6 times in the spleen and kidney when treated with a higher dose of PG compared to the control. The reduction in MMPs expression due to the effect of PG aqueous crude extract was observed to act against the effect of AFB1 on various living organs involved in AFB1 metabolism. IHC analysis indicated a more significant reduction efficiency observed in the expression of MMP-7 compared to both studied markers in the mice's liver.

Keywords: Ginseng, AflatoxinB1, Matrix metalloproteinase.

INTRODUCTION

Ginseng refers to the dried roots of several plants of the species Panax sp. (Family Araliaceae). The three major commercial ginsengs are Panax ginseng Meyer (Korean ginseng), which has been used as an herbal medicine for more than 2000 years, Panax quinquifolium L. (American ginseng), and Panax notoginseng (Chinese ginseng) [1]. These ginsengs have a long history of use as food or medicinal products in China, Korea, Japan, and other Asian countries. Ginseng is well-known as an adaptogen and a

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restorative tonic and is widely used in Traditional Chinese Medicine (TCM) and Western herbal preparations [2]. The therapeutic potential and antioxidant properties of ginseng have been widely explored. Research suggests that ginseng may possess anti-inflammatory properties, potentially beneficial for managing conditions related to chronic inflammation, and for regulating blood pressure, metabolism, and immune system function [3,4]. Furthermore, ginseng treatment can reduce the severity of histopathological, histochemical, and serological alterations, but it does not completely eliminate them [5,6]. Ginsenosides are secondary ginseng metabolites, representing the most critical components of ginseng, and are involved in the modulation of various cellular functional activities.

Aflatoxins (AFs) are secondary metabolites predominantly produced by Aspergillus fungi. Approximately 20 types of AFs, produced by Aspergillus flavus and Aspergillus parasiticus, have been detected in food and feed [7,8]. Aflatoxin B1 (AFB1) is a highly hazardous mycotoxin with potent carcinogenic and mutagenic properties. The presence of aflatoxins in food and feed leads to significant physiological effects such as reduced white and red blood cell counts, hemoglobin, hematocrit, alanine aminotransferase activity, and body and organ weight [9,10]. Subpar food and feed processing and storage conditions can allow the growth and contamination by AFs [5]. Because warm temperatures and humidity provide favorable conditions for fungal growth, AF food contamination is widespread, particularly in tropical regions where temperature and humidity levels are high [11,12]. The carcinogenic, genotoxic, and cytotoxic effects of Aflatoxin B1 (AFB1) are well-documented [13]. The International Agency for Cancer Research categorizes AFs as Class I human carcinogens. In natural systems, all cell membranes mainly consist of polyunsaturated fatty acids. These act as the primary target for AF reactions. Therefore, cellular damage mediated by AFs could relate to free radical liberation through AF metabolism, initiating lipid peroxidation and cell damage [13]. Many investigations have focused on improving food and animal feed by adding medicinal plants or their extracts in various aspects, including reducing the effects of toxins [13,14].

Matrix metalloproteinases (MMPs) are a type of zinc-dependent extracellular matrix (ECM) degrader capable of degrading every component of the ECM. The ECM is a fundamental network involved in embryonic development, angiogenesis, cell repair, and tissue regeneration. MMPs have been associated with several types of cancer, including colon, lung, head and neck, and breast cancer [15]. MMPs are identified by names starting with MMP-1 and ending with MMP-28. They are classified into various categories based on the substrate they attack and their chemical formula and structure. MMP-1 belongs to the interstitial collagenases and degrades types I, II, and III of fibrillar collagens, the main structural components of the ECM. It is postulated to be the reason why collagenases do not participate in the initial stages of carcinogenesis [16,17,18]. MMP-7, also known as elastin, laminin, and fibronectin, is a protein that cleaves collagen, matrilysin, osteopontin, proteoglycans, and entactin. MMP-7 is primarily associated with tissue remodeling in biliary atresia-related liver fibrosis. Increased MMP-7 expression has been observed in various human primary malignancies, including lung, breast, ovarian, and prostate cancer. Both benign and malignant colorectal tumors have been reported to up-regulate MMP-7 [19]. This study aimed to investigate the effect of PG aqueous crude extract on mice orally administered with aflatoxin B1 by monitoring the changes in the expression of Matrix Metalloproteinase, specifically MMP-1 and MMP-7, in the liver, spleen, and kidney using immunohistochemistry (IHC).

MATERIALS AND METHODS

Production, extraction, and quantification of Aflatoxin B1(AFB1)

A pure culture of Aspergillus flavus (A. flavus) was cultivated after isolating A. flavus from 22 Iraqi patients with aspergillosis. A. flavus isolates were microscopically diagnosed before initiating AFB1 production. These isolates were grown on Sabouraud Dextrose Agar medium

(SDA), and subsequently, sterilized rice medium was prepared and distributed in 250 mL Erlenmeyer flasks (25 mL in each flask).

A 5 mm diameter disk was cut from the SDA growth using a sterile cork borer and inserted into the rice medium to create the inoculum. The flasks were then incubated at 28 °C for 21 days [19,20]. After incubation, the moldy rice was soaked for 24 hours in 75 cc of 99.45% chloroform in a shaded room. The soaking medium was then homogenized for 15 minutes using an electric mixer. The extracted solution was sieved using gauze and then passed through Whatman filter paper (grade 1, 110 mm), with the moldy rice deposit rinsed with 50 mL of chloroform before filtering.

The combined chloroform portions were evaporated to dryness at 50 °C. The resultant extract, thickened into a pasty substance, was stored in a refrigerator at 4 °C until use [20].

Purified Aflatoxin B1 (AFB1) Standard

A 10 mg/mL solution of AFB1 was prepared by diluting it in 100% methanol. A 100 µL sample of the dilution was placed in a 2 mL vial, stirred thoroughly, and then further diluted 100,000 times in a mixture of water and methanol (7:3 v/v). The resulting solution was stored in a deep freezer at -70 °C until use [22].

Both thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were utilized for the quantification and qualification testing of the isolated AFs [21].

According to Park and Troxell [22], a harmful impact dosage of 9 mg/kg body weight (B.W.) of pure aflatoxin B1 extract, reflecting a 90% aflatoxin concentration, was determined based on previous experimental data. For further concentrations, Al-Mudallal, N. H. (2023) [23] utilized 30% and 60% of pure aflatoxin B1, corresponding to 3 mg/kg B.W. and 6 mg/kg B.W., respectively, and confirmed its inductive effect on MMP-1 and MMP-7 expression [24]. Based on the above studies, 60% of pure aflatoxin B1 was used, equivalent to 6 mg/kg body weight.

Preparation of *Panax ginseng* extract and detection of ginsenosides using high-performance liquid chromatography (HPLC)

Powdered roots of Korean ginseng (Panax ginseng) (100 g) were soaked for 8 hours in 700 mL of distilled water (1:7 w/v) and subjected to a water bath at 85–90 ºC with gentle agitation; this step was repeated five times [25,26]. The mixture was then centrifuged at 2000 rpm for 10 minutes. The clear supernatant was filtered through Whatman No.1 paper and concentrated in an oven at 50–55 ºC until it reached a dark brown syrupy consistency. This was then stored in the dark at 4 ºC until use.

Experimental animals

We obtained twelve male Swiss albino mice, ranging in age from 10 to 12 weeks, from the National Center for Drug Control and Research in Baghdad. They were housed in the regulated animal house of Al-Nahrain University in plastic cages, covered with a layer of sawdust, at a temperature of 25 °C, subject to a light/dark cycle of 4-10 hours, and provided with water. The study was approved by the Ethical Committee of Al Iraqi University's College of Medicine in Baghdad, Iraq, with approval No: FM.SA/119.

Experimental design

Animals were exposed to different treatment conditions as suggested by the study carried out by El-Din et al. [27]. The animals were divided into four groups as follows, with three replicates for each group:

Group 1: For two weeks, the animals in the control group were fed pure AFB1 extract (6 mg/kg bw), representing 60% of the concentration twice a week.

Group 2: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week and then administered crude ginseng extract (50 mg/kg bw) orally once a day for three weeks.

Group 3: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week. They were then administered crude ginseng extract (100 mg/kg bw) orally once a day for three weeks.

Group 4: For two weeks, the animals were given pure AFB1 extract (6 mg/kg bw, at 60% concentration) twice a week. They were then administered crude ginseng extract

(150 mg/kg bw) orally once a day for three weeks.

Preparation of the tissue for the immunohistochemistry

A histopathological study was conducted following the procedures outlined by Bancroft and Gamble [28]. After the animals were sacrificed, various organs (liver, spleen, and kidney) were removed and immersed in a physiological solution (pH 7), prepared according to Benson's method [29]. To attach tissue slices to slides, Mayer's albumin was used. The slides were then placed in a 37 °C oven for 1–2 hours. The washing step was repeated three times, with each wash using 10%, 70%, and 95% concentration solutions. Eosin and Hematoxylin were used to stain the slides, which were left for 10 seconds before being rinsed in water and immersed in 1% concentration acid alcohol. Following these steps, the slides were coated with Distyrene-Plasticizer-Xylene (DPX) and then immersed in Xylene for 15–30 minutes. The histological examination was performed using a light microscope.

Evaluation of matrix metalloproteinases (MMP) 1 and 7 using immunohistochemistry

The damaged liver, kidney, and spleen tissues were embedded in paraffin and incubated in an 80°C hot air incubator for seventy minutes, utilizing positively charged adhesion microscope slides. Subsequently, the slides were rehydrated in graded alcohol. The treated slides were then immersed in xylene for 30 minutes, followed by fresh xylene for an additional 5 minutes. The slides were further processed in 50% ethanol for 5 minutes and distilled water for 5 minutes, allowing for suitable cooling.

For IHC, 40 µL of primary antibodies, including anti-MMP-1 (cat. no. ab52631; Abcam, Cambridge, UK) and anti-MMP-7 (cat. no. ab5706; Abcam, Cambridge, UK), were applied in accordance with the manufacturer's instructions [30, 31].

Evaluation of immunostaining for matrix metalloproteinases (MMP-1 and 7)

Evaluation of immunostaining for matrix metalloproteinases (MMP-1 and 7) MMP-1 and MMP-7 expression were quantified by counting the number of positive cells, identified through cytoplasmic staining with the brown light "3,3'-Diaminobenzidine" stain (DAB) using a 40X light microscope [29, 32]. The evaluation of MMP-1 and MMP-7 immunostaining utilized a scoring system that assessed the extent and intensity of staining in three hotspot areas at an optimal magnification of 40X. Scores ranged from 0 to 100 percent and were classified into weak, moderate, and strong groups [32].

Table 1. Immunostaining expression of the MMP-1 scoring system

MMP-1	Score	Intensity	Stained cells (%)
Negative	0	No staining	<10
Positive	+	Weak	<70
	++	Moderate	>70
	+++	Strong	>80

Table 2. Immunostaining expression of the MMP-7 scoring system

MMP-7	Score	Intensity	Stained cells (%)
Negative	0	No staining	<10
Positive	+	Weak	<40
	++	Moderate	>=40
	+++	Strong	>50

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Packages for Social Sciences (SPSS) version 27. Significance was considered when the p-value was equal to or less than 0.05. The data were presented using simple measurements of means. Additionally, one-way ANOVA was utilized to compare the different organs subjected to variable treatments.

RESULTS

AFB1 production, extraction, and quantification

HPLC investigation of AFs in rice plantation extract showed that the accumulated solvents of acetonitrile: H2O (40:60 v/v) in the system were very suitable after growing A. flavus in rice cultivation extract. AFB1 purification by HPLC and TLC techniques revealed only one peak at an 8.5-minute retention period for AFB1 compared to standard AFs, with a concentration of 0.05 ppm. The rate of retention of every component in the column determines the separation of the mixture into its components.

Estimation of MMP-1 and MMP-7 using immunohistochemistry technique

IHC technique provided definitive data and was presented as counts and percentages. Immunostaining was observed in the AFB1-treated slides as well as in slides treated with aqueous ginseng extract, indicating specificity in the immunohistochemical signals. Immunostaining for MMP-1 and MMP-7 in various mouse tissues was carried out based on the ability of these tissues to absorb DAB cytoplasmic staining. IHC analysis demonstrated positive expression of MMP-1 and MMP-7 in different AFB1-treated tissue organs, as evidenced by brown staining in the cytoplasm of cells, reflecting the effect of DAB pigment (as demonstrated in figures 1, 2, 3, 4, 5, and 6). The stained cells for MMP-1 and MMP-7 expression in 60% AFB1-treated liver sections were counted as 26.12% and 44.33% (number of staining cells according to the DAB and scoring system), respectively. However, their expression in the liver section reduced to 14.00% and 40.03%, respectively, after the addition of 50 mg/kg bw ginseng, compared with the control group. Ginseng extract at 100 mg/kg bw decreased MMP-1 and MMP-7 expression to 10.00% and 37.20%, respectively, when compared to the control group, whereas ginseng extract at 150 mg/kg bw took the upper hand in reducing MMP-1 and MMP-7 expression to 9.76% and 3.30%, respectively, compared to the control group (as listed in table 3).

Table 3. Comparison of MMP-1 and MMP-7 expression in mice liver after being treated with AFB1 and different concentrations of crude PG extract

	Group 1 Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1 + (50 mg/kg PG extract)	60% AFB1 + (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract
MMP-1	26.12	14.00	10.00	9.76
MMP-7	44.33	40.03	37.20	3.30

Figures [1] and [2] illustrate the differences between liver tissues subjected to only AFB1 and those exposed to AFB1 + ginseng crude extract. AFB1-treated liver tissues exhibited a higher degree of MMP-1 and MMP-7 expression ([Figure 1A], [2A]). In contrast, liver tissues treated with AFB1 for 10 days, followed by post-treatment with ginseng crude extract twice a day for two weeks, demonstrated a decrease in MMP-1 and MMP-7 expression levels ([Figure 1B], [2B]).

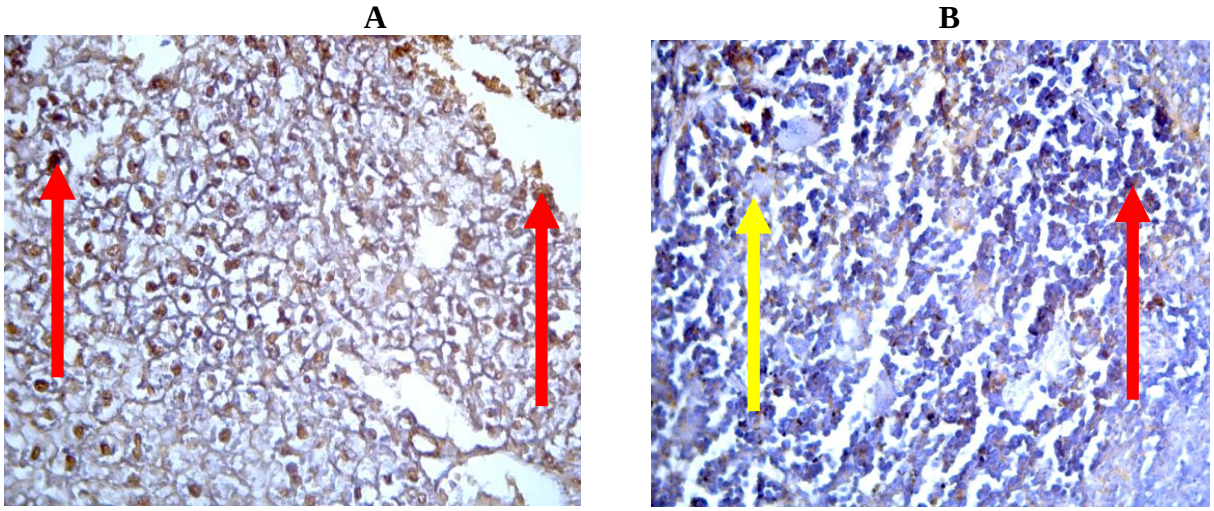

Figure 1. MMP-1 expression in mice liver sections in (A) 60% AFB1treated mice as positive control, (B) AFB1-treated mice liver section then treated with 150 mg/kg bw PG extract (weak expression). Read raw: over expression, yellow raw: downregulated.

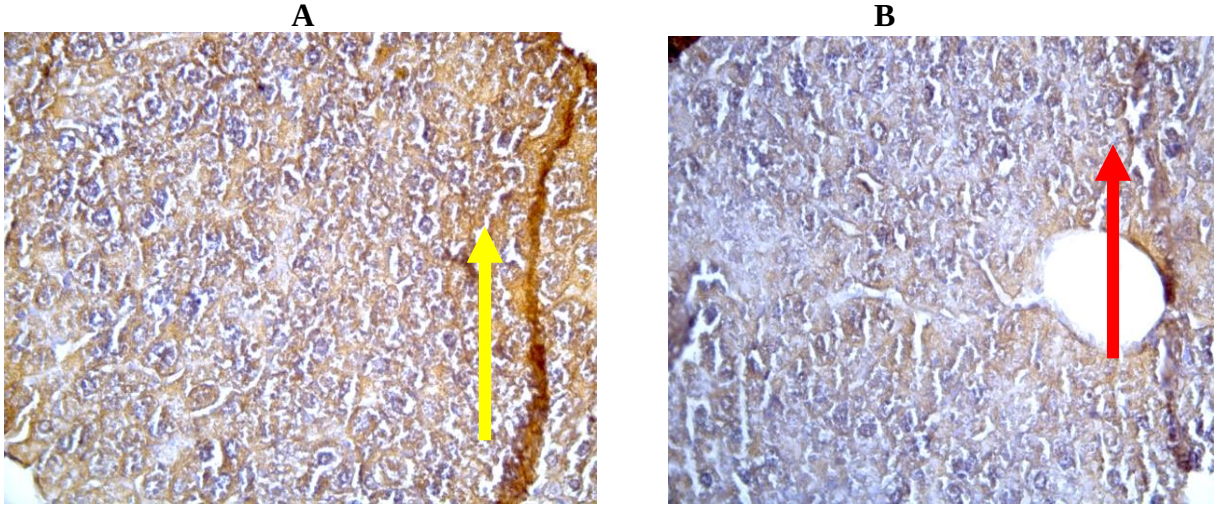

Figure 2. MMP-7 expression in mice liver sections in (A) 60% AFB1 treated mice as positive control, (B) AFB1 + 150 mg/kg bw crude PG extract-treated mice (Moderate expression). Read raw: over expression, yellow raw: downregulated.

A significant reduction in MMP-1 expression was observed in AFB1-administered mice spleen tissues after treatment with different concentrations of crude ginseng extract. In spleen sections, MMP-1 and MMP-7 were expressed at 34.00% and 19.33%, respectively, after treatment with 60% AFB1. The reductions in MMP-1 and MMP-7 expression in group 2 mice were 22.00% and 15.04%, respectively, following treatment with 50 mg/kg bw ginseng, compared to the control group. Ginseng extract at a concentration of 100 mg/kg bw downregulated MMP-1 and MMP-7 expression to 19.12% and 12.20%, respectively, while 150 mg/kg bw of ginseng extract decreased the expression in MMP-1 and MMP-7 to 6.06% and 8.22%, respectively, as compared to the control group (listed in table 4).

Table 4. Comparison of MMP-1 and MMP-7 expression in mice spleen after being treated with AFB1 and different concentrations of crude PG extract

	Group 1Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1 + (50 mg/kg PG extract)	60% AFB1 + (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract)
MMP-1	34.00	22.00	19.12	6.06
MMP-7	19.33	15.04	12.20	8.22

Figure 3 illustrates the differences among spleen sections from tissues subjected to the same treatment by counting the cells that absorbed DAB cytoplasmic staining. Strong MMP-1 expression was observed in the 60% AFB1-treated mice section (as demonstrated in Figure 3A), whereas the highest reduction in MMP-1 expression was demonstrated in spleen tissues treated with 60% AFB1 + 150 mg/kg bw crude extract of ginseng (as shown in Figure 3B).

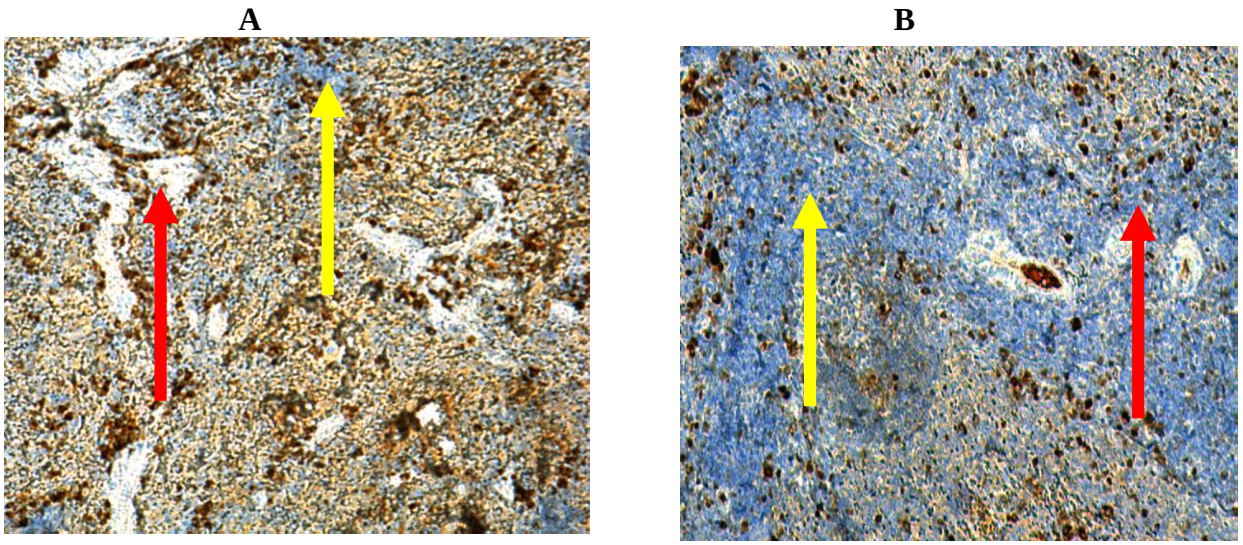

Figure 3. Matrix metalloproteinases 1 (MMP-1) expression in mice spleen section (A) CONTROL MMP-1 expression on 60% AFB1 treated mice (weak expression), (B) spleen section of AFB1 + 150mg/kg bw crude PG extract treated mice (weak expression). Read raw: over expression, yellow raw: downregulated.

In a comparison between MMP-1 and MMP-7 expressions, MMP-7 was expressed as 19.33% after treatment with 60% AFB1, while the expression decreased to 8.22% when treated with 150 mg/kg bw ginseng crude extract (Figure 4).

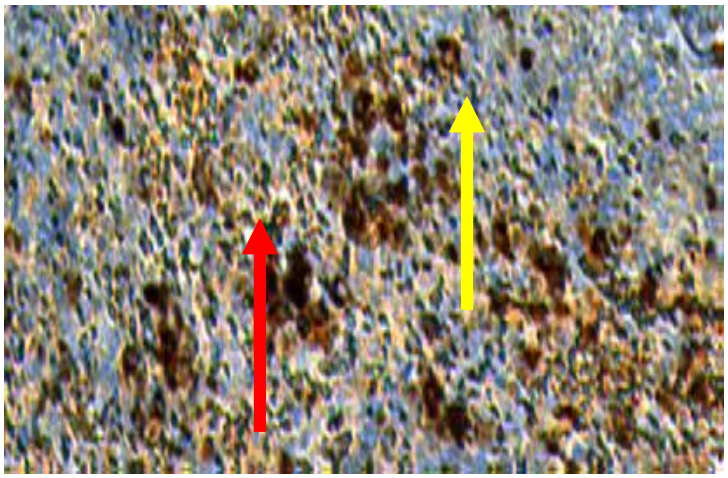

Figure 4. (MMP-7) expression on 60% AFB1-treated mouse spleen (weak expression). Read raw: over expression, yellow raw: downregulated.

In contrast, mice kidney sections exhibited a noticeable downregulation of MMP-1 and MMP-7 expression, with all being graded under weak expression (Table 5).

Table 5. Comparison of MMP-1 and MMP-7 expression in mice kidneys after being treated with AFB1 and different concentrations of crude PG extract.

	Group 1 Control	Group 2	Group 3	Group 4
	60% AFB1	60% AFB1+ (50 mg/kg PG extract)	60% AFB1+ (100 mg/kg PG extract)	60% AFB1 + (150 mg/kg PG extract)
MMP-1	18.00	13.00	7.12	2.23
MMP-7	22.65	9.02	4.23	4.00

Following treatment with 60% AFB1 (Group 1), MMP-1 and MMP-7 expression in kidney tissue appeared to be 18.00% and 22.65% respectively. Post-treatment with 50 mg/kg bw PG aqueous extract (Group 2) resulted in a reduction of MMP-1 and MMP-7 expression to 13.00% and 9.02% respectively.

Further reduction in MMP-1 and MMP-7 expression to 7.12% and 4.23% respectively was observed in Group 3 when mice were post-treated with PG aqueous extract of 100 mg/kg bw. Finally, when PG aqueous extract concentration of 150 mg/kg bw was used for post-treatment in Group 4, a tremendous reduction of MMP-1 and MMP-7 expression was noticed, scoring 2.23% and 4.00% respectively, indicating downregulation compared to control (Group 1) where no post-treatment of PG aqueous extract was applied (Table 5).

Figures 5 and 6 illustrate the immunostaining expression of MMP-1 and MMP-7 in different kidney sections. MMP-1 and MMP-7 were overexpressed when subjected to 60% AFB1, while the expression reduced to weak or negative expression after crude ginseng extract treatment.

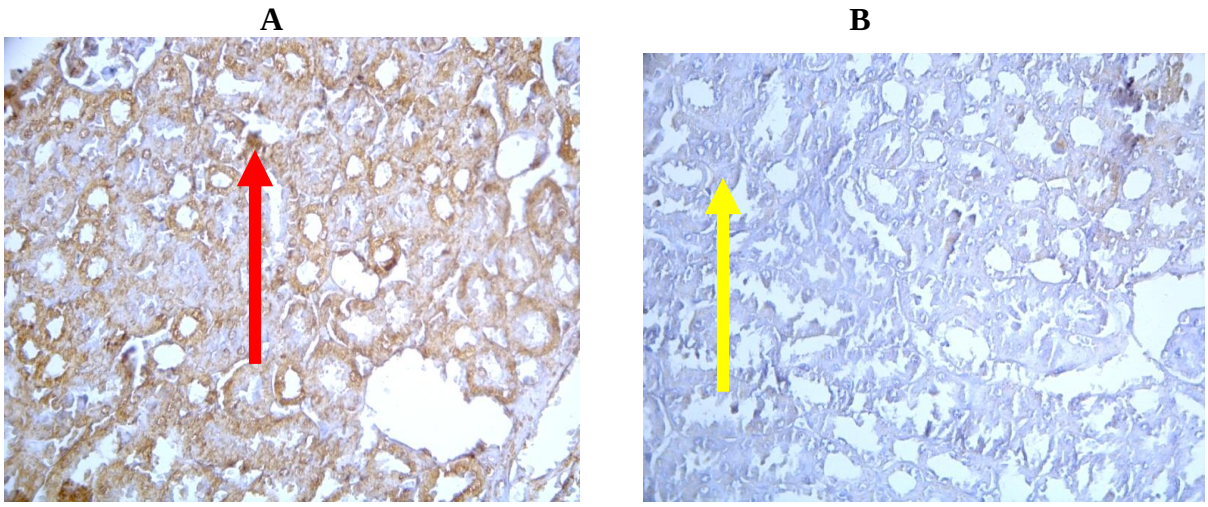

Figure 5: (MMP-1) expression in mice kidney section (A) Control group MMP-1 expression on 60% AFB1 treated mice (strong expression). (B) Kidney section of AFB1 treated mice then post- PG crude treated mice at 150 mg/kg bw (weak expression). Read raw: over expression, yellow raw: downregulated.

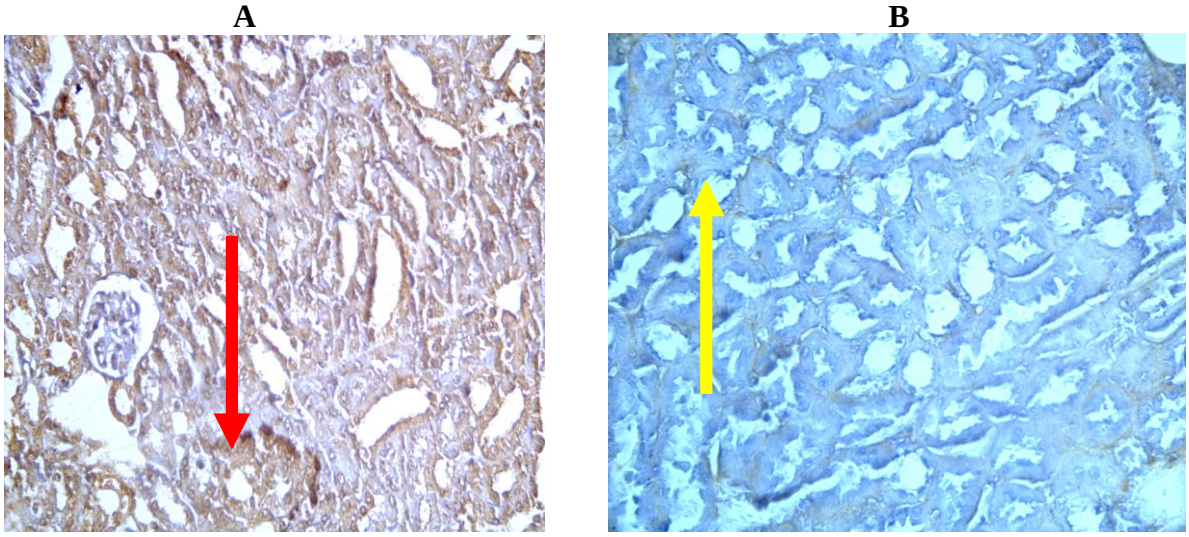

Figure 6. (A) Control: (MMP-7) expression in kidney section of 60% AFB1 treated mice, (B) Kidney section of AFB1-treated mice then post-treated with 150 mg /kg bw crude PG extract (weak expression). Read raw: over expression, yellow raw: downregulated.

For a comparative study of MMP-1 and MMP-7 expression in the above-studied organs, One-Way ANOVA was applied to analyze the relationships between treatment concentrations and marker expression in different organs (Table 6).

Table 6. Comparative study between MMP-1 expression in mice liver, spleen and kidneys after being treated with AFB1 and different concentrations of crude PG extract.

	Control (60%AFB1)	Group 1 60% Aflatoxin B1 + (50 mg/kg PG extract)	Group 2 60% Aflatoxin B1+ (100 mg/kg PG extract)	Group 3 60% Aflatoxin B1 + (150 mg/kg PG extract)
MMP-1 liver	30.36	13.00	14.00	9.69
	24.00	14.00	7.00	10.30
	24.00	15.00	9.00	8.79
MMP-1 Spleen	39.00	22.00	18.24	2.18
	24.00	22.00	18.12	9.00
	39.00	22.00	21.00	7.00
MMP-1 Kidney	18.00	20.00	7.36	1.10
	18.00	10.00	13.00	1.37
	18.00	9.00	1.00	4.10
P		0.294	0.207	0.02*

MMP-1 expression was significantly reduced in the mice liver organ by approximately 3-fold after treatment with a higher experimental dose of PG extract. In contrast, the reduction in effectiveness increased to 9 and 8 folds, respectively, in the spleen and kidney compared with the control. However, the expression reduction with the other PG experimental doses appeared insignificant.

MMP-7 expression was significantly reduced in the mice liver organ by approximately 13 folds, while the reduction in effectiveness decreased to 2.3 and 5.6 folds in the spleen and kidney when treated with a higher dose of PG compared with the control (Table 7).

Table 7. Comparative study between MMP-7 expression in mice liver, spleen and kidneys after being treated with AFB1 and different concentrations of crude PG extract

	Control (60%AFB1)	Group 1 60% AFB1+ (50 mg/kg PG extract)	Group 2 60% AFB1 + (100 mg/kg PG extract)	Group 3 60% AFB1+ (150 mg/kg PG extract)
MMP-7 Liver	45.99	40.00	37.20	5.80
	46.00	40.60	37.20	1.10
	41.00	40.30	37.20	3.00
MMP-7 Spleen	15.99	16.12	12.26	10.63
	14.00	16.00	12.20	10.20
	28.00	13.00	12.20	4.10
MMP-7 Kidney	22.70	7.02	10.00	4.00
	22.79	8.02	1.08	4.00
	22.54	12.02	1.60	4.00
P		0.323	0.088	0.00

DISCUSSION

According to the results obtained from this study, the amount of PG aqueous extract was found to be directly linked to the reduction of AFB1 toxicity. AFB1 metabolism has historically been considered to take place in two stages known as Phase I and Phase II [34]. The CYP450 enzyme systems mediate Phase I metabolism, which consists primarily of enzyme-mediated hydrolysis, reduction, and oxidation reactions that convert AFB1 to more toxic epoxide forms (AFBO). GST enzyme systems mediate Phase II metabolism, involving conjugation reactions of the AFBO intermediates, converting them into more hydrophilic compounds readily extractable in urine or bile [35,36].

In this study, we focused on the effect of AFB1 on the liver and kidneys, as these are the main organs involved in AFB1 metabolism. Additionally, we used the spleen as an example to observe its effect on other body organs. IHC findings confirmed the ability of AFB1 to disrupt the oxidant-antioxidant balance inside affected cells in different body organs at graded levels [37,38].

AFB1 undergoes biotransformation to diverse metabolites that interact with various biomolecules such as nucleic acids, resulting in the formation of AFB-N7-guanine adducts, representing a major DNA damaging event following exposure to AFs. Failure of the repair process leads to alterations in somatic development, especially at the transcriptional level. AFB1 carcinogenicity is mediated by changes in gene expression leading to mutation induction. AFB1 cytotoxicity may be associated with the disruption of cellular membrane integrity by stimulating the lipid peroxidation process in cells [39,40].

Scientifically approved evidence indicates the oxidative and carcinogenic effects of AFB1. Up-regulation of MMPs has been observed in each type of cancer and found to be associated with poor prognosis among cancer patients [41]. MMP-1 overexpression is associated with an increase in hepatocellular carcinoma cells, most likely as a result of ECM degradation during the epithelial-mesenchymal transition (EMT). MMP-7 has also been demonstrated to trigger MMP-1 [42].

Al-Mudallal, N. H. A. L. confirmed that different concentrations of AFB1 and the exposure time were linked to the severity of liver injury and the expression levels of MMP-1 and MMP-7 in the liver [23].

Different types of MMPs are located in the cytosol, subcellular organelles, nucleus, and extracellular regions, serving different roles at different stages, including cell growth, differentiation, survival, and motility. MMPs induced ECM degradation not only support tumor invasion but also alter the behavior of tumor cells, leading to cancer metastasis and ultimately disease progression. Therefore, inhibition of MMPs activity can serve as a useful therapeutic strategy in combating this life-threatening cancer [43,44]. Inhibition of MMPs through downregulation of MMPs expression reduces mRNA expression of MMPs via modulating the Akt signaling pathway [45,46,47].

The crude extract of PG was found to induce potent protective action in laboratory animals and may play a role in the prevention of hepatic, renal, and splenic injury produced by aflatoxins [34]. Several previous studies were carried out on the analysis of PG aqueous crude extract active constituents; PG aqueous extract contained alkaloids, ginsenosides, and another protective component that directly acts as inhibitors of MMPs [48].

Many previous histopathological studies revealed that AFB1 administration induces degenerative changes in the liver, spleen, and kidney.

In this study, we focused on the effect of PG aqueous extract and how it acts against AFB1 toxicity on the liver, spleen, and kidneys. A higher reduction effectiveness was proved in liver tissues after monitoring the expression of MMP-7 in orally 60% AFB1-administered mice, being reduced from 44.33% to 3.30% after treatment with 150mg/kg of PG.

Hashim, S. S. et al. (2016) [48] studied the effect of

PG against AFB1-mediated toxicity on different body organs, and the histological examination of AFB1-treated kidneys showed the appearance of apoptotic cells, degenerative cells, and shrinkage of glomeruli [50]. On the other hand, the kidney sections treated with PG extract appeared to be normal tissue [34].

The hepatotoxic, mutagenic, and carcinogenic effects of AFB1 on several animal models and humans explain the strong efforts of researchers to find any detoxification mechanisms that can be easily applicable [51]. In this study, AFB1 detoxification might be conducted by lowering MMP-1 and MMP-7 marker expression using PG aqueous extract [52], which is an effective strategy against carcinogenesis, mutagenesis, and other kinds of toxicity mediated by carcinogens [49]. The sample size was restricted and considered as one of the limitations in this study.

CONCLUSION

The toxicity reduction effects of PG aqueous crude extract were confirmed against the impact of AFB1 on different living organs involved in AFB1 metabolism. Immunohistochemistry (IHC) analysis indicated a higher reduction effectiveness for both studied markers (MMP-1 and MMP-7), particularly in the expression of MMP-7 in the liver of mice.

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تأثر تعبير MMP-1 وMMP-7 بالجينسينوسيدات في الفئران المعرضة للأفلاتوكسين: B1 في دراسة على الجسم الحي

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ملخص

تعد الجينسينغ الباناكس (PG) واحدة من الأدوية العشبية الأكثر استخدامًا بشكل عام، وقد أظهرت أن لديها مجموعة متنوعة من التأثيرات الإيجابية مثل التأثير المضاد للالتهابات والمضاد للأكسدة ومضاد للسرطان. يعمل الجينسينويدات الطبيعية الموجودة في نبات الجينسنغ على تثبيط انتشار الخلايا وتقليل بوضوح الضرر الكبدي الناجم عن بعض المواد الكيميائية. يعد الأفلاتوكسين ب1 (AFB1)واحدة من أبرز السموم الفطرية، نظرًا لتأثيراتها الضارة على الكبد والمناعة وتسببها في تكون الأورام في النماذج الحيوانية والبشر. في هذه الدراسة، تم دراسة تأثيرات مستخلص الجينسنغ الخام بتراكيز مختلفة على تعبير المصفوفة المعدنية البروتينية 1 و 7 (MMP-1) و (MMP-7) في الكلى والطحال والكبد لفئران تعرضت تجريبيًا لمادة الأفلاتوكسين ب1 باستخدام تقنية التصبيغ المناعي الكيميائي للانسجة (IHC) ، أُعطيت الفئران 6 ملغ/كغ من وزن الجسم (bw) من الأفلاتوكسين ب1 المنقى المعزول والمستخلص من فطر الاسبرجلاس فلافس *Aspergillus flavus* ، تركيز 0.05 جزء في المليون عن طريق الفم مرتين في الأسبوع لمدة أسبوعين. ثم تمت مقارنة تأثيرات ثلاث جرعات مختلفة (50 , 100, 150) ملغ/كغ من وزن الجسم من الجينسنغ الخام. تم تقدير تعبير المصفوفة المعدنية البروتينية MMP-1وMMP-7 في الأعضاء باستخدام تقنية IHC، تم استخدام 6 ملغ/كغ من الأفلاتوكسين ب1 المنقى، الذي يمثل تركيزًا بنسبة 60٪، كمجموعة سيطرة. أظهرت تحليل IHC أن التعبير عن المصفوفة المعدنية البروتينية MMP (1,7) في الطحال والكبد والكلية للفئران قد قل عقب العلاج بمستخلص الجينسنغ الخام. انخفض تعبير MMP-1 في الكبد بنسبة تقريبية 2.6 مرة، في حين بلغ الانخفاض الفعال ل MMP-1 إلى 9 و8 مرات على التوالي في الطحال والكلية عند معالجتها بجرعة أعلى من الجينسنغ مقارنة بالسيطرة. انخفض تعبير MMP-7في الكبد بنسبة تقريبية 13 مرة، في حين انخفض التعبير الفعال ل MMP-7 إلى 2.3 و5.6 مرات في الطحال والكلية عند معالجتها بجرعة أعلى من الجينسنغ مقارنة بالسيطرة. لوحظ تقليل تعبير MMPs بفعالية بفعل مستخلص الجينسنغ الخام في مواجهة تأثير الأفلاتوكسين ب1 على الأعضاء الحية المشاركة في أيض الأفلاتوكسين ب1 ،أشار تحليل IHCإلى وجود تقليل اكثر فعالية في تعبير MMP-7في مقارنة بين كلا المؤشرين المدروسين في كبد الفئران.

الكلمات الدالة: الجينسنغ، الأفلاتوكسين ب1، المصفوفة المعدنية البروتينية.

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