

Protective efficiency of *Chelidonium majus* extract against hepatoimmune and DNA changes induced by aflatoxin B1

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ARTICLE INFO

Received on: 26/06/2021

Accepted on: 12/11/2021

Available Online: 05/03/2022

Key words:

Aflatoxin B1, *Chelidonium majus*, oxidative stress, liver, antioxidant.

ABSTRACT

Aflatoxin B1 (AFB1) has potent hepatotoxic and carcinogenic effects. The objective of the present study was to evaluate the protective effect of the *Chelidonium* ethanolic extract (CEE) against the AFB1-induced hepatotoxicity in rats. Thirty-two male albino rats (5 months old) were grouped and treated intragastrically for 28 days as follows: 1) control: each rat was given 1.0 ml vehicle daily [Dimethyl sulfoxide –phosphate saline (DMSO-PBS) buffer containing 44 mg DMSO]; 2) animals received the CEE (300 mg/kg/day); 3) animals received AFB1 (80 µg/kg/day), and 4) animals received both AFB1 (80 µg/kg/day) and the CEE (300 mg/kg/day). The results declared that AFB1 intoxication induced significant increases in alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, triglycerides, total cholesterol, low density lipoprotein (LDL)- cholesterol, creatinine, urea, inflammatory cytokines (interleukin-1beta and tumor necrosis factor-alpha), tumor markers (alpha-fetoprotein and carcinoembryonic antigen), DNA fragmentation, nitric oxide, and malondialdehyde coupled with a significant reduction in high density lipoprotein (LDL)- cholesterol, glutathione peroxidase, superoxide dismutase, catalase, and glutathione values, while the group that received the CEE alone showed insignificant changes in these parameters as compared with the control one. Interestingly, coadministration of the CEE besides AFB1 significantly prevented the above mentioned deteriorations; moreover, the histopathological examination of the liver in group four revealed a better ameliorative pattern by CEE treatment. In conclusion, the CEE exhibits a pronounced antioxidative activity, making it promising as a protective and immunomodulatory agent against aflatoxins' health risks.

INTRODUCTION

Aflatoxin B1 (AFB1), a mycotoxin, is a secondary metabolite that is mostly produced by the *Aspergillus parasiticus* and *Aspergillus flavus* fungi (Ismail *et al.*, 2018). AFB1 is present in foods, grains, and feedstuffs as a natural contaminant (Alm-Eldeen *et al.*, 2015). It exerts deleterious effects on both humans and animals (Ishikawa *et al.*, 2017). Ingestion of AFB1-contaminated products can cause immunosuppressive (Zhang

et al., 2019) and teratogenic and mutagenic disturbances (Diao *et al.*, 2013). It is a strong carcinogenic agent due to its potential in inducing oxidative stress and disturbance in the antioxidant defense mechanisms (Alm-Eldeen *et al.*, 2015), so it was classified among the first group of carcinogens (Eaton *et al.*, 1994).

Also, AFB1 is potently hepatotoxic as its ingestion causes hepatocellular hyperplasia, cirrhosis, hepatic necrosis, and acute liver damage in both humans and animals (Murphy *et al.*, 2006). AFB1 is metabolized by cytochrome P450 in the liver, resulting in the extreme production of free radicals such as hydrogen peroxide and superoxide ion leading to oxidative exertion that could be neutralized by the antioxidants (Mohajeri *et al.*, 2018). Many investigators have confirmed the beneficial role

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of antioxidant agents as a granulated strategy for liver protection from the toxicity of aflatoxins (El-Bahr, 2015).

The *Chelidonium majus* L (*C. majus*) plant is a member of the Papaveraceae family and is known as greater celandine (Gilca *et al.*, 2010). The extracts of *C. majus* have been shown to exhibit antispasmodic (Hiller *et al.*, 1998) and anti-inflammatory (Jang *et al.*, 2004) activities. It showed also antitumor and antigenotoxic effects (Biswas *et al.*, 2002). In addition, it is used in the treatment of liver disorders (Vahlensieck *et al.*, 1995) and gallbladder and digestive dysfunctions (Barnes *et al.*, 2007). Furthermore, the alkaloids of *C. majus* exhibit an analgesic action as in morphine (Hiller *et al.*, 1998). The medicinal attention to *C. majus* is due to its pharmaceutical components, such as phenolic compounds, flavonoids, alkaloids, and proteins (Nawrot *et al.*, 2014). Ukrain, an alkaloid, which is derived from *C. majus*, was found to have antineoplastic, antitumor, and immunomodulatory properties (Bozeman *et al.*, 2012). This study was conducted to determine (*in vitro*) the phenolic constituents, radical scavenging activity (RSA), and reducing power potential of the *C. majus* ethanolic extract for exploring (*in vivo*) its antioxidant and protective efficiencies against oxidative stress and hepatotoxicity in AFB1-intoxicated rats.

MATERIALS AND METHODS

Chemicals

AFB1 was purchased from the Cayman Chemical Co. (USA). DMSO was obtained from Heiltropfen Co. (Germany). All other chemicals were of the highest analytical grade and were obtained from national research center stores.

Extraction

The aerial parts of *C. majus* were obtained from Abd El-Rahman Harraz (Bab El-Khalk Zone, Cairo, Egypt). The plant was authenticated in the Botany Department, Faculty of Science, Cairo University. The plant carries the taxonomic serial number 501481. The plant was ground and mixed with 70% ethyl alcohol (1:5 w/v), and the mixture was kept at room temperature for 48 hours in a closed container and then filtered, and the ethanol was evaporated from the filtrate using a rotary evaporator and freeze-dried to remove water residues (Filipiak-Szok *et al.*, 2017). The resulting extract yield (%) was calculated and stored at -20°C until further use.

In vitro assessments of the extract

The assessment of the RSA of the CEE by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out using the Nogala-Kalucka *et al.* (2005) method. The RSA was calculated as the percentage of the scavenged DPPH for three replicates.

The estimation of the ferric-reducing power of the CEE was carried out using the modified method described by Sethiya *et al.* (2014). The reducing power of the CEE was calculated as ascorbic acid equivalent from the standard curve of ascorbic acid.

In vivo study and experimental design

Five-month-old male albino rats (150–170 g) were purchased from the Animal Colony, Research Institute of

Ophthalmology, Egypt. One week before the experiment, the animals were maintained in plastic cages for acclimatization. All animals were treated ethically according to the Ethical Committee of the National Research Centre (FWA 00014747) that in turn approved the protocol of this study. After adaptation, the rats were divided randomly into four groups (eight rats each) and were orally treated by a gastric tube as follows: each animal in group one was daily given 1.0 ml DMSO-PBS buffer (pH 7.2) containing 44 mg DMSO and this served as the control group, the animals in group two were given the CEE (300 mg/kg/day) (Zarei *et al.*, 2015) dissolved in the DMSO-PBS buffer, the animals in group three were administered with AFB1 (80 $\mu\text{g}/\text{kg}/\text{day}$) (Abdel-Wahhab *et al.*, 2015) dissolved in the DMSO-PBS buffer, and the animals in group 4 were given AFB1 (80 $\mu\text{g}/\text{kg}/\text{day}$) in combination with the CEE (300 mg/kg/day). All groups were treated for 28 days.

Blood and tissue sampling

After 28 days, fasting blood samples were collected from the retro-orbital plexus of rats (Schermer, 1967) into polyethylene tubes and cool-centrifuged. The serum was separated, divided into aliquots, and stored at -80°C till analysis. After the blood collection, the animals were killed by sudden decapitation; then, the liver was removed, washed in saline, and dried on filter paper. A certain weight from each liver was homogenized in phosphate buffer (pH 7.4) to give 10% w/v homogenate. Then, the homogenate was centrifuged at 1,700 rpm at 4°C for 10 minutes (Lin *et al.*, 1998), and the supernatant was stored at -70°C until the analysis of oxidative stress markers. One part from each liver was rolled in aluminum foil and stored at -80°C for the determination of DNA fragmentation level. Another part of the liver was immersed in a formaldehyde-saline (10% v/v) solution for histopathological examination.

Serum biochemical measurements

The activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) was assessed spectrophotometrically using reagent kits purchased from CliniSciences, France. The concentration of creatinine and urea was measured in the serum using reagent kits purchased from BioVision, Inc., USA. The levels of triglycerides, total cholesterol, LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C) were tested using reagent kits obtained from Cell Biolabs, USA. The determination of serum carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), tumor necrosis factor-alpha (TNF- α), and interleukin-1beta (IL-1 β) was carried out using rats' reagent ELISA kits purchased from Sinogeneclon Biotech Co., Hangzhou, China.

Estimation of oxidative stress markers

Hepatic lipid peroxidation (measured as malondialdehyde, MDA), nitric oxide (NO), and reduced glutathione (GSH) levels and also the enzymatic activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were determined spectrophotometrically using kits purchased from Biodiagnostic Co., Giza, Egypt.

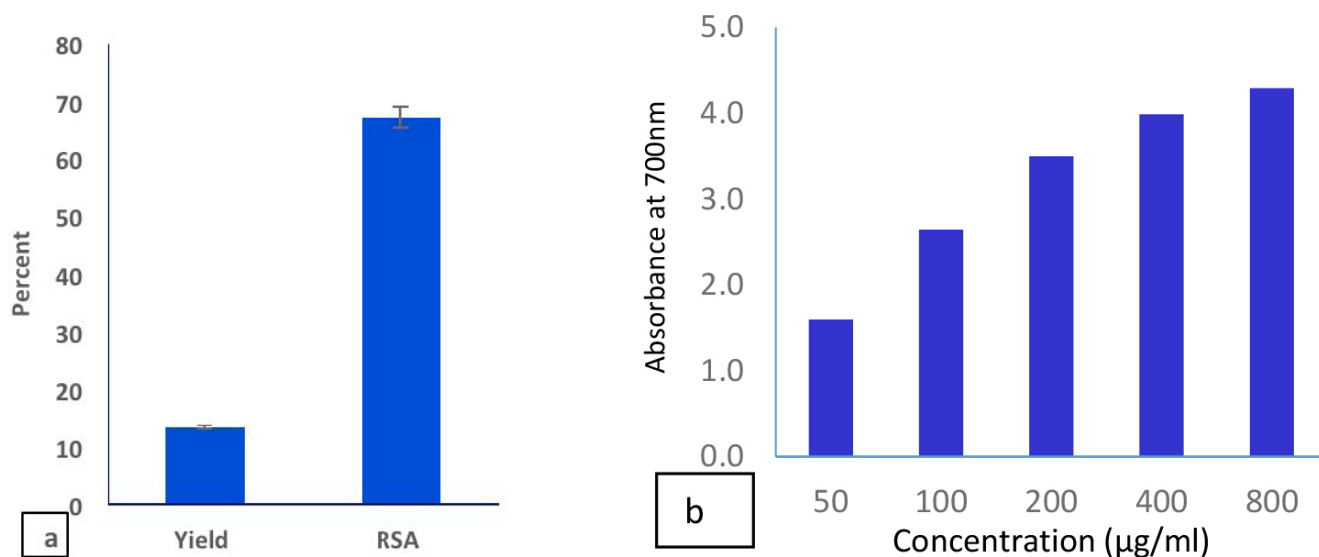


Figure 1. Yield and RSA (a); reducing power (b) of the *C. majus* ethanolic extract.

Table 1. HPLC analysis of phenolic ingredients of the *C. majus* ethanolic extract.

	Area	Conc. (µg/ml = µg/25.5 mg)	Conc. (µg/g)
Gallic acid	940.88	91.45	3,586.09
Chlorogenic acid	1,151.19	93.75	3,676.49
Methyl gallate	16.16	0.23	8.99
Caffeic acid	661.30	22.76	892.47
Syringic acid	694.45	29.13	1,142.33
Ellagic acid	120.72	11.90	466.85
Coumaric acid	45.09	0.76	29.66
Vanillin	565.17	12.25	480.27
Ferulic acid	848.59	27.88	1,093.26
Naringenin	247.07	13.63	534.43
Taxifolin	208.53	18.06	708.14
Cinnamic acid	863.66	8.16	319.89
Kaempferol	217.91	11.77	461.44

Determination of DNA fragmentation

The quantitative determination of the percentage of the fragmented DNA was carried out using the method of Perandones *et al.* (1993).

Histopathological examination

Liver sections of 5 µm thickness were processed and stained for histological examination under light microscopy (Drury and Wallington, 1980).

Statistical analysis

The obtained data were subjected to the one-way analysis of variance followed by Duncan's multiple *post hoc* test at a significance level of $p \geq 0.05$. This was carried out using the Statistical Analysis System (SAS, 1998) program software [copyright (c) by SAS Institute Inc. (Cary, NC)].

Results

The *in vitro* study indicated the antioxidant properties of the *C. majus* ethanolic extract (CEE), which appeared from testing the RSA and reducing power of the extract (Fig. 1). The extract scavenged 67% of the DPPH free radical. We noticed also that the reducing power increases with increasing the concentration of the extract. The phytochemical analysis of the CEE using HPLC revealed the identification of 13 phenolic compounds. Among these, gallic acid, chlorogenic acid, syringic acid, and ferulic acid were the major phenolic components in the extract (Table 1 and Fig. 2). The intoxication with AFB1 resulted in serious degeneration in the rats' livers, where a significant elevation was observed in serum ALT, AST, ALP, and GGT activities; also, urea and creatinine levels increased significantly, while these parameters showed nonremarkable changes in the rats' group that received the CEE alone relative to the control group, indicating the safety of the CEE, at the used dose, on hepatic

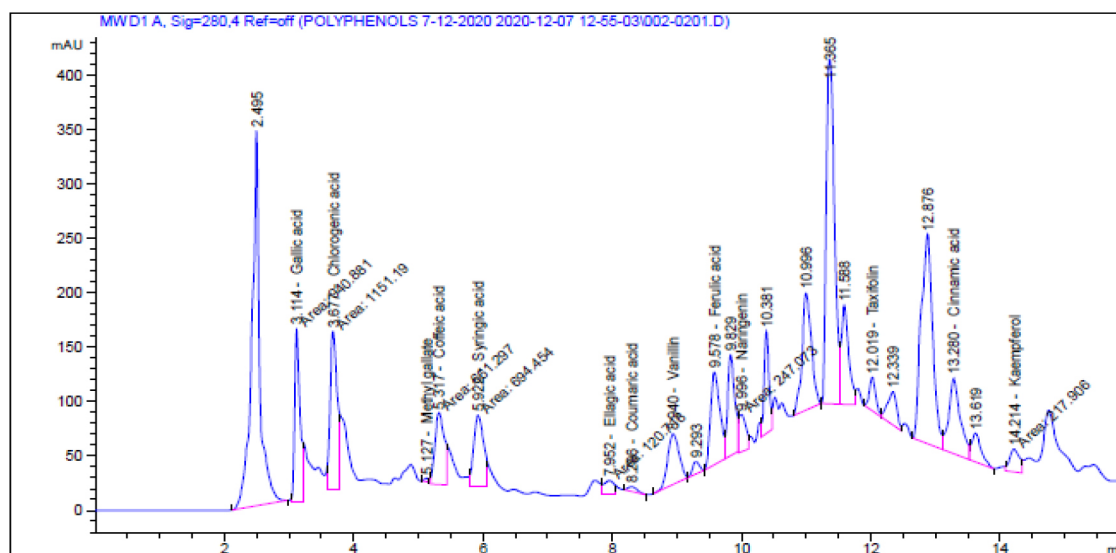


Figure 2. HPLC imprint profile of the *C. majus* ethanolic extract.

Table 2. Serum biochemical measurements of CEE- and AFB1-treated rats.

Parameter	Control	CEE	AFB1	AFB1 + CEE
ALT (IU/l)	32 ± 0.3.6	31 ± 2.7	184 ± 8.3 ^a	85.3 ± 6.56 ^b
AST (IU/l)	32 ± 0.3.6	28 ± 3.1	121 ± 5.5 ^a	78.6 ± 9.6 ^b
ALP (IU/l)	84 ± 4.38	83 ± 7.4	256.4 ± 11.4 ^a	375.4 ± 12.5 ^b
GGT (IU/l)	53 ± 2.8	49 ± 2.4	152 ± 8.9 ^a	142.1 ± 3.8 ^b
Urea (mg/dl)	32 ± 1.97	29 ± 2.1	41 ± 3.1 ^a	35.1 ± 4.9 ^b
Creatinine (mg/dl)	0.73 ± 0.17	0.71 ± 0.07	0.93 ± 0.21 ^a	0.85 ± 0.12 ^b
Total-C (mg/dl)	81.7 ± 4.1	78.3 ± 3.9	187.2 ± 5.9 ^a	107.6 ± 5.1 ^b
Triglyceride (mg/dl)	75.8 ± 3.3	74.8 ± 4.1	191.5 ± 6.3 ^a	92.3 ± 4.1 ^b
LDL-C (mg/dl)	27.2 ± 1.7	26.5 ± 1.9	56.7 ± 2.8 ^a	33.4 ± 4.4 ^b
HDL-C (mg/dl)	46.3 ± 2.6	45.8 ± 2.7	31.3 ± 2.2 ^a	40.5 ± 2.9 ^b

Values are mean ± SE for eight rats.

AFB1: aflatoxin B1; CEE physiological markers, *Chelidonium majus* ethanolic extract.

^a Compared with the control group while ^b compared with the AFB1 group at $p \leq 0.05$.

and renal functions. Favorably, treatment of rats with the CEE together with AFB1 produced a significant improvement in the above mentioned physiological markers, as the extract succeeded in restoring the elevated values close to those of the control group (Table 2).

Table 2 also shows the serum lipid profiles in the different studied groups. In comparison with the control group, the obtained data indicated that AFB1 ingestion significantly increased the concentrations of triglycerides, total cholesterol, and LDL-C, whereas it significantly decreased the concentration of HDL-C, whereas the ingestion of the CEE alone did not change the levels of lipid profile parameters. Interestingly, animals treated with the CEE in line with AFB1 showed a significant increase in HDL-C level associated with a significant decrease in the levels of triglycerides, total cholesterol, and LDL-C as compared to the AFB1-treated group.

Table 3 depicts the results of serum tumor and inflammatory markers in the different studied groups. AFB1 ingestion produced a significant increase in serum tumor

markers (AFP and CEA) and inflammatory cytokines (IL-1 β and TNF- α). The CEE never deteriorated these parameters when it was administrated separately; favorably, it markedly succeeded in downregulating the elevation of the measured tumor and inflammatory markers that resulted from AFB1 intoxication.

With respect to the redox status of the hepatic tissue, AFB1 intoxication displayed significant increases in NO and MDA, concomitant with a significant drop in GPx, SOD, and CAT activities as well as GSH level compared to the control group, while these values of oxidative stress markers did not negatively change in animals that received the CEE alone. Coadministration of the CEE with AFB1 showed a considerable protecting action against the oxidative stress deterioration induced by AFB1 intoxication (Table 4).

Concerning the effect of AFB1 on DNA (Fig. 3), animals intoxicated with AFB1 showed a significant rise in the ratio of fragmented DNA compared to the control rats. Cotreatment of rats with the CEE in line with AFB1 induced a moderate protective

Table 3. Serum tumor and immunoinflammatory markers of CEE- and AFB1-treated rats.

Parameter	Control	CEE	AFB1	AFB1+CEE
CEA (ng/ml)	1.66 ± 0.06	1.64 ± 0.16	7.57 ± 0.72 ^a	3.13 ± 0.08 ^b
AFP (ng/ml)	0.62 ± 0.19	0.67 ± 0.07	2.81 ± 0.11 ^a	1.69 ± 0.44 ^b
IL-1β (ng/ml)	7.6 ± 1.65	7.8 ± 1.44	30.2 ± 2.41 ^a	18.6 ± 1.89 ^b
TNF-α (μg/ml)	10.4 ± 0.24	11.08 ± 0.94	41.9 ± 5.32 ^a	27.5 ± 0.65 ^b

Values are mean ± SE for eight rats.

AFB1: aflatoxin B1; CEE: *Chelidonium majus* ethanolic extract.

^aCompared with the control group while ^bcompared with the AFB1 group at $p \leq 0.05$.

Table 4. Hepatic oxidative stress markers of CEE- and AFB1-treated rats.

Parameter	Control	CEE	AFB1	AFB1+CEE
MDA (nmol/g tissue)	154 ± 8.1	152 ± 7.7	354 ± 12.4 ^a	208 ± 9.9 ^b
NO (μmol/g tissue)	60 ± 4.2	59 ± 5.8	183 ± 8.7 ^a	84 ± 6.6 ^b
GSH (mmol/g tissue)	5.11 ± 0.44	5.21 ± 0.46	2.11 ± 0.32 ^a	4.3 ± 0.47 ^b
GPx (U/g tissue)	5,930 ± 106	5,870 ± 117	2,174 ± 94 ^a	4,142 ± 109 ^b
SOD (U/g tissue)	9,684 ± 151	9,768 ± 125	4,572 ± 127 ^a	8,022 ± 105 ^b
CAT (U/g tissue)	91 ± 2.9	93 ± 4.2	47 ± 3.9 ^a	77 ± 6.6 ^b

Values are mean ± SE for eight rats.

AFB1: aflatoxin B1; CEE: *Chelidonium majus* ethanolic extract.

^aCompared with the control group while ^bcompared with the AFB1 group at $p \leq 0.05$.

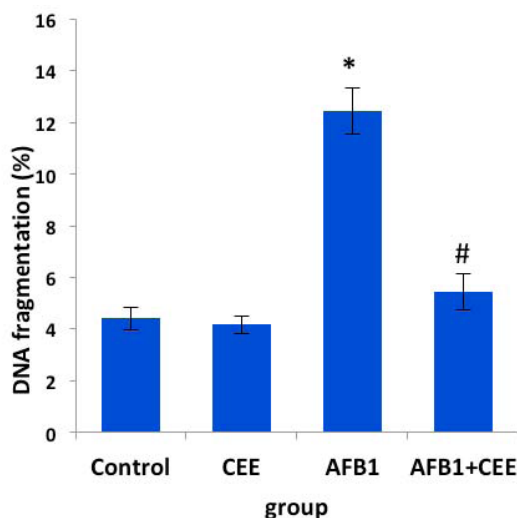


Figure 3. Effect of oral ingestion of the CEE on hepatic DNA fragmentation. Values are mean ± SE for eight rats. *Compared with the control group while #compared with the AFB1 group at $p \leq 0.05$. AFB1: aflatoxin B1; CEE: *C. majus* ethanolic extract.

effect against the AFB1-induced DNA damage; this was achieved from the significant drop in the fragmented DNA amount.

The hepatic histopathological findings of the control group, as well as the CEE-treated one, revealed normal hepatocytes architecture (Fig. 4A and B). In contrast, the liver sections of the

AFB1-intoxicated rats showed damaged livers with the dense portal and lobular inflammation associated with fibrosis (Fig. 5), while sections obtained from rats that received the CEE besides AFB1 showed liver tissues having preserved architecture and negligible sight of inflammation (Fig. 6).

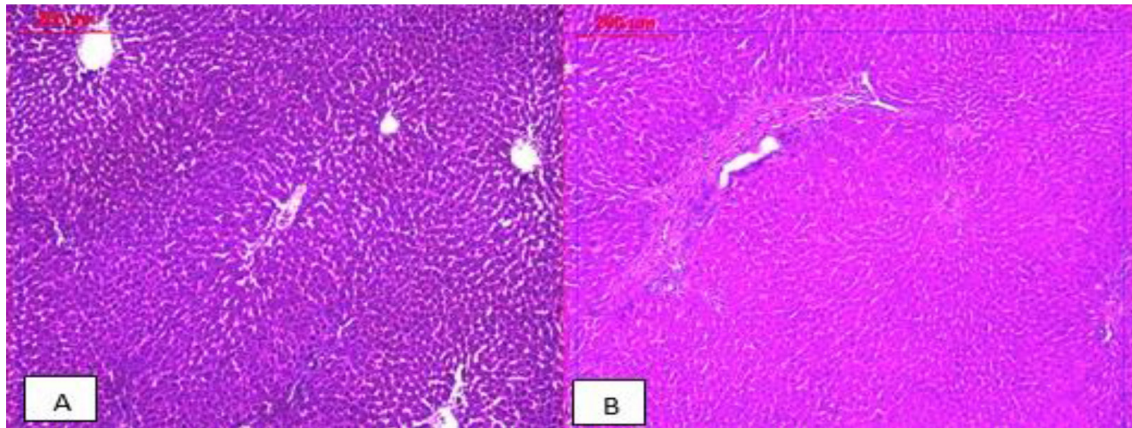


Figure 4. Photomicrographs of the livers' sections of healthy control rats (A) and rats orally administrated with the *C. majus* ethanolic extract (B). Both sections show preserved architecture with normal hepatocytes and central vein ($\times 100$).

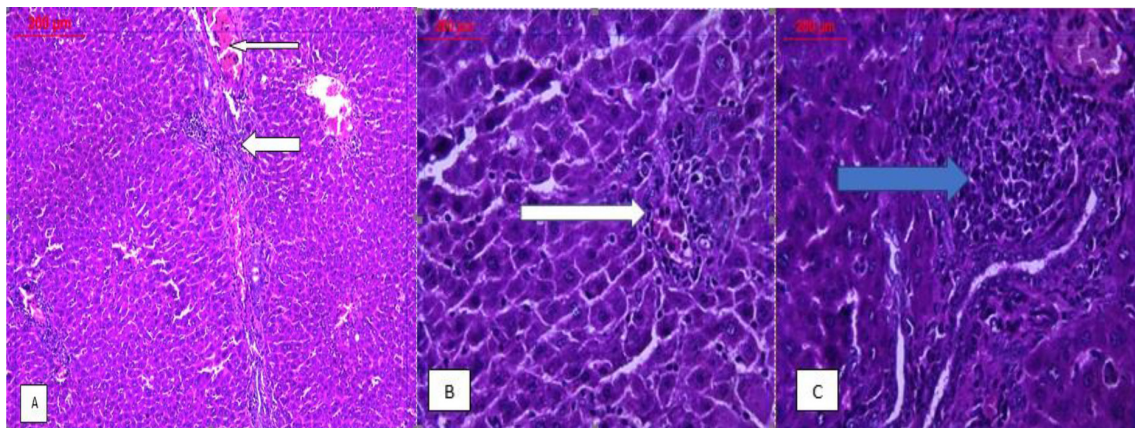


Figure 5. Photomicrographs of livers' sections of rats intoxicated with AFB1; (A) low power ($\times 100$) view demonstrating the portal inflammation (thick arrow), central vein congestion and portal fibrosis (thin arrow), (B) high power ($\times 400$) view showing damaged liver with lobular inflammation (white arrow), and (C) another high power ($\times 400$) view illustrating the dense portal inflammation associated with fibrosis (blue arrow).

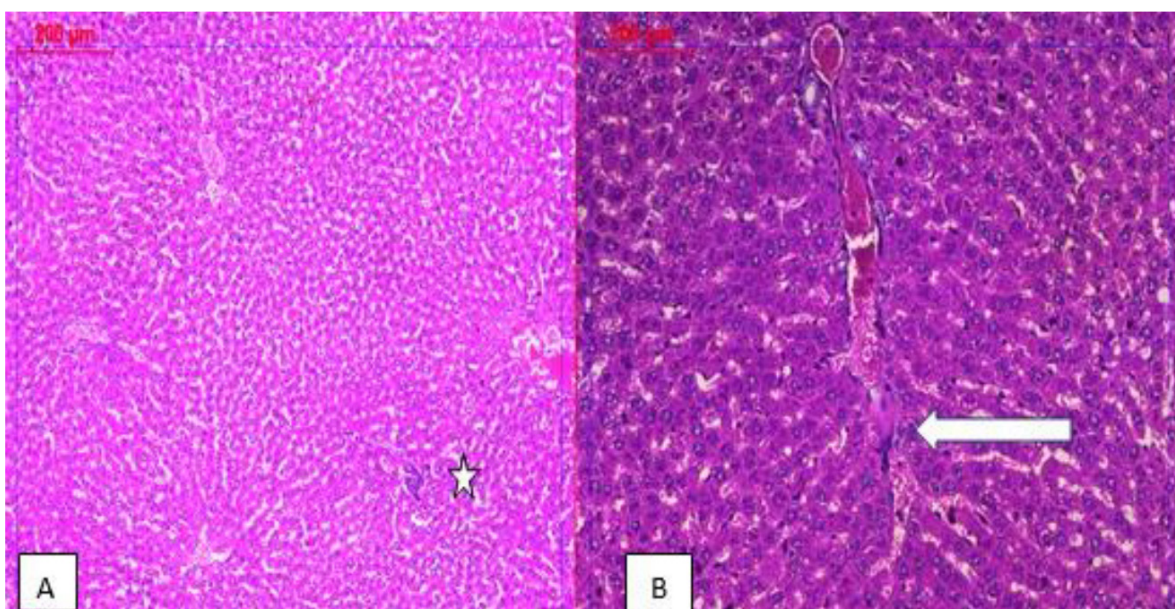


Figure 6. Photomicrographs of livers' sections of rats that received the *C. majus* ethanolic extract in combination with AFB1; low power ($\times 100$) view showing liver tissue with preserved architecture and negligible inflammation within normal limits (white star) (A); medium power ($\times 200$) view illustrating that mild vascular congestion was also noted (B).

DISCUSSION

The liver is the major site of xenobiotic metabolism (Wang *et al.*, 2007). It is considered one of the important body organs, where it detoxifies or eliminates toxins and foreign substances (Khan, 2006). In the present study, AFB1 intoxication induced liver dysfunction that appeared from the significant elevation in the activities of the serum enzymes ALT, AST, ALP, and GGT compared to the control group. These enzymes are liberated into the bloodstream after cellular damage, so the leakage of these enzymes into the blood may be related to the injury in the hepatic cell membrane (Recknagel *et al.*, 1991). These results are in agreement with the findings of Eftekhari *et al.* (2018) and Monmeesil *et al.* (2019). Moreover, the increases in urea and creatinine levels in the AFB1-treated group demonstrated the deleterious and stressful effect on kidney tissue; this result is in line with those obtained by Hamzawy *et al.* (2013).

AFB1 toxicity mainly targets the liver because AFB1 is metabolized largely in the liver, by the cytochrome P450 enzyme system, to form reactive 8,9-epoxide (Choy, 1993; McLean and Dutton, 1995; Sergent *et al.*, 2008), and it is also metabolized to some extent in extrahepatic tissues (Sergent *et al.*, 2008). AFB1 toxicity is mainly attributed to the binding of the epoxide to DNA to form DNA adducts. The induction of high levels of DNA adducts leads to cellular injury (Ellis, 2009; McLean and Dutton, 1995) and causes hepatic damage such as hemorrhage, cellular necrosis, injury of intrahepatic bile ducts, and elevation in liver enzymes (Rawal *et al.*, 2010).

Treatment of AFB1-intoxicated rats with the CEE significantly reduced the markers of liver and kidney functions. This indicated the hepato- and renoprotective potential of the CEE, which may be due to the presence of phytochemicals. Previously, it has been shown that treatment with the *C. majus* extract could reduce the increased values of transaminases and bilirubin induced by carbon tetrachloride exposure (Mitra *et al.*, 1992 and 1996).

We observed in our study significant increases in the concentrations of triglycerides, total cholesterol, and LDL-C concomitant with a significant decrease in HDL-C level in rats given AFB1 alone, while treatment with the CEE besides AFB1 reversed these values and returned these levels towards those of the control values. The marked increases in triglycerides, cholesterol, and HDL-C reported in the AFB1-intoxicated group and the significant reduction in HDL-C level agree with those reported previously by Abdel-Wahhab *et al.* (2007 and 2010). The increments in serum levels of cholesterol and triglycerides are correlated with hepatic dysfunction and biliary obstruction (Edrington *et al.*, 1995). Also, Marcus and Milton (1982) reported that cholesterol concentration is linked with metabolic functions, which are affected by the liver and kidney integrity.

In the current study, AFB1 significantly increased CEA and AFP levels, and treatment with the CEE besides AFB1 reduced these levels. CEA and AFP are specific markers for liver cancer. AFB1 enhances the formation of reactive oxygen species that cause DNA damaging, which leads to carcinogenesis (Yang *et al.*,

2000). On the other hand, AFB1 induced hepatocarcinogenesis and DNA damage by promoting apoptosis through the increment in caspase-3, Bax, and p53 expression and the decrement in Bcl-2 expression (Liao *et al.*, 2014). The elevation in AFP and CEA levels is in accordance with a previous study which indicated that AFB1 ingestion could increase AFP and CEA levels in rats (Abdel-Wahhab *et al.*, 2010).

The participation of inflammatory mediators in mycotoxin toxicity has been reported (Akinrinmade *et al.*, 2016). IL-1 β and TNF- α levels exhibited a marked increase in the serum of AFB1-intoxicated animals while treatment with CEE reduced the levels of these cytokines implying that the CEE has an anti-inflammatory activity against AFB1 toxicity. Previous studies revealed that *C. majus* reduces certain cytokines' synthesis and modulates the values of immune cells and immunoglobulins in mice (Lee *et al.*, 2007), and it was reported that the crude extract and its purified compounds derived from *C. majus* have anti-inflammatory properties in *in vivo* and *in vitro* studies (Biswas *et al.*, 2002). A similar elevation in TNF- α and IL-1 β levels in animals receiving AFB1 has been reported in a previous study (Hamzawy *et al.*, 2013). The increased IL-1 β level manifests the presence of inflammation (Safieh-Garabedian *et al.*, 1995). TNF- α has an important role in carcinogenesis (Balkwill, 2009; Moor *et al.*, 1999) and in the induction of hemorrhagic necrosis of the cells challenged with DNA damage (Balkwill, 2009). This suggestion was asserted by the results of DNA fragmentation in our study, where the fragmentation and damage of liver DNA markedly increased in AFB1-intoxicated animals. AFB1 toxicity may increase TNF- α and IL-1 amounts via activation of caspase-3 (an apoptotic marker) (Meik *et al.*, 2001).

Previously, it was stated that the elevation in oxidative stress results in the damage of liver DNA that contributes to apoptosis and necrosis of the hepatic cells (Mukhopadhyay *et al.*, 2011). This damage is achieved as a distinctive event in cell apoptosis (Mourad *et al.*, 2017). Administration of the CEE to the AFB1-treated animals showed a significant decrease in hepatic DNA degeneration as compared to the AFB1-intoxicated group. The reduction of DNA deterioration by CEE treatment may be due to the antioxidant capacity of this extract.

As expected, AFB1 administration produced a state of oxidative stress. This appeared from the significant elevation in nitric oxide and MDA and the significant reduction in the values of glutathione and the antioxidant enzymes (GPx, SOD, and CAT) in the AFB1 group compared to the control group. The antioxidant enzymes which include GPx, SOD, and CAT play an important role in the conversion of oxidative products into harmless molecules (Nimse and Pal, 2015). Our results agreed with the results of El-Kady *et al.* (2010) and Kanbur *et al.* (2011) who reported that the oxidative stress may be due to the direct action of AFB or by its reactive 8,9-epoxide metabolites and the free radicals generated during the formation of these metabolites. AFB1 increases free radicals' production and elevates lipid peroxidation and hence leads to DNA, lipid, and protein damage and loss of biological functions such as membrane permeability and calcium influx and consequently affects the stability of DNA that leads to cancer (Klaunig *et al.*, 2011; Smela *et al.*, 2001).

The histopathological examination showed damaged livers with the dense portal and lobular inflammation associated with fibrosis in the group that ingested AFB1 similar to those observed previously (Hamzawy *et al.*, 2013; Poapolathep *et al.*, 2015). Administration of the CEE in combination with AFB1 reduced these pathological changes confirming the protective effect of the CEE on the hepatic cells from degenerative effects induced by the free radicals generated during AFB1 metabolism.

The hepatoprotective effects of the CEE against aflatoxicosis were demonstrated in the current study by suppressing oxidative stress and inflammation that cause DNA and membrane damage. Since the CEE has antioxidant compounds which can inhibit lipid peroxidation and prevent the exhaustion of the endogenous glutathione and antioxidant enzymes, this in turn prevents DNA damage and also improves liver integrity and function and consequently improves lipid metabolism. Another mechanism that participates in the protection against aflatoxicosis is the inhibition of cytochrome P450 enzymes or enhancing GST-mediated in AFB1 detoxification (Hayes *et al.*, 1998), since Liu *et al.* (2019) proved that chelidonine which is a major constituent of *C. majus* inhibits cytochrome P450 enzymes mediating the conversion of AFB1 to the reactive 8,9-epoxide.

Based on the obtained results, the CEE showed strong antioxidant activity demonstrated from testing of DPPH scavenging activity and further confirmed by the data of reducing power ability. This may be due to the rich and diverse phytochemicals present in the extract. The phytochemical analysis of the CEE revealed the presence of phenolic compounds such as chlorogenic acid, gallic acid, syringic acid, ferulic acid, vanillin, caffeic acid, and coumaric acid. These phenolic compounds are considered strong antioxidants which directly scavenge free radicals and inhibit lipid peroxidation (Lee *et al.*, 2003) thus preventing these free radicals from reaching and assaulting the critical molecules such as lipids, DNA, and proteins. For instance, chlorogenic acid, ferulic acid, and vanillin have DPPH and ABTS^{•+} free radical scavenging ability (Yang *et al.*, 2018). In addition, some of the phenol derivatives, such as ferulic acid, caffeic acid, and coumaric acid, have been found to contain decent antioxidant activities (Andreasen *et al.*, 2001).

Other alkaloid compounds have been identified in the ethanolic dry extracts of *C. majus*, such as chelidonine, protopine, and coptisine (Hiller *et al.*, 1998). It was reported that nano chelidonine administration after cadmium chloride toxicity in mice markedly diminished lipid peroxidation and restored the levels of triglyceride, cholesterol, glutathione, and antioxidant enzymes (Paul *et al.*, 2013). Besides that, *C. majus* coptisine has been found to reduce oxidative phosphorylation in rats' liver mitochondria (Kamins'kyi *et al.*, 1999).

Our results showed that the CEE, when administered alone, did not significantly affect all studied parameters as compared to normal values. These results demonstrate the safety of this extract on liver tissue. Our findings are consistent with those obtained by Mazzanti *et al.* (2009 and 2013) who reported that the prolonged ingestion of the *C. majus* extract by Wistar rats appeared to be safe and they excluded cellular toxicity,

cholestasis, inflammation, and other diseases that could influence liver function. Conversely, the *C. majus* alkaloid induced hepatotoxic effects in some human cases (Tarantino *et al.*, 2009; Teschke *et al.*, 2011). It has been hypothesized that these toxic effects are attributed to the simultaneous treatment with *C. majus* and other pharmaceutical products such as hormones (estrogens and thyroid hormones) and anti-inflammatory drugs (Benninger *et al.*, 1999).

CONCLUSION

The present study has demonstrated that the *C. majus* ethanolic extract exhibits pronounced antioxidative activity making it promising as a hepatoprotective agent against hepatotoxicity induced by aflatoxins. At the same time, it is important to point out that the extract dose applied in the present study is safe at that used dose and duration; therefore, further studies are needed to explore the possible toxicity of extra doses.

ACKNOWLEDGMENTS

The authors thank the National Research Centre (NRC) for its unlimited allowance and for letting the group use the laboratories and instruments during the development of the study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICT OF INTERESTS

The authors declare they have no conflicts of interest.

FUNDING

There is no funding to report.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

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How to cite this article:

Gomaa HF, Fadl NN, Elmashad WMA, Abouelfadl DM, Mannaa FA, Abdel-Wahhab KG. Protective efficiency of *Chelidonium majus* extract against hepatotoxic and DNA changes induced by aflatoxin B1. *J Appl Pharm Sci*, 2022; 12(03):140–149.