Pumpkin *(Cucurbita moschata)* against *Aspergillus flavus* and aflatoxin B1 induced lung cyto-morphological damage in rats

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Abstract: The protective efficacy of pumpkin (*Cucurbita moschata*) fruit aqueous extract against either aflatoxin B1 (AFB1) toxicity and *Aspergillus flavus* fungus infection induced lung histolomorphological damage in rats was investigated. AFB1 and *A. flavus* were administered intraperitoneally (0.2mg/Kg body weight) for 15 successive days. The result demonstrated that intoxication of rats with AFB1 induced lung damage as observed by alveolar hyperplasia, pulmonary hemorrhage and fibrosis. Infection with *A. flavus* also showed damaging impact on the rat lung as observed by fibrosis of bronchioles and alveolar hyperplasia. Oral co-administration of aqueous extract of pumpkin fruits (1.0 mg / kg of body weight) to either rat groups intoxicated with AFB1 or infected with *A. flavus* for 20 consecutive days showed more or less normal histological structure of rat lungs. In conclusion, aqueous extract of pumpkin fruits has a protective role against AFB1 or *A. flavus* induced lung damage which may be related to the antioxidant constituents of the plant extract.

Keywords: *Aspergillus flavus*, aflatoxin B1, lung damage.

INTRODUCTION

Aspergillus flavus (Aspergillaceae) is one of the causal agent of food poisoning by producing aflatoxin B1 that can cause certain diseases in gastrointestinal tract, respiratory system and earring in human and animal, in addition to liver cancer (Stewart *et al.*, 1996, Gursoy *et al.*, 2008, Al-Hizab *et al.*, 2015). Aflatoxin B1 (AFB1) is a cytotoxic and carcinogenic mycotoxin (Poapolathep *et al.*, 2015).

Although AFB1 is well known for its carcinogenic effect on the liver, the lung is also another target organ of AFB1 toxicity through the inhalation of dusts or ingestion of food contaminated with this mycotoxin (Eaton and Groopman, 1994, Massey *et al*., 1995). AFB1 can induce lung carcinogenesis directly or indirectly through blood circulation. Epidemiological evidence had showed that exposure to AFB1 can cause lung cancer in humans (Dvorackova *et al*., 1981, Cusumano, 1991) and the capability of AFB1 to cause pulmonary cancer has been shown in experimental animal models (Wieder *et al*., 1968, Stoner, 1991, Guindon *et al*., 2008).

AFB1 toxic mechanism has been previously studied. It has found that AFB1 is transformed in lung to a highly reactive intermediate by cytochrome P450 (CYPs 1A2 and 3A4) enzymes (Heber, 2004) which is responsible for initiation of lung carcinogenesis (Preston and Williams, 2005). Although the mechanism of aflatoxin toxicity is not fully understood, some reports have suggested that toxicity may be caused by the overproduction of intracellular reactive oxygen species (ROS) like hydroxyl radical, superoxide anion and hydrogen peroxide (H_2O_2) during the biotransformation of AFB1 in the liver by cytochrome P450 (Towner *et al*., 2003). These reactive species may attack soluble cell components as well as cell membranes, leading eventually to the deterioration of cell functions and cell death (Berg *et al*., 2004).

Although human CYPs activate AFB1 to the electrophilic exo- AFBO, there are some ambiguities as to which isoform is the most important in human liver (Souza *et al*., 1999, Klein *et al*. 2002). CYPs 1A2 and 3A4 have been found to be the major CYPs isoforms for AFB1 biotransformation in human liver. Also, these isoenzymes beside their mRNA, have been detected in lung tissue of human (Cullen and Newberne, 1993, Heber , 2004).

Treatment with the antimicrobial synthetic drugs was greatly effective, but their application has led to environmental problems and undesirable side effects, including residual toxicity, teratogenicity, carcinogenicity, spermatotoxicity, hormonal imbalance, etc. (Pandey, 2003). Natural compounds of plant origin as a source for the discovery of a new drug to treat different diseases have attracted attention in recent decades. Herbs safely proved to be effective against many human diseases. So, natural agent that can protect against AFB1 toxicity may be useful for human health with minimal or no side effects. Traditional therapeutic plants were used by some authors for their anti-aflatoxigenic, antifungal, and antioxidant properties (Joseph *et al*., 2005, Kumar *et al*., 2007).

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Pumpkin, belongs to the genus of Cucurbita and family Cucurbitaceae, is frequently refers to any one of the species, namely *Cucurbita mixta, Cucurbita pepo, Cucurbita moschata* and *Cucurbita maxima* (Aliu *et al.*, 2011). Pumpkin was reported to have medicinal properties. *C moschata* was found to possess components with antioxidant activity, including vitamins A and E, xanthophylls, carotenes and phenolic compounds which have beneficial impacts in protecting tissues against oxidative damage (Chanwitheesuk *et al*., 2005). The plant contains phosphorus, iron, zinc, vitamin E and vitamin B complex (Shaaban, 2005). Tetra-saccharide glyceroglycolipids were obtained from *C. moschata* and showed significant hypoglycemic effects in diabetic mice (Jiang and Du, 2011). PG105 isolated from the stem of *C. moschata* showed anti-obesity in obese mice induced by a high fat diet (Choi *et al*., 2007). In addition, pharmacological studies on the properties of several squashes (*C. moschata, C. pepo, C. Maxima, C. Mixta, C. Ficifolia and Telfairia occidentalis* Hook) have showed that they have antidiabetic, antimicrobial, anti-oxidant, anticancer, antimutagenic, anthelmintic and immunomodulatory activities (Caili *et al*., 2006).

The aim of the current study was to investigate the prophylactic effect of aqueous extract of *C. moschata* fruit against either AFB1 toxicity *or Aspergillus flavus* infection induced histolopathological changes in rat lungs.

MATERIALS AND METHODS

Chemicals

Chemicals used in this study were of a high purity, product of Sigma and Merck Companies. AFB1 was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant

The fruits of pumpkin (*Cucurbita moschata*) were obtained from the local market in Jeddah, Saudi Arabia, during summer 2015. The fruits were identified by a taxonomist in the Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Preparation of C. moschata aqueous extract

250 g of dried fruits, were mixed with one liter of distilled water and the obtained mixture was boiled under reflux for 30 min and then centrifuged, filtered, frozen at -20°C, and then lyophilized. The obtained lyophilized fruit extract was dissolved in water before *in vivo* ingestion to rats (Xia and Wang, 2006).

Preparation of AFB1

AFB1 was dissolved in dimethyl sulfoxide (2mg/ml) and then 0.1ml/100g body weight was administered to experimental animals intraperitoneally (Ha *et al*., 1999).

Growth and preparation of the pathogenic fungi

Aspergillus flavus toxigenic strain was obtained from the MERCIN unit, College of Agriculture, Ein-Shams University, Cairo, Egypt. The tested strain of *A flavus* was isolated from grain samples ($2.5\text{-}3.5\times10^{-2}$ propagates / g grain) on May 2015. 0.1ml suspension of laboratory fungus spores was added to 50ml of sabouraud dextrose agar in sterile Petri dishes and then incubated at a temperature of 25°±2°C for 6 days.

Animal and experimental design

Animal experiment was carried out according to the local ethics committee. Sixty female albino rats (150-170g) were used for this study. They were purchased from the Experimental Animal Center, Faculty of Science, King Abdulaziz University. The animals were kept in clean polypropylene cages under standard conditions (20±2°C, 12h light/dark cycle and 50-70% relative humidity). The animals were fed commercial pellet diet and water *ad libitum*. One week after acclimation, the rats were divided into six groups, each of ten rats, as follow G1: Normal healthy rats, G2: Normal rats ingested with pumpkin fruit aqueous extract, G3: Rats intoxicated with AFB1 G4: Rats intoxicated with AFB1 and co-administered with pumpkin fruit aqueous extract, G5: Rats infected with *A. flavus*, G6: Rats infected with *A. flavus* and co-administered with pumpkin fruit aqueous extract.

AFB1 was dissolved in dimethyl sulfoxide and administered intraperitoneally (0.2mg/Kg body weight) to the rats of AFB1 intoxicated groups. *A. flavus* fungus suspension was injected intraperitoneally (0.2mg/Kg) to *A. flavus* rat infected groups. AFB1 and *A. flavus* fungus were administered to rats for 15 consecutive days. Pumpkin fruit aqueous extract was administered orally (1.0mg/Kg body weight) along with either AFB1 or *A. flavus* administration for 20 consecutive days.

After the experimental period (20 days), the animals were sacrificed under anesthesia and the lung samples were removed for histopathological studies.

Histopathological examination

Histopathological exmination of lung tissue was carried out using standard histopathological technique. A small pieces of lungs were fixed in 10% formalin and then embedded into paraffin wax. Sections of 5µm thick were obtained using a microtome and then mounted on the glass microscope slides. The sections were stained with hematoxylin-eosin and examined by a light microscopy.

RESULTS

Histopathological observation

The protective role of pumpkin fruit aqueous extract against histomorphological lung damage induced by either AFB1 or *A. flavus* infection is shown in fig. 1. AFB1 induced lung damage as observed by alveolar

hyperplasia, pulmonary hemorrhage and fibrosis (figs. 1c, d and e). Co-administration of pumpkin fruit aqueous extract to AFB1 intoxicated rats (fig. 1f) showed more or less normal histological structure of lung. Infection of rats with *A. flavus* showed damaging deleterious impact on rat lungs as observed by fibrosis of bronchioles (fig. 1g) and alveolar hyperplasia (fig. 1h). Co-administration of pumpkin fruit aqueous extract to rats infected with *A. flavus* showed more or less normal histological structure of lung.

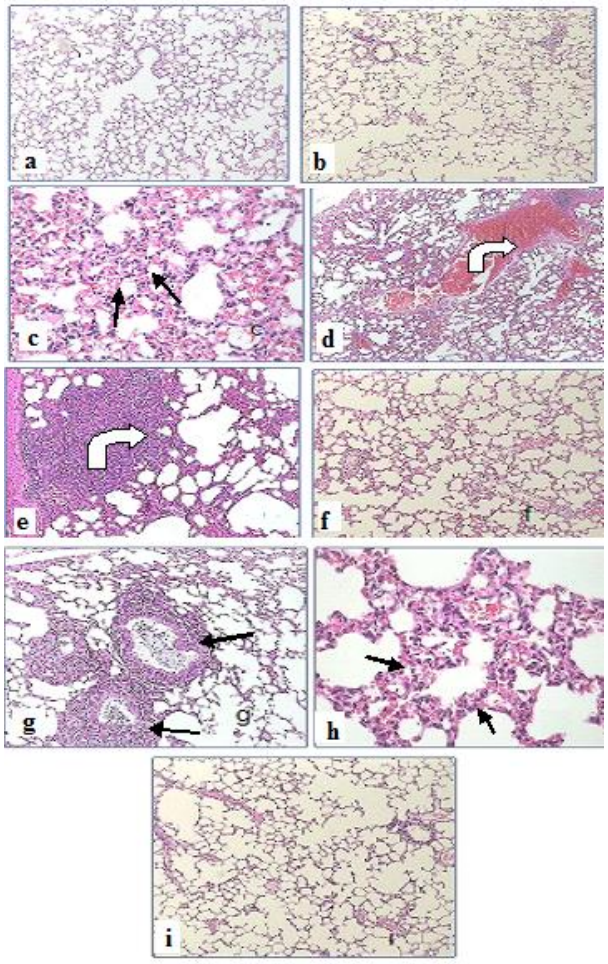

Fig. 1: Sections of rat lungs of different experimental groups, (a) Lung of normal rat showing thin-walled alveoli with a single layer of squamous epithelium and a thin layer of connective tissue between the alveoli, bronchioles are lined by ciliated columnar epithelium (larger bronchioles) or by cuboidal epithelium (smaller bronchioles leading to alveoli). (b) Lung section of rat ingested with pumpkin fruit water extract showing normal architecture of lung. (c, d and e) Lung sections of rat intoxicated with AFB1, showing alveolar hyperplasia (c, arrows), hemorrhage (d, curved arrow) and fibrosis (e, curved arrow). (f) Section of rat lung intoxicated with AFB1 and co-administered with pumpkin fruit water extract, showing more or less normal histology of lung. (g&h) Sections of rat lung infected with *A. flavus*, showing fibrosis of bronchioles (g, arrows) and alveolar hyperplasia (h, arrows). (i) Section of rat lung infected with *A. flavus* and co-administered with pumpkin fruit water extract, showing more or less normal architecture of lung (H&E × 400).

DISCUSSION

The major target organ for AFB1 toxicity is the liver; however, there are many evidences demonstrated that AFB1 toxicity can also induce lung damage in both humans and in experimental animals (Stoner, 1991; Massey *et al*., 1995).

The current study revealed that intoxication of rats with AFB1 or infection with *A. flavus* fungus caused lung histomorphological damage as observed by alveolar hyperplasia, hemorrhage and fibrosis in AFB1 intoxicated group as well as in *A. flavus* infected group. This result may indicate an oxidative damage induced in rat lungs by either AFB1 toxicity or *A. flavus* infection. This observation is supported by previous clinical study revealed that aspergillosis can induce necrotic pneumonia (Hofman *et al*., 2010). Also, a previous study has reported that infection with *A. flavus* induced pro-inflammatory cytokines, including tumor necrosis factor –α (TNF- α) and interleukin -6 (IL-6) which have the principle role in pulmonary inflammation and damage and a decrease in the anti-inflammatory cytokine, IL-10 (Anand and Tiwary, 2010). In addition, some authors have reported that chronic exposure to low concentration of AFB1 in foods causes tissue fibrosis (Al-Hizab *et al*., 2015). Brahmi *et al*.,(2011) have reported that oxidative damage caused by AFB1 may impair the cell membrane integrity by inducing phospholipid A2 to promote lipid per oxidation in the cells. Souza *et al*., (1999) had found that the oxidative stress is the principle cause of AFB1 toxicity which could be prevented by antioxidants.

Administration of aqueous extract of pumpkin fruits to rats intoxicated with AFB1 or infected with *A. flavus* could protect the lungs of rats from the damaging effect of either AFB1 or *A. flavus* as observed by normal architecture of histomorphological pictures of lung tissues. Our result may indicate that the used extract has potential antimicrobial effect and/or antioxidant beneficial action. Pumpkin was found to possess components with antioxidant activities, including vitamins E and A, xanthophyll's, carotenes, and phenolic compounds which have the principle role in protecting against oxidative tissue injury (Chanwitheesuk *et al*., 2005). Also, some authors have reported that pumpkin contains polysaccharide that has significant cytoprotective effect and antioxidative activity (Yang *et al*., 2007).

Estimation of the natural product safety, which accepted as remedies, is important for public health. The current

work ensured that the administered dose of *Cucurbita moschata* fruit aqueous extract (1.0mg/Kg body weight) to normal healthy rats has no any adverse side effects as observed by normal lung histological picture.

CONCLUSION

Aqueous extract of pumpkin fruits has a cytoprotective role against the deleterious impact of *A. flavus* or AFB1 - induced lung damage which may be related to the antioxidant effect of the plant extract.

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