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The evaluation of curcumin effects on some acute phase proteins in aflatoxin B1 applied rats

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Abstract: This study was designed to determine the effects of curcumin on some acute phase proteins in rats treated with aflatoxin B1. In this study, healthy 38 male Wistar Albino rats were used. The animals in control group were given food and distilled water. The animals in DMSO group were orally given 1 ml 10% DMSO daily for 60 days, animals in Cur group was orally given 300mg/kg curcumin dissolved in 10% DMSO daily for 60 days. Animals in AFB1 group were orally given 250µg/kg aflatoxin B1 dissolved in 10% DMSO daily for 60 days. Animals in AFB1+Cur group was orally given 250µg/kg aflatoxin B1 dissolved in 10% DMSO and 300mg/kg curcumin dissolved in 10% DMSO daily for 60 days. In blood samples taken from all animals, fibrinogen, prothrombin, albumin and CRP levels were determined. In this study, fibrinogen, prothrombin and CRP levels with AFB1 group were found to be significantly higher than the control group ($p<0.05$). In the AFB1+Cur group, fibrinogen and CRP levels were lower than in the aflatoxin group ($p<0.05$). In the study, it was observed that albumin level in rats in AFB1 group significantly decreased compared to the control group ($p<0.05$) and this difference was found to be eliminated depend on the application of curcumin together with aflatoxin B1 ($p<0.05$). In conclusion, our findings regarding the ameliorating effects of curcumin on acute phase protein abnormalities caused by aflatoxicosis will contribute to future research.

Keywords: Aflatoxin, curcumin, fibrinogen, prothrombin, albumin, rats.

INTRODUCTION

Aflatoxins are secondary metabolites and these metabolites are synthesized by *Aspergillus flavus* and *Aspergillus parasiticus* (Aggarwal and Harikumar, 2009, Aggarwal, Kumar *et al*., 2003). The naturally occurring aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2) could easily contaminate food. Aflatoxin B1 (AFB1) is the most common and most toxic; also, the liver is its crucial target organ (Aggarwal *et al*., 2007, Ahmadi 2010, Anand *et al*., 2008, Azuine and Bhide, 1992).

It is stated that aflatoxin B1 causes mutations in the host gene structure due to its toxic and mutagenic properties and causes hepatocellular necrosis by metabolically active and reactive intermediates (Ates, *et al*., 2022, Hatipoglu and Keskin, 2022) The liver is responsible for many complex and vital functions such as detoxi fication, bile formation, carbohydrate and fat metabolism, urea formation, inactivation of polypeptide hormones in the organism. Therefore, it is the target organ of the toxic effects of various compounds (Bansal *et al*., 2011, Hatipoğlu and Keskin, 2022). In addition, the liver plays a significant role in the acute phase response (Bar-Sela *et al*., 2010). Numerous physiological mechanisms provide the continuation of homeostasis. Poisoning, infections, mechanical or thermal injuries occurred by different compounds, including aflatoxin B1, leading to an organism's complex, early and general response called acute phase response (Bowman, 2014). The acute phase response includes changes that ensure survival during the emergency period after injury. In an organism exposed to various traumas, there is seen increment in the synthesis of group plasma proteins synthesized in the liver and called acute-phase proteins (Cray, 2012, Cray *et al*., 2009, Davalos and Akassoglou, 2012). This acute response increases the concentrations of proteins such as ceruloplasmin, C-reactive protein, amyloid A, haptoglobin, α-acid glycoprotein, α1-antitrypsin and α-anti chymotrypsin are seen (Dowton and Colten, 1988). Although the functions of some of these increased proteins in inflammation are not fully known, it is suggested that protease inhibitors such as α-acid glycoprotein, α1-antitrypsin and α-anti chymotrypsin act against the damaging effects of proteolytic enzymes released from cells during the inflammation (Duvoix *et al*., 2005, El-Agamy, 2010). The increase of ceruloplasmin level may provide antioxidant protection. The rise of complement factors serves to form opsonin and chemotactic elements and to counteract foreign matter. The rise in coagulation parameters such as plasminogen, Factor VIII, prothrombin and fibrinogen is intended to accelerate coagulation formation and prevent bleeding in the inflammation area (El-Agamy, 2010) . The increase in the synthesis of acute-phase proteins in the liver during the acute response is accomplished by an increase in the transcription of these proteins. It is increased serum concentrations (Davalos and Akassoglou, 2012, El-Barbary, 2016).

Natural chemicals' chemopreventive properties have attracted extensive attention in the last two decades (Tras

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et al., 2022). Curcumin is one of the most researched chemicals due to its possible medicinal actions and low toxicity concerns. Since ancient times, curcumin has been utilized as a folk medicine (Farooqui and Farooqui, 2019). Curcuminoids (curcumin I, 75%; curcumin II, 20%; and curcumin III, 5%) obtained from the rhizomes of Curcuma longa (turmeric) are the active components (El-Mahalaway, 2015). There is various reports related to the effect of curcumin on enzymes such as glutathione S-transferase, epidermal growth factor receptor, cytochrome 1A1 (CYP1A1), CYP3A4, protein kinase C, 5-LOX, COX-2 protein kinases like IκB kinase, , human epidermal growth factor receptor 2, AKT, , transcription factors like nuclear factor kappaB, activator protein-1, Janus activated kinase, nuclear factor E2-related factor 2 in cell culture and animal studies. Curcumin's influence on signal transducer and transcription factor activation refers to its powerful antioxidant, anti-inflammatory and anti-proliferative properties (El-Nekeety, Abdel-Azeim *et al*., 2014, Fouad, Mamer *et al*., 2001, Fulop, 2007, Gabay and Kushner, 1999). The objective of the present study was to investigate the effects of curcumin on some acute-phase proteins in aflatoxin applied rats.

MATERIALS AND METHODS

Animals

For the experiment, 38 male Wistar albino rats (weighing 34–36 g) were used. Selcuk University's Experimental Application and Research Center provided the animal material. Before the study, the animals were weighed, their overall health was evaluated and they were separated into five groups based on average body weight. The rats were housed ad libitum in an environment with a relative humidity of 50-10%, 12/12 day-night light cycles and plastic rat cages for the duration of the study (60 days).

Experimental procedure

The animals were grouped into five (5) groups. The control group (Control, n=6) were not expose to any treatment. The Dimethyl sulfoxide group (DMSO, n=6) were orally given 1 ml 10% DMSO daily for 60 days. The curcumin group (Cur, n=6) were orally given 300mg/kg curcumin dissolved in 10% DMSO daily for 60 days. The aflatoxin B1 group (AFB1, n=10) were orally given 250µg/kg aflatoxin B1 dissolved in 10% DMSO daily for 60 days. The aflatoxin B1+curcumin group (AFB1+Cur, n=10) were orally given 250µg/kg aflatoxin B1 dissolved in 10% DMSO and 300mg/kg curcumin dissolved in 10% DMSO daily for 60 days (Irene and Onyechi, 2004, Reeta *et al*., 2011).

Measurements

At the end of 60 days, blood was taken from the animals in all groups and the blood samples were analyzed to determine the levels of fibrinogen, prothrombin, albumin and CRP levels. Fibrinogen and prothrombin levels were determined using the Siemens BFT2 device via Siemens kits. Albumin and CRP levels were determined in Siemens Abbott C8000 autoanalyzer using Siemens kits.

Ethical approval

The Selcuk University Experimental Medicine Research and Application Center Ethics Committee accepted this study protocol (Report No. 2018-29).

STATISTICAL ANALYSIS

The data obtained from the study were analyzed by one-way ANOVA (SPSS 19). Duncan's multiple range test to determined differences among the groups. Differences were considered significant at $p<0.05$.

RESULTS

In this study, fibrinogen, prothrombin and CRP levels with AFB1 group administration were significantly higher than in the control group ($p<0.05$). In the group in which AFB1+Cur group, fibrinogen and CRP levels were lower than in the AFB1 group ($p<0.05$). In the study, it was observed that albumin level decreased significantly in AFB1 group compared to the control group ($p<0.05$) and this difference disappeared in AFB1+Cur group ($p<0.05$) (fig. 1).

DISCUSSION

The acute phase response was made up of a series of systemic changes and physiologic brought on by infection and tissue injury. Infection, burns, trauma, inflammatory disorders, tissue infarction and advanced cancer are among circumstances that cause the acute phase response to be activated (Gong *et al*., 2016). The plasma levels of acute-phase proteins increase or decrease at least 25% during an inflammatory disorder. In humans, 39 distinct acute phase proteins have been identified including coagulation and fibrinolysis proteins, complement system members, transport proteins, antiproteases and inflammatory mediators (Gong *et al*., 2016, Gruys *et al*., 2005).

The increase in CRP level, which can be determined in the initial stages of infection, trauma, or organ injuries, was found to be significantly higher in the AFB1 group in this study ($p<0.05$, fig. 1). CRP activates the complement system and phagocytosis, acting as an opsonin by binding to cell debris, degenerate cells and polysaccharides on bacteria, fungi and parasites. It also regulates cytokine production and helps chemotaxis (Gupta *et al*., 2010, Hadrup *et al.*, 2020, Irene and Onyechi, 2004). The increased CRP level in the AFB1 group was significantly lower in the AFB1+Cur group ($p<0.05$, fig. 1).

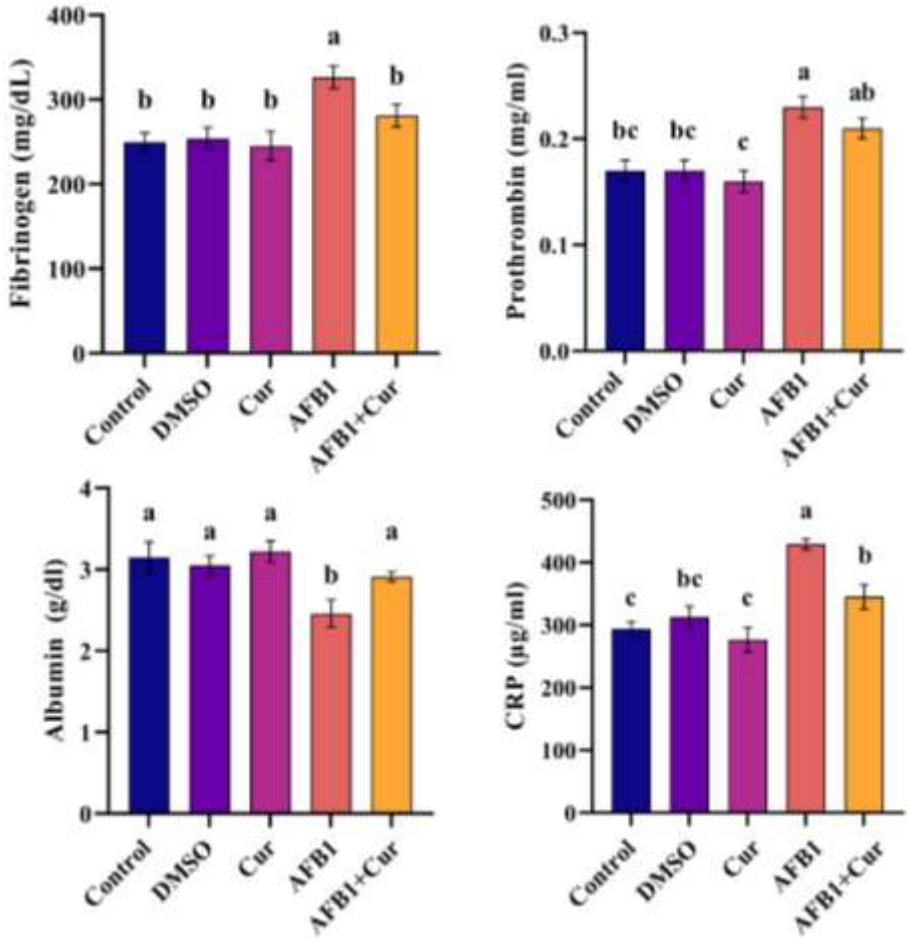

Fig. 1: The effects of curcumin on fibrinogen, prothrombin, albumin and CRP levels in aflatoxin-applied rats (Mean±SE). [a-c] The difference between mean values with different superscripts in the same column is significant at the $p<0.05$ level.

Fibrinogen, a large protein consisting of six polypeptide chains, is converted to fibrin by thrombin and is essential for coagulation (Jaeschke *et al*., 2002, Joe *et al*., 1997). Fibrinogen and derivatives can stimulate a large part of the immune cells during inflammation. In the cases such as infection, inflammation and tissue damage during the acute phase response, an increase in the amount of prothrombin is observed and fibrinogen to trigger coagulation and prevent any bleeding in the inflammation site (El-Agamy, 2010, Irene and Onyechi, 2004, Kaneko, 1997). The study determined that the amount of fibrinogen and prothrombin in the AFB1 group was higher than in the control group as expected ($p<0.05$, fig. 1). In contrast, the level of fibrinogen in rats with curcumin application together with aflatoxin was significantly lower than in the AFB1 group ($p<0.05$, fig. 1). The change in prothrombin level was not important.

Albumin, the major negative acute-phase protein in all species, is the most abundant protein in serum. It serves both as a source of food and as a regulator of osmotic pressure. The decreased albumin level in infection, inflammation and malnutrition conditions are attributed to edema, the decrease of liver synthesis, protein loss from the gastrointestinal tract and kidneys (Joe *et al*., 1997, Kelloff *et al*., 1993). In this study, it was observed that albumin levels decreased significantly in the AFB1 group compared to the control group ($p<0.05$, fig. 1), whereas this difference disappeared in the AFB1+Cur group ($p<0.05$, fig. 1).

This study, determined increases in CRP, fibrinogen, prothrombin levels and determined decrease in albumin level are considered important in showing the hazardous effect of aflatoxin B1 on especially liver and other organs. In this study, based on the positive effects mentioned in infection, trauma and inflammatory events, significant changes in acute-phase proteins were determined with oral administration of curcumin together with aflatoxin for 60 days. The positive changes determined in CRP, fibrinogen and albumin levels with the application of curcumin in the study support the studies reporting that curcuminoids have positive effects on hepatic functions in inflammatory conditions (Koj, 1974, Leray *et al*., 2011,

Lin and Lin-Shiau, 2001). Although a very limited number of studies have been conducted on curcumin and aflatoxicosis or aflatoxin B1, some findings show the protective effects in both toxins and cancer cell culture studies in humans and animals (Magić *et al*., 1995, Mahfouz, 2015, Moriguchi *et al*., 2003, Mosesson, 2005). However, it has been stated that curcumin has anti-inflammatory, antioxidant, free radical scavenger, radio-protective, chemotherapeutic and anti-tumorigenic effects (Nayak and Sashidhar, 2010, O'reilly and Eckersall, 2014). It is also reported that the anti-inflammatory effect of curcumin is performed by preventing neutrophil infiltration and suppressing proinflammatory cytokines in macrophages (Poapolathep *et al*., 2015, Rao *et al*., 1993). It is also claimed that curcumin reduces its toxicity by changing microsomal activation of aflatoxin B1 (Mosesson, 2005, Reeta *et al*., 2011).

CONCLUSION

The data obtained data from the present study showed that curcumin application to the rats administered with aflatoxin B1 ameliorated abnormal changes in the acute phase proteins (CRP, fibrinogen, albumin) due to aflatoxicosis.

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