

Full Length Research Paper

Antifungal and prophylactic activity of pumpkin (*Cucurbita moschata*) extract against *Aspergillus flavus* and aflatoxin B1

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The antifungal effect of pumpkin (*Cucurbita moschata*) fruit aqueous extract against *Aspergillus flavus* was investigated *in vitro*. The result showed that incubation of different pumpkin aqueous extract (0.5 to 2%) with living mass of the fungus has suppressive effect on the fungal growth after 6 days compared with untreated control sample. However 2% of pumpkin aqueous extract was the most effective in inhibiting the fungal growth. The protective efficacy of pumpkin fruit aqueous extract against either *A. flavus* fungus infection or aflatoxin B1 (AFB1) toxicity induced renal damage in rats was also investigated. *A. flavus* and AFB1 were administered intraperitoneally (0.1 ml/100 g of body weight) for 15 consecutive days. The result revealed that infection of rats with *A. flavus* or intoxication with AFB1 significantly induced renal damage as indicated by marked increased levels of serum urea, uric acid and creatinine as well as histopathological pictures compared with normal healthy rats. Oral co-administration of aqueous extract of pumpkin fruits (1.0 mg / kg of body weight) to either rat groups infected with *A. flavus* or intoxicated with AFB1 for 20 consecutive days effectively normalized the serum kidney function biomarkers and confirmed by histomorphologic pictures which showed normal histological structure.

Key words: *Aspergillus flavus*, aflatoxin B1, antifungal, renal damage.

INTRODUCTION

Fungal infections are mainly caused by opportunistic fungi and are usually associated with immunosuppression (Shoham and Levitz, 2005). Aflatoxins (AFs) are highly toxic secondary fungal metabolites produced by the species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow on a wide variety of foods and feeds under favorable temperature and humidity (Giray et al., 2007). There are four naturally occurring aflatoxins, the most toxic being aflatoxin B1 (AFB1), and three structurally similar compounds namely aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Aflatoxins are not only contaminant our food stuffs, but also are found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals

(Fink-Gremmels, 1999; Bennett and Klich, 2003; Aycicek et al., 2005).

Aflatoxins are well known to be potent mutagenic, carcinogenic, teratogenic and immunosuppressive agent; also inhibit several metabolic systems, causing liver, kidney and heart damage (Wogan, 1999; Bintvihok, 2002; Wangikar et al., 2005). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Salunkhe et al., 1987).

The mechanism of AFB1 toxic effect has been extensively studied. It has been shown that AFB1 is activated by hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB1-8,9-epoxide, which subsequently binds to nucleophilic sites in

DNA, and the major adduct 8,9-dihydro-8-(N7guanyl)-9-hydroxy-AFB1 (AFB1 N7-Gua) is formed (Sharma and Farmer, 2004). The formation of AFB1- DNA adducts is regarded as a critical step in the initiation of AFB1-induced carcinogenesis (Preston and Williams, 2005). Although the mechanism underlying the toxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H₂O₂) during the metabolic processing of AFB1 by cytochrome P450 in the liver (Towner et al., 2003; Naaz et al., 2007; Shi et al., 2012). These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Berg et al., 2004).

The use of synthetic drugs as antimicrobials was greatly effective, but their application has led to a number of ecological and medical problems due to residual toxicity, carcinogenicity, teratogenicity, hormonal imbalance, spermatotoxicity, etc. (Pandey, 2003). Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. So, natural substances that can prevent AFB1 toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional medicinal plants were applied by some authors for their antifungal, anti-aflatoxin and antioxidant activity (Joseph et al., 2005; Kumar et al., 2007).

Pumpkin, belonging to the genus of *Cucurbita* and family Cucurbitaceae frequently refers to any one of the species *Cucurbita pepo*, *Cucurbita mixta*, *Cucurbita maxima*, and *Cucurbita moschata* (Itis.gov., 2009). Pumpkin was reported to have medicinal properties. *C. moschata* was reported to have antioxidant components including vitamin A, vitamin E, carotenes, xanthophylls and phenolic compounds which have the principal role in protecting against oxidative tissue damage (Chanwitheesuk et al., 2005).

Tetrasaccharide glyceroglycolipids were obtained from *Cucurbita moschata* and showed significant glucose-lowering effects in streptozotocin- and high-fat-diet-induced diabetes in mice (Jiang and Du, 2011). Water-soluble extract, named PG105, prepared from stem parts of *C. moschata*, contains potent anti-obesity activities in a high fat diet-induced obesity mouse model (Choi et al., 2007). The plant is also used as laxative, and in the treatment of headaches, heart disease, high blood cholesterol and high blood pressure (AL-Sayed, 2007).

The aim of this study was to evaluate antimicrobial impact of *C. moschata* fruit aqueous extract against toxigenic *A. flavus* and also to investigate the *in vivo* protective effect of the extract against *A. flavus* infection

and AFB1 toxicity induced renal dysfunction and histopathological structural changes in rats.

MATERIALS AND METHODS

Preparation of aflatoxin B1 standard (AFB1)

AFB1 was obtained from Sigma-Aldrich (St. Louis, MO, USA). A concentration of 2 mg/ml of AFB1 was prepared using dimethyl sulphoxide. AFB1 was administered to experimental animals intraperitoneally using a dosage of 0.2 mg/Kg of body weight (Ha et al., 1999)

Organism under study

A. flavus toxigenic strain was obtained from The MERCIN unit, College of Agriculture, Ein-Shams University- Cairo, Egypt.

Preparation of *Aspergillus flavus* cultures

Fifty ml of Sabouraud dextrose agar were added to sterile Petri dishes and 0.1 ml spore suspension were inoculated. The inoculated media were incubated at 25 ± 2°C for 6 days.

The effect of different concentrations of pumpkin fruit aqueous extract on *Aspergillus flavus* bio mass

The effect of pumpkin fruit aqueous extract on the mycotic spindle formation of the experimental fungus was studied on the liquid Sabouraud Dextrose media. Exactly 1 ml of different concentrations (0.5, 1.0, 1.5 and 2.0%) of pumpkin fruit aqueous extract was added to a flask contained 50 ml of the liquid media. Control group flasks with no added pumpkin fruit aqueous extract were also prepared. All prepared media were sterilized by bacterial filtration and then fertilized with disks of *A. flavus* fungus with diameter of 5 mm. Cultures were filtered after incubation at 25 ± 2°C for 3 and 6.

Plant specimens

Pumpkin (*Cucurbita moschata*) fruits were purchased from the local market in Jeddah, Saudi Arabia during summer 2011.

Preparation of plant aqueous extract

The fruit extract was prepared according to the method of Xia and Wang, (2006b). One hundred grams of dried fruits were mixed with 1000 ml of distilled water and the mixture was boiled at 100°C under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at -20°C, and then lyophilized. The lyophilized product of plant extract obtained was either dissolved in water before oral administration to rats or sterilized by bacterial filter for the antifungal studies.

Animal and treatments

Animal experiment was performed in accordance with legal ethical guidelines of the Medical Ethical Committee of King Abdelaziz University, Jeddah, KSA. Sixty healthy female albino rats (150 to

Table 1. Effect of various concentrations of pumpkin fruit aqueous extract on bio mass of *Aspergillus flavus* grown on liquid media after 6 days (mg \pm SE).

Pumpkin fruit aqueous extract concentration %	Radial growth after 3 days	% of inhibition	Radial growth after 6 days	% of inhibition
0.0	8.10 \pm 0.11 ^a	00.00	7.33 \pm 0.30 ^a	00.00
0.5	7.30 \pm 0.10 ^b	9.877	5.51 \pm 0.13 ^b	24.829
1.0	5.10 \pm 0.00 ^c	30.423	5.22 \pm 1.02 ^b	35.556
1.5	4.69 \pm 0.31 ^c	42.099	3.84 \pm 0.82 ^c	47.613
2.0	3.72 \pm 0.00 ^d	54.074	2.31 \pm 1.06 ^d	68.486

Different letters in the same column are significantly different $P \leq 0.001$.

170 g) were obtained from the Experimental Animal Center, Faculty of Science, King Abdulaziz University. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at $20 \pm 2^\circ\text{C}$, 50 to 70% relative humidity and 12-h light/dark cycle. The animals were provided with commercial rat pellet diet and deionized water *ad libitum*. After one week acclimation, the rats were randomly divided into 6 groups (each of 10 rats) as follow: G1: Normal healthy rats, G2: Normal rats ingested with pumpkin fruit aqueous extract, G3: Rats infected with *A. flavus*, G4: Rats infected with *A. flavus* and co-administered with pumpkin fruit aqueous extract, G5: Rats intoxicated with AFB1, G6: Rats intoxicated with AFB1 and co-administered with pumpkin fruit aqueous extract.

AFB1 was dissolved in dimethyl sulphoxide and administered intraperitoneally (0.1 mg/100 g body weight) to the rats of AFB1 intoxicated groups. *A. flavus* spore suspension was injected intraperitoneally (0.2 mg/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 (1.0 mg / kg of body weight) to either rat groups treated with AFB1 or infected with *A. flavus* fungus for 20 consecutive days.

After the experimental period (20 days), the blood samples were collected from each animal in all groups into sterilized tubes for serum separation. Serum was separated by centrifugation at 3000 rpm for 10 min and used for biochemical serum analysis of kidney function. After blood collection, rats of each group were sacrificed under ether anesthesia and the kidney samples were collected for histopathological examination.

Histopathological examination

A small pieces of kidneys were fixed by 10% formalin and then embedded into paraffin, sectioned for 5 to 6 μm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy.

Biochemical serum analysis

Urea (Orsonneau et al., 1992), uric acid (Fossati et al.,1980) and creatinine (Henry, 1974) were measured as indicators of kidney function.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean \pm S.D. The significant differences among

values were analyzed using analysis of variance (one-way ANOVA) followed by Bonferroni as a post-ANOVA test. Results were considered significant at $P < 0.05$ (Hill, 1971).

RESULTS

The effect of different concentrations of pumpkin fruit aqueous extract on the vitality of bio mass of *A. flavus* fungus was illustrated in Table 1. The result showed that incubation of different concentrations of pumpkin aqueous extract (0.5 to 2%) with living mass of the fungus has suppressive effect on the fungus growth after 6 days compared with untreated control sample. 2% of the plant extract was the most effective in inhibiting the fungal growth.

The protective role of pumpkin fruit aqueous extract on kidney serum function biomarkers against *A. flavus* infection and AFB1 toxicity in rats is depicted in Table 2. The result showed that infection of rats with *A. flavus* (G3) or treated with AFB1 (G5) for 15 consecutive days, dramatically causes renal tissue damage as indicated by marked increases in serum urea, uric acid and creatinine compared with normal rats ($P \leq 0.001$). Oral co-administration of pumpkin fruit aqueous extract to either groups (G3 or G5), effectively protected the kidney from the damaging effect of either *A. flavus* (G4) or AFB1 (G6) as documented by the normalization of the studied kidney function biomarkers. Histopathological observation supported the biochemical result. kidney section of rat infected with *A. flavus* indicated its damaging deleterious impact on rat kidney as observed by the damaged architecture of proximal tubular epithelia, such as cell swelling and lysis, cytoplasm vacuolation, nuclear membrane breakdown, cell shrinkage, nuclear condensation, and necrosis of some glomeruli and tubules (Figure 1c). Co-administration of pumpkin fruit aqueous extract to rats infected with *A. flavus* showed more or less normal histological structure of kidney (Figure 1d). Injection of rats with AFB1 showed sever damage to histomorphological picture of rat kidneys (Figure 1e and f) as observed by marked dilatation and congestion of renal blood vessel (Figure 1e), degeneration of most renal tubules, swelling and lysis of

Table 2. Levels of serum kidney function biomarkers (mean \pm SE) in different experimental animals.

Group	Urea	Uric acid	Creatinine
Normal	23.26 \pm 2.1 ^b	3.13 \pm 0.15 ^b	0.45 \pm 0.05 ^b
Normal + pumpkin fruit extract	23.16 \pm 1.5 ^b	2.98 \pm 0.10 ^b	0.5 \pm 0.02 ^b
Infected with <i>A. flavus</i>	30.56 \pm 1.25 ^a	3.9 \pm 0.1 ^a	0.83 \pm 0.023 ^a
Infected with <i>A. flavus</i> + pumpkin fruit extract	20.16 \pm 2.6 ^b	3.0 \pm 0.1 ^b	0.54 \pm 0.02 ^b
Intoxicated with AFB1	33.13 \pm 2.9 ^a	5.3 \pm 0.12 ^a	0.88 \pm 0.022 ^a
Intoxicated with AFB1 + pumpkin fruit extract	21.23 \pm 0.96 ^b	3.4 \pm 0.08 ^b	0.49 \pm 0.015 ^b

Different letters in the same column are significantly different $P \leq 0.001$.

cells, cytoplasm vacuolation and nuclear pyknosis. Co-administration of pumpkin fruit aqueous extract to AFB1 intoxicated rats (Figure 1g) showed more or less normal histological structure of kidney. Administration of pumpkin fruit aqueous extract to normal rats showed no side effects on rat kidneys as indicated by normal kidney function and supported by histomorphologic picture of rat kidneys which showed normal histological structure

DISCUSSION

Plants have been used in medicine for a long period of time, since they are easy to obtain and used in treatment of various diseases (Sardi et al., 2011). It contains phosphorus, iron and zinc, vitamin E and vitamin B complex, very poor in sodium (Shaaban, 2005). Regarding the search for new antifungal ideal agent must have a broad spectrum of fungicidal activity without causing toxicity to the host (Carrillo-Muñoz et al., 2006). The treatment of fungal infections is not always effective because of resistance to drugs in addition to presenting high toxicity for human cells. For this reason, there is a continuing search for new drugs which are more potent antifungal, but safer, than existing drugs (Fenner et al., 2006).

The antifungal activity of aqueous extract of pumpkin fruits against *A. flavus* fungus was studied. The result revealed that different concentrations of pumpkin aqueous extract (0.5 to 2%) showed inhibitory beneficial effect on the growth of fungus living mass after 6 days compared with untreated control. 2% of the plant extract was the most effective one in inhibiting the fungal growth. This is consistent with the results of Saddiq, (2010) who reported high ability of the pumpkin fruits and seeds alcohol extract to inhibit the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* as well as against the toxigenic fungus *A. flavus*. Also this result is supported by previous study by Wang and Ng. (2003) who isolated antifungal peptide from *C. moschata* seeds namely cucurmoschin which has abundant arginine, glutamate and glycine residues. Cucurmoschin inhibited mycelial growth in the fungi *Botrytis cinerea*, *Fusarium oxysporum* and *Mycosphaerella oxysporum*. The authors

also stated that the cucurmoschin showed a translation-inhibitory activity (IC₅₀ = 1.2 μ M) which was more effective than some of the antifungal proteins (Lam et al., 2000, Wang and Ng, 2000, 2002) and the antifungal peptides from red bean, pinto bean (Ye and Ng, 2001) and chickpea (Ye et al., 2002). Ribosome-inactivating proteins inhibit translation in rabbit reticulocyte lysate with a much higher potency (IC₅₀ in pM concentration) (Barbieri et al., 1993) and they also inactivate fungal ribosomes (Roberts and Selitrennikoff, 1986).

The protective role of aqueous extract of pumpkin fruits on kidney serum function biomarkers against *A. flavus* infection and AFB1 toxicity in rats was investigated. The result showed that either infection of rats with *A. flavus* or intoxication with AFB1 dramatically induce nephrotoxicity in rats, as demonstrated by the significantly increased levels of serum urea, uric acid and creatinine. The alteration in these kidney function biomarkers was more evidence in rats intoxicated with AFB1. The reno-toxic effect of *A. flavus* or AFB1 was further confirmed by the severely destructed renal tissue, as shown in the histological analysis. Histomorphological picture of rat kidneys infected with *A. flavus* showed damaged of renal proximal tubular epithelia, such as cell swelling and lysis, cytoplasm vacuolation, nuclear membrane breakdown, cell shrinkage, nuclear condensation, and necrosis of some glomeruli and tubules. Injections of rats with AFB1 showed sever damage to histomorphological picture of rat kidney indicated by vacuolar degeneration and necrosis of the renal tubular cells. Our results are similar to other studies indicated impairment of kidney functions and abnormal pathological changes with severe inflammatory response of kidney in animals intoxicated with AFB1 (Valdivia et al., 2001; Salim et al., 2011). Although the mechanism underlying the toxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H₂O₂) during the metabolic processing of AFB1 by cytochrome P450 in the liver (Towner et al., 2003). These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Berg et al., 2004).

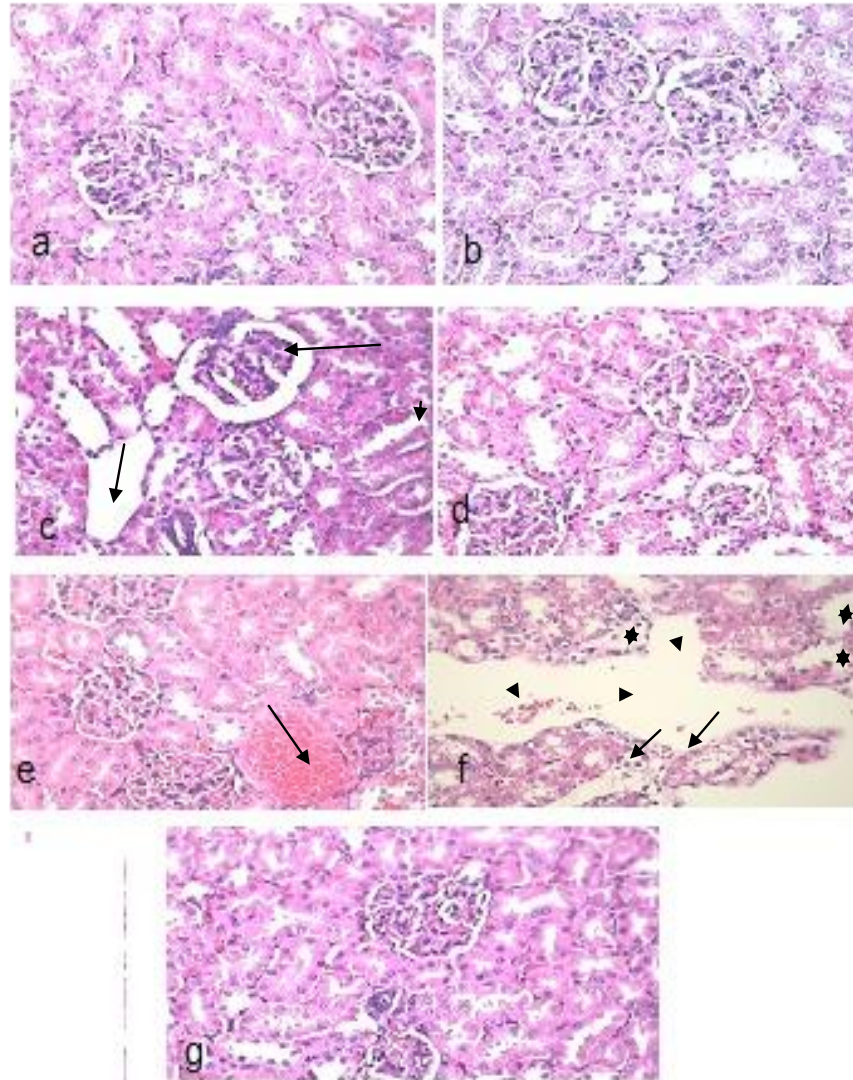


Figure 1. Sections of rat kidneys of different experimental groups, (a) kidney of normal healthy rats showing normal histological structure of renal parenchyma, (b) kidney section of rat ingested with pumpkin fruit aqueous extract showing normal histological structure of renal parenchyma, (c) kidney section of rat infected with *A. flavus* showing various degrees of damage to the architecture of proximal tubular epithelia, such as cell swelling and lysis, cytoplasm vacuolation (arrow head), nuclear membrane breakdown, cell shrinkage, nuclear condensation, and necrosis of some glomeruli and tubules (thin arrows), (d) section of rat kidney infected with *A. flavus* and co-administered with pumpkin fruit aqueous extract showing more or less normal histological structure of kidney, (e and f) sections of rat kidney intoxicated with AFB1 showing severe damage to the renal architecture such as marked dilatation and congestion of renal blood vessel (e, arrow), degeneration of most renal tubules (arrow heads), swelling and lysis of cells, cytoplasm vacuolation (stars) and nuclear pyknosis (f, arrows), (g) section of rat kidney intoxicated with AFB1 and co-administered with pumpkin fruit aqueous extract showing more or less normal histological structure of kidney.

Souza et al. (1999) reported that the oxidative stress is the principle manifestations of AFB1-induced toxicity which could be mitigated by antioxidants. Administration of aqueous extract of pumpkin fruits to rats infected with *A. flavus* or intoxicated with AFB1 markedly protected the

kidney from the damaging deleterious impact on kidney tissue indicated by normalization of kidney function biomarkers in rats administered *A. flavus* or AFB1 simultaneously with the used plant extract. The beneficial effect of this extract on kidney function biomarkers was

supported by histomorphologic picture of kidney which showed more or less normal histological structure. The ameliorative effect of the used plant extract on kidney dysfunction and its histopathological pictures induced by fungal infection or AFB1 may indicate to its antimicrobial effect or antioxidant potential action. Pumpkin was reported to have antioxidant components including vitamin A, vitamin E, carotenes, xanthophylls and phenolic compounds which have the principle role in protecting against oxidative tissue damage (Chanwitheesuk et al., 2005; Saddiq, 2010).

Evaluation of the adverse effects of the natural products, accepted as remedies, is important in implementing safety measures for public health. The present work proved that the administered dose of *C. moschata* fruit aqueous extract (1.0 mg / kg of body weight) to normal healthy rats has no adverse effects which was indicated by the normal levels of the serum renal tested function in comparison to normal untreated rats. This result was confirmed by normal renal histomorphological picture.

In conclusion, aqueous extract of pumpkin fruits has a protective role against *A. flavus* or AFB1-induced renal damage which may be related to the antioxidant constituents of the plant extract.

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REFERENCES

- AL-Sayed AM (2007). Nutrition in Islam. First Printing, Publisher Library thousand for the post. Arab Republic of Egypt. p. 33.
- Aycicek H, Aksoy A, Saygi S (2005). Determination of aflatoxin levels in some dairy and food products consumed in Ankara, Turkey. *Food Control* 16:263-266.
- Barbieri WK, Battelli MG, Stirpe F (1993). Ribosome-inactivating proteins from plants. *Biochem. Biophys. Acta* 1154:237-282.
- Bennett JW, Klich M (2003). Mycotoxins. *Clin. Microbiol. Rev.* 16:497-516.
- Berg D, Youdim M B, Riederer P (2004). Redox imbalance. *Cell Tissue Res.* 318:201-213.
- Bintvihok A (2002). New insights to controlling mycotoxin danger in ducks. *Feed Techn.* 6(1):28-29.
- Carrillo-Muñoz AJ, Giusiano G, Ezkurra PA, Quindós G (2006). "Antifungal agents: mode of action in yeast cells," *Revista Espanola de Quimioterapia* 19:130-139.
- Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005). Screening of Antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem.* 92:491-497.
- Choi H, Eo H, Park K, Jin M, Park EJ, Kim SH, Park JE, Kim S (2007). A water-soluble extract from *Cucurbita moschata* shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. *Biochem. Biophys. Res. Commun.* 359(3):419-425.
- Fenner R, Betti AH, Mentz LA, Rates SMK (2006). "Plants with potential antifungal activity employed in Brazilian folk medicine," *Braz. J. Pharm. Sci.* 42:369-394.
- Fink-Gremmels J (1999). Mycotoxins: their implications for human and animal health. *Vet. Q.* 21:115-120.
- Fossati P, Prencipe Land Berti, G (1980). Use of 3,5-dichloro-2-hydroxybenzene sulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin. Chem.* 26(2):227-231.
- Giray B, Girgin G, Engin AB, Aydin S, Sahin G (2007). Aflatoxin levels in wheat samples consumed in some regions of Turkey. *Food Control* 18:23-29.
- Ha TG, Jang JJ, Kim S G, Kim N D (1999). (Allylthio) Pyrazine inhibition of aflatoxin B-induced hepatocarcinogenesis in rat. *Chemico – Biol. Interact.* 121:209-222.
- Henry R J (1974). In "Clinical Chemistry", Principles and Techniques, 2nd ed, Harper and Row p. 525.
- Hill H B (1971). Principles of Medical Statistics, ninth ed. Oxford Univ.
- Jiang Z, Du Q (2011). Glucose-lowering activity of novel tetrasaccharide glyceroglycolipids from the fruits of *Cucurbita moschata*. *Bioorg. Med. Chem. Lett.* 21(3):1001-1003.
- Joseph GS, Jayaprakasha GK, Selvi AT, Jena BS, Sakariah KK (2005). Antiaflatoxic and antioxidant activities of *Garcinia* extracts. *Int. J. Food Microbiol.* 101:153-160.
- Kumar R, Mishra AK, Dubey NK, Tripathi YB (2007). Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxic and antioxidant activity. *Int. J. Food Microbiol.* 115:159-164.
- Lam YW, Wang HX, Ng TB (2000). A robust cysteine-deficient chitinase-like antifungal protein from inner shoots of the edible chive *Allium tuberosum*. *Biochem. Biophys. Res. Commun.* 279:74-80.
- Naaz F, Javed S, Abdin MZ (2007). Hepatoprotective effect of ethanolic extract of *Phyllanthus amarus* Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. *J. Ethnopharmacol.* 113:503-509.
- Orsonneau JL, Massoubre C, Cabanes M, Lustenberger P (1992). Simple and sensitive determination of urea in serum and urine. *Clin. Chem.* 38(5):619-623.
- Pandey R (2003). Pesticides and sterility. *Everyman's Sci.* 38:84-86.
- Preston RJ, Williams GM (2005). DNA-reactive carcinogens: mode of action and human cancer hazard. *Crit. Rev. Toxicol.* 35:673-683.
- Roberts WK, Selitrennikoff CP (1986). Isolation and partial characterization of two antifungal proteins from barley. *Biochim. Biophys. Acta* 880:161-170.
- Saddiq AA (2010). Antimicrobial and therapeutic effects of the Pumpkin plant (*Cucurbita moschata*) against some harmful microbial. *Egypt. J. Exp. Biol.* 6(1):41-49.
- Salim AB, Zohair A, Hegazy AE, Said A (2011). Effect of some strains of probiotic bacteria against toxicity induced by Aflatoxins *in vivo*. *J. Am. Sci.* 7:772-783.
- Salunkhe DK, Adsule RN, Padule DN (1987). Occurrence of aflatoxin. In *Aflatoxin in Foods and Feeds* pp. 44-92.
- Sardi JCO, Almeida AMF, Mendes Giannini MJS (2011). "New antimicrobial therapies used against fungi present in subgingival sites—a brief review," *Arch. Oral Biol.* 56:951-959.
- Shaaban MJ (2005). *Medicine and Life*, First Edition, Knowledge House, Beirut, Lebanon.
- Sharma RA, Farmer PB (2004). Biological relevance of adduct detection to the chemoprevention of cancer. *Clin. Cancer Res.* 10:4901-4912.
- Shi D, Guo S, Liao S, Su R, Pan J, Lin Y, Tang Z (2012). Influence of selenium on hepatic mitochondrial antioxidant capacity in ducklings intoxicated with aflatoxin B₁. *Biol. Trace Elem. Res.* 145(3):325-329.
- Shoham S, Levitz MS (2005). "The immune response to fungal infections," *Br. J. Haematol.* 129:569-582.
- Souza MF, Tome AR, Rao VS (1999). Inhibition by the bioflavonoid termination of aflatoxin B1-induced lipid peroxidation in rat liver. *J. Pharm. Pharmacol.* 51:125-129.
- Towner RA, Qian SY, Kadiiska M B, Mason RP (2003). *In vivo* identification of aflatoxin-induced free radicals in rat bile. *Free Radic. Biol. Med.* 35:1330-1340.
- Valdivia AG, Martínez A, Damián FJ, Quezada T, Ortiz R, Martínez C, Llamas J, Rodríguez ML, Yamamoto L, Jaramillo F, Loarca-Piña MG, Reyes JL (2001). Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B1 intoxication in broiler chickens. *Poult. Sci.* 80:727-734.
- Wang HX, Ng TB (2000). Ginkbilobin, a novel antifungal protein with sequence similarity to embryo-abundant protein. *Biochem. Biophys. Res. Commun.* 279:407-411.

- Wang H, Ng TB (2002). Isolation of lilin, a novel arginine- and glutamaterich protein with potent antifungal and mitogenic activities from lily bulbs. *Life Sci.* 70:1075-84.
- Wang H.X, Ng T.B (2003). Isolation of cucurmoschin, a novel antifungal peptide abundant in arginine, glutamate and glycine residues from black pumpkin seeds. *Peptides* 24:969-972
- Wangkar PB, Dwivedi P, Sinha N, Sharma AK, Telang AG (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. *Toxicology* 215:37-47.
- Wogan GN (1999). Aflatoxin as a human carcinogen. *Hepatology* 30:573-575.
- Xia T, Wang Q (2006 a). Antihyperglycemic effect of *Cucurbita ficifolia* fruit extract in streptozocin induced diabetic rats. *Fitoterapia* 77:530-533.
- Xia T, Wang Q (2006b). D-chiro-inositol found in *Cucurbita ficifolia* (Cucurbitaceae) fruit extracts plays the hypoglycemia role in streptozocin diabetic rats. *Pharm. Pharmacol.* 58(11):1527-1532.
- Ye XY, Ng TB (2001). Peptides from pinto bean and red bean with sequence homology to cowpea 10-kDa protein precursor exhibit antifungal, mitogenic and HIV-1 reverse transcriptase-inhibitory activities. *Biochem. Biophys. Res. Commun.* 285:414-419.
- Ye XY, Ng TB, Rao PF (2002). Cicerin and arietin, novel chickpea peptides with different antifungal potencies. *Peptides* 23:817-822.