

## Protective Effects of Captopril against Aflatoxin B1-Induced Hepatotoxicity in Isolated Perfused Rat Liver

Amir Moghaddam-Jafari,\*<sup>1</sup> Mohammad Kazem Koohi,<sup>1</sup> Mahmood Ghazi-Khansari,<sup>2</sup> Parvin Pasalar<sup>3</sup>

1. Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Medical Biochemistry, Tehran University of Medical Sciences, Tehran, Iran

| Article information  | Abstract  |
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| <p>Article history:<br/>Received: 23 Sep 2012<br/>Accepted: 30 Oct 2012<br/>Available online: 21 Jan 2013<br/>ZJRMS 2014; 16(2): 29-32</p> <p>Keywords:<br/>Hepatotoxicity<br/>Aflatoxins<br/>Captopril</p> <p>*Corresponding author at:<br/>Department of Toxicology,<br/>Faculty of Veterinary<br/>Medicine, University of<br/>Tehran, Tehran, Iran<br/>E-mail:<br/>moghadamjafari@gmail.com</p> | <p><b>Background:</b> The liver is the major target organ for aflatoxin B1 (AFB1). Ingestion of aflatoxin causes hepatotoxicity. In this study, captopril as new agent to help the hepatotoxicity induced by aflatoxin was suggested.</p> <p><b>Materials and Methods:</b> The isolated perfused rat liver (IPRL) was chosen for evaluating hepatic function. Sixteen rats were divided randomly into four experimental groups: control, captopril, AFB1 and AFB1 + captopril. The level of glutathione content and lipid peroxidation, as marker of oxidative stress and lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST) activities and pH of the perfusate medium were measured.</p> <p><b>Results:</b> There was a significant decrease in lipid peroxidation and same increase was observed in glutathione level. Treatment with captopril also modulated the enzymes activity and pH of perfusate.</p> <p><b>Conclusion:</b> This study showed that captopril protects the hepatotoxicity induced by AFB1. Therefore, this drug may provide an effective new strategy to reduce of aflatoxins toxicity.</p> |

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### Introduction

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most hepatotoxic being aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and three structurally similar compounds namely aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) and aflatoxin G<sub>2</sub> (AFG<sub>2</sub>). Ingestion of aflatoxin in contaminated food and feed is known to cause hepatotoxicity [1]. Various reports suggest the role of oxidative stress in hepatotoxicity. Intracellular reactive oxygen species (ROS) like superoxide anion, hydroxylradical and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is produced by cytochrome P450 during the metabolic processing of AFB<sub>1</sub> in the liver. These species attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis.

Peroxidative damages induced in the cell are encountered by elaborate defense mechanisms, including enzymatic and nonenzymatic antioxidants. Several compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of ROS and other free radicals. During recent years, diverse biological and synthetic chemical structures are studied with hepatoprotective effects, for instance, angiotensin-converting enzyme (ACE) inhibitors, which have both an antihypertensive and cardioprotective action,

are commonly used in the treatment of hypertension and most forms of heart failure.

The beneficial effects of ACE inhibitors were thought to be primarily due to the inhibition of angiotensin II formation. A number of studies have shown that ACE inhibitors like captopril improve hepatic damages like oxidative stress. Captopril is rapidly absorbed from the gastrointestinal tract, with detectable plasma concentrations apparent as early as 15 minutes. The extent of absorption is between 60 and 75% of an oral dose and peak plasma concentrations occur after approximately one hour [2]. Treatment with captopril showed to increase antioxidant enzymes and nonenzymatic antioxidants defenses [3]. This effect appears to be attributable to the Sulfhydryl groups (SH) in the compound which may be due to captopril abilities to scavenge reactive oxygen species [4].

To address the protective effects of captopril on AFB<sub>1</sub> toxicity, we have established an isolated perfused rat liver (IPRL). IPRL is a useful experimental system for evaluating hepatic function without the influence of other organ systems, undefined plasma constituents, and neural-hormonal effects. Hepatic architecture, cell polarity, and bile flow are preserved in the IPRL. Furthermore, the IPRL allows repeated sampling of the live to different concentrations of test chemicals.

## Materials and Methods

**Animal selection:** In this experimental interventional study, male Albino Wistar rats (200-300 g) were obtained from the vivarium section of the department of pharmacology, Tehran University of Medical Sciences, Tehran, Iran. Animals were housed in stainless steel cages covered by wood chips in a temperature controlled room (22°C) and subjected to a 12 hour light-dark cycle. They were allowed to have free access to tap water and standard diet for the duration of the study. All experiments were performed according to the institutional guidelines for animal care and use.

**Study groups:** Sixteen rats were allocated to four treatment groups (4 rats each): (1) Control (KHBB) without additional agents, (2) AFB<sub>1</sub> (1ppm), (3) Captopril (1mM), (4) Pretreatment group: after 30 min of stabilization with Krebs buffer, captopril (1mM) was added to the perfusion for 10 min. Then AFB<sub>1</sub> (1ppm) was added to the perfusion fluid. The doses of AFB<sub>1</sub> and captopril were chosen based on our experience in our previous investigation that determined the hepatotoxic dose of aflatoxin (led to a release of cytosolic enzymes and lipid peroxidation of hepatocytes) and the submaximal concentration of captopril (the concentration without any effect on hepatic viability like control group). AFB<sub>1</sub> was dissolved in dimethyl sulfoxide (DMSO) and further diluted with distilled water to the required concentration. The final gavage solution of AFB<sub>1</sub> contained 1% DMSO. Captopril was dissolved in KHBB for reaching to desired concentration.

To evaluate the effects of aflatoxin and captopril on the liver, perfusate samples were collected after 120 min of perfusion. At the end of perfusion, the middle lobe of liver was obtained to thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) assays. They were frozen in liquid nitrogen until further analysis.

**Chemicals:** All reagents were of the highest quality available. Aflatoxin B<sub>1</sub> and captopril were obtained from sigma (St. Louis, MO). Other chemicals used in this study were from Merck, Germany.

**Liver surgery and perfusion:** Animals were anesthetized by intraperitoneal injection of 90 mg/kg ketamine HCl and 10 mg/kg xylazine. The abdomen was opened through a midline and one transversal incision and the bile duct was cannulated. Heparin sodium solution was injected via the abdominal vena cava to prevent blood clotting. The portal vein was cannulated and the liver was perfused with Krebs-Henseleit buffer (KHBB) at a flow rate of 25 ml/min. Glucose (0.1% w/v) and bovine serum albumin (BSA) were added to a final concentration. After cannulating the superior vena cava through the right atrium of the heart, the inferior vena cava was ligated and the liver was carefully removed.

As rapidly as possible, the excised liver was connected to the recirculating perfusion system (for details of the perfusion system see reference [5]). In most experiments, the liver was perfused with 200 ml krebs- Henseleit buffer supplemented with 0.25% BSA (w/v) at a flow rate of 40 ml/min at 37°C in a recirculating system. The perfusate

was collected from a polyethylene tube (PE-240). In all experiments, the liver was allowed to equilibrate in the perfusion system for 30 min before treatment was initiated.

**Enzyme measurement:** Lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST) activities in the perfusion medium were measured using kits purchased from Teb Gostaran Hayan (Tehran, Iran) as UV methods.

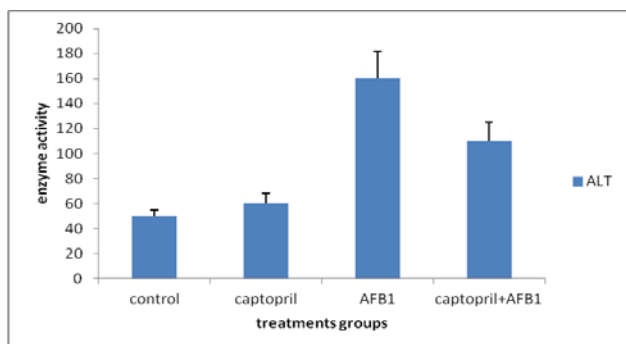
**Lipid peroxidation:** Lipid peroxidation was determined by the amount of thiobarbituric acid reacting substances (TBARS). The perfused liver was used for obtaining a 10% homogenate in cooled 0.15 M KCl. This was centrifuged for 10 min at 600g to obtain a post-nuclear homogenate. The amount of TBARS in the post-nuclear homogenates (mg protein/ ml 0.15M KCl, pH=7.2) was measured according to previous method [6]. The mixture of 0.6 ml of 2.8% trichloroacetic acid + 5N HCl + 2% thiobarbituric acid in 50 mM NaOH (2:1:2 v/v) was added to the homogenates. The samples were heated a 100°C for 15 min. The absorbance was read at 532 nm against appropriate blanks. The values were expressed in nmoles malondialdehyde per mg protein, using a molar extinction coefficient of  $1.56 \times 10^5$ /M cm.

**Determination of total glutathione (GSH) content:** Glutathione (GSH) content was determined as described by Kuo and Hook [7]. Briefly, the tissue was homogenized in 20% (w/v) trichloroacetic acid and centrifuged at 6000 rpm for 20 min. To determine GSH concentration in the tissue, an aliquot of the deproteinized supernatant fraction was added to 2 ml of 0.3 M Na<sub>2</sub>HPO<sub>4</sub> solution followed by the addition of 0.5 ml of 0.04%, 5, 5-dithiobis-[2-nitrobenzoic acid] dissolved in 10% sodium citrate. The absorbance at 412 nm was measured immediately after mixing and the GSH values were determined by extrapolation from the standard curve. Total protein was determined in a sample of liver tissue homogenate after 120 min of perfusion by Bradford method.

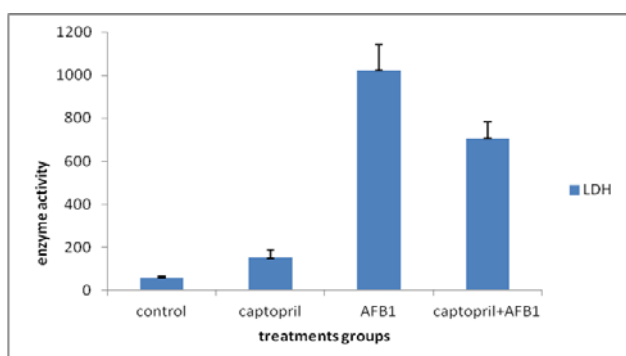
**Statistical analysis:** The results analyzed using SPSS-17. All values were expressed as the mean±standard error (SEM). Data were analyzed by repeated measures analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons. The differences were considered significant at  $p < 0.05$ .

## Results

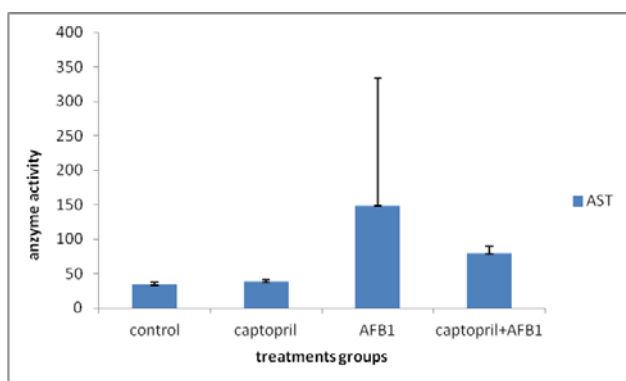
**Enzyme release:** To assess liver toxicity, enzymes (AST, ALT and LDH) leakage into perfusion medium was monitored throughout the experimental studies. In control and captopril experiments without AFB<sub>1</sub>, the release of these enzymes into the perfusate was small. The addition of AFB<sub>1</sub> to the perfusate resulted in a statistically significant increase of AST, ALT and LDH releases into the perfusate at the end of experiment (Fig. 1, 2 and 3). This increase in enzyme releases was attenuated in the presence of captopril in pretreatment group. The analysis of variance showed significant effects for AST and ALT ( $p < 0.05$ ), while this incidence was lower for LDH fall in pH induced by AFB<sub>1</sub> in pretreatment group.



**Figure 1.** The mean of ALT activity (U/L) after 120 Min. Concurrent of administration prevent an increase in ALT activity ( $p \leq 0.05$ )



**Figure 2.** The mean of LDH activity (U/L) after 120 Min. Concurrent of administration prevent an increase in LDH activity. The activity of enzyme in group of captopril + AFB1 decreased compared with group of AFB1



**Figure 3.** The mean of AST activity (U/L) after 120 Min. Concurrent of administration prevent an increase in AST activity. This different was statistically significant ( $p \leq 0.05$ )

**Table 1.** Effects of captopril on level of lipid per-oxidation and glutathione content

| Concentration                                 | Oxidative stress parameters*        |                                   |
|---|-------------------------------------|-----------------------------------|
|   | Lipid per-oxidation (nmol/ $\mu$ g) | Glutathione level (nmol/ $\mu$ g) |
| Control group                                 | 0.405 $\pm$ 0.134                   | 569 $\pm$ 85.1                    |
| 1ppm AFB1 (positive control)                  | 2.172 $\pm$ 0.17                    | 91.71 $\pm$ 8.2                   |
| 1 mM captopril                                | 0.65 $\pm$ 0.13                     | 501 $\pm$ 32.2                    |
| 1ppm AFB1+1 mM captopril (Pretreatment group) | 0.99 $\pm$ 0.2                      | 402 $\pm$ 38.2                    |

\* Each value represents the mean  $\pm$ SE per group. The glutathione content and total lipid peroxidation markedly changed under the experimental condition ( $p \leq 0.05$ )

**Lipid peroxidation:** The TBARS concentration was significantly greater in tissue homogenate treated with AFB<sub>1</sub> alone compared to control, captopril and AFB<sub>1</sub> + captopril treated groups,  $p < 0.05$  (Table 1).

**GSH concentration:** As shown in table 1, GSH levels of AFB<sub>1</sub>-treated livers were significantly lower than those of control, captopril and AFB<sub>1</sub> + captopril treated groups ( $p < 0.05$ ).

**pH of perfusate:** In the control and captopril experiments, pH of the perfusate medium lay between 7.38 and 7.33 over the whole perfusion period. Statistically significant reductions of pH occurred after perfusion with AFB<sub>1</sub>. Captopril reduced the fall in pH induced by AFB<sub>1</sub> in pretreatment group.

## Discussion

Major scientific interest in the poisonings is therapeutic strategies. In this study, we used the IPRL system to investigate the ameliorative effects of captopril in aflatoxin B<sub>1</sub> induced enzyme changes and peroxidative hepatic damages. The findings confirmed that captopril has a protective effect against aflatoxins toxicity with different pathways.

AFB<sub>1</sub> is readily absorbed from the gut of the rat and can be detected in the liver of the poisoned rat within 30 min [8]. It induces hepatotoxicity through oxidative stress [9]. Malondialdehyde (MDA) is a product of lipid peroxidation that has been used as an indicator in oxidative damages. Several authors reported that mycotoxins can cause lipid peroxidation. In present study, there was a significant decrease in MDA by captopril treatment. Our data of the antioxidant effects of captopril were in agreement with other researchers who observed a significant valuable effect of captopril on hepatic oxidative stress induced by arsenite in rats [10].

Diabetes induces oxidative stress. In a study, captopril could modulate the production of ROS in circulating neutrophils in diabetic people [11]. Furthermore, captopril increases endogenous antioxidant enzyme catalase and superoxide dismutase (SOD) activity in pressure-overload rats [12]. Conjugation of AFB<sub>1</sub> to glutathione (mediated by glutathione transferase) and its subsequent excretion is regarded as an important detoxification in animals. In most animals, AFB<sub>1</sub> is detoxified primarily by glutathione S-transferases (GSTs) which attach, by nucleophilic substitution, the endogenous thiol glutathione (GSH) to the electrophilic AFB<sub>1</sub> to form the non-toxic and safely-excreted AFB<sub>1</sub>-GSH adduct [13]. Resistance to AFB<sub>1</sub> toxicity has been interpreted in terms of levels and activities of these detoxifying pathways. GSH is known to play an important role in protecting cells from oxidant-induced tissue injury. Data suggested that the bioactive properties of biological thiols (GSH, cystein and captopril) might contribute to their effects of antioxidant activities. In our study, GSH showed an increasing after treating with captopril. Therefore, captopril could confer resistance to AFB<sub>1</sub>. According to this hypothesis, we have also shown previously that paraquat (PQ) lung toxicity (thiol-oxidizing agent) can be

prevented by chronic captopril treatment in vivo mice. Moreover, we reported that captopril alleviates the biochemical alterations caused by PQ in isolated perfused rat lung and also captopril ameliorates toxicity induced by PQ in mitochondria isolated from the rat liver [4].

There was a significant reduce in pH observed in aflatoxicosis. Due to structural damages to the liver, urea was released into the perfusate. Therefore, we found the decrease in pH in isolated perfused rat liver. Captopril modulated the changes of pH that induced by AFB<sub>1</sub>. pH slowly recovered by captopril treatment. Free radicals can cause damage in structural components in cells and tissues. This ultimately results structural damage [14]. The protective components are classified as the antioxidant such as captopril can effectively removes the excess levels of free radicals. Therefore, captopril will prevent damages of AFB<sub>1</sub> to the structure of liver cells by this pathway.

The levels of ALT, AST, and LDH are main indices of liver injury [15]. Therefore, in this experimental these general marker enzymes of liver were estimated by standardized methods. Other general signs of aflatoxicosis are changing of enzyme activity of liver. Serum ALT, AST, LDH, marker enzymes for hepatocellular necrosis, was significantly elevated in aflatoxicosis [13]. The

effects of captopril on various enzyme activities of the liver are described in figure 1, 2 and 3. In this study, the enzymes activities are significantly normalized. Our similar results have also been observed. A study indicates that LDH activity levels were also reduced in patients diagnosed with hypertension by captopril treatment [16].

Collectively, captopril would be advised to prevent the toxicity of AFB<sub>1</sub>. Many of population are exposed to toxic doses of AFB<sub>1</sub>. Captopril seems to be a potentially useful agent to decrease AFB<sub>1</sub> and other component that have similar toxicity mechanisms.

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### Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of Interest

The authors declare no conflict of interest.

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