**Introduction**

Peanut (*Arachis hypogaea* L.) is belonging to Family Fabaceae. Egypt is a major exporting country of peanut and the European markets account for 68% of its exports. In May, 1999 the European Commission suspended the import of peanuts from Egypt due to the presence of aflatoxins in concentrations exceeding the safe levels specified in the EU regulations.

Many soil borne fungi are serious pathogens of pod and seeds in the field and many storage fungi infect peanut during storage and transportation. This causing pod and seeds rot and their invasion caused various damages including yield loses in both quality and quantity of seeds, discoloration, decay and mycotoxin production. Peanuts are important substrates for the growth of mold and subsequent aflatoxins production by different species of *Aspergillus* (Abdel-Wahhab et al., 2011).

Mycotoxins are secondary metabolites which have low molecular weights and produced by mycelia or spores of filamentous fungi (González et al., 2001). They are classified as the most important chronic, non-antigenic and non-infectious foodborne risk factor. They are more injurious than synthetic contaminants, plant toxins, food additives, and pesticide residues. Both humans and animals may show acute or chronic toxification caused by mycotoxin ingestion and the pathological condition results from this ingestion is called mycotoxicosis (Nierman et al., 2008).

Aflatoxins are the most toxic group of...
mycotoxins that are produced by some *Aspergillus* species (*Aspergillus flavus*, *A. parasiticus* and the rare *A. nomius*) (Park et al., 2002). The International Cancer Research Institute identified aflatoxins (AFs) as class 1 carcinogens (Asim et al., 2011 and Prabu et al., 2013). AFB1 is the most potent genotoxic and carcinogenic form of AFs (Park et al., 2002). The risk of contamination by aflatoxins of food and feed in Africa is increased due to environmental, agronomic, socio-economic factors, temperature, food substrate, strain of the mold and other environmental factors that affect mycotoxin production (Guchi, 2015).

In recent years, using mycotoxin-adsorbing agents to bind mycotoxins in the gastrointestinal tract of animals lead to decrease their bioaccumulation, toxicities and bioavailability and results in promising improvement in feed industrial applications. Adsorption agents are very useful to prevent aflatoxicosis but may not very effective for adsorption of other mycotoxins (Cheng et al., 2016).

Agricultural wastes such as peel of various fruits and vegetables is applied in food and other industries as an adsorption agents for various contaminants (Mohamed et al., 1994). Peels of fruits and vegetables such as *Mangifera indica*, *Solanum tuberosum*, *Lagenaria siceraria*, *Luffa acutangula*, *Ananas comosus*, *Momordica charantia*, *Carica papaya*, *Moringa oleifera*, *Raphanus sativa*, *Punica granatum*, *Chrysophyllum albidum* have broad antitoxic activities (Kulkarni, 2005; Chanda et al., 2010; Kamba & Hassan, 2010; Mohd et al., 2012 and Janjua et al., 2013). Furthermore, several plant wastes have been shown to have ameliorative effects against induced toxicity of AFB1 in animals (Liu et al., 1995; Gyamfi & Aniya, 1998; Oluwafemi & Taiwo, 2004; Farombi et al., 2005 and Choi et al., 2011).

Banana peels are agricultural waste that discarded all over the world as useless material, although, they have high adsorbent potentiality (Hossain et al., 2012). They are very useful for refining processes and purification. They have absorption capabilities for some elements and ions in liquid or solution, such as adsorption capacities to eliminate chromium from wastewater (Memon et al., 2008), copper (Hossain et al., 2012) in addition to some dyes (Velmurugan et al., 2011). It is abundantly available, low cost, and environment friendly bio-material. This agricultural waste is also cheap, inexhaustible and non-hazardous, and is specifically selective for heavy metals adsorption and can be easily disposed by incineration (Das et al., 2008). It could be used as similar to charcoal and activated charcoal as common adsorbent (Mopoung, 2008).

The modification of banana and orange peels with acid treatment significantly improve color adsorption capacity as compared to raw banana and orange peels (Mane & Bhusari, 2012). Banana peel has proven to be a promising material for the removal of contaminants from olive mill wastewaters by adsorption (Mandi et al., 2009). Zheng & Wang (2013) concluded that banana peel containing functional groups as adsorbent which is responsible for the outstanding adsorption performance for Au (III). The alkaline fractions of peel were evaluated as a multi-functional antibacterial, dye adsorption, and UV protective agent on the cotton substrate (Salah, 2012 and Chabuck et al., 2013).

The objective of this study aimed to investigate the effect of banana peel as adsorbent agent to the aflatoxins produced from *A. flavus* in *in vitro* experiment as well as to study its potential to reduce the injurious effect on liver and kidney of albino rats, feed with AFs contaminated diet.

**Materials and Methods**

**Fungal isolation from raw peanut**

Five grams from raw peanut sample, obtained from local markets, were disinfected using 2.5% sodium hypochlorite for one minute, rinsed three times in 10ml sterile distilled water, dried and homogenized with 90ml of sterilized distilled water in stomacher for 2min. Serial dilutions of $10^{-1}$, $10^{-2}$ and $10^{-3}$ were prepared from peanut suspension. Each dilution was shaken and 1ml aliquot was transferred onto surface of petri-dishes containing rose bengal chloramphenicol agar medium (NMKL Report, 2005). The dishes were rotated by hand to disperse on the surface of medium then incubated at 25°C for 7 days. The resulting colonies were counted, isolated and identified using standard identification manuals (Gilman, 1957; Barnett & Hunter, 1972; Raper & Fennell, 1965; Moubasher, 1993; Watanabe, 2002; Samson, 1979 and Samson et al., 1995, 2010). The identified fungal cultures were maintained on PDA medium (Shotwell et al., 1966).
Molecular identification of the most frequent fungus

This experiment was carried out to confirm identification of the most frequent fungal species in isolation plates Aspergillus flavus which nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing was used. DNA extracted by Quick-DNA™ Fungal/Bacterial Micro prep Kit (Zymo research #D6007) Then PCR was made by using Maxima Hot Start PCR Master Mix (Thermo K1051). GeneJET™ PCR Purification Kit (Thermo K0701) was used to clean up PCR product. Internal transcribed spacer (ITS) region of 5.8S rRNA was amplified using the primers ITS1 (5’TCCGTAGGTGAACCTGCGG3’) and ITS4 (5’TCCCTCGCTTAATGATATGC 3’). Sequencing of PCR amplified product at GATC Company by use ABI 3730xl DNA sequencer using forward and reverse primers. Combining the traditional Sanger technology with the new 454 technologies. The closely related phylogenetic sequences were obtained after entering the resulting sequence into BLAST (Basic Local Alignment Search Tool) of the National Center for Biotechnology Information (NCBI) data base search program (http://WWW.ncbi.nlm.nih.gov/blast/). A phylogenetic tree was constructed using MEGA 6 software.

Determination of aflatoxins concentration

The concentration of AFs extracted from A. flavus fermentation medium as described by Abdel-Rahman et al. (2017) was determined using HPLC technique (Agilent 1200 Series U.S.A with column C18, Lichrospher 100 RP-18, 5µm×25cm). The mobile phase constituted of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460nm (Florescence detector), respectively, (Roos et al., 1997 and AOAC, 2016).

In vitro adsorption capacity of AFs using banana peel powder

Adsorption equilibrium experiments were carried out by adding 250, 500, 750, 1000µL of A. flavus aflatoxins mother solution, then diluted to 100ml by distilled water in polyethylene bottle to obtain solutions having concentrations of (1080,1158,1191.6, and 1436.3ppb), respectively. Seven levels of the adsorbent banana peel powder, 0.0 (control) 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% w/v distilled water, were added to each AFS diluted solutions which shake using horizontal shaker for 1and 2h. The suspension was filtered and extracted with chloroform (1:2, v/v) (Abd El-mageed, 1987) then evaporated until obtaining dry film in rotary evaporator. The obtained dry film containing AFs was reconstituted with water: methanol: acetonitrile (54:29:17, v/v/v) the final concentration of aflatoxins in distilled water was measured using HPLC technique (Agilent1200 series). Histopathological alterations under the effect of AFs singly or in combination with banana peel powder

Six groups of albino rats were used as shown in Table 1. Animal housing and treatment conditions were approved by the ethics committee that determines the ethical and animal welfare practices (approval number: CU/1/F/48/18).

Histopathological technique was performed according to the method of Bancroft & Stevens (1996). Samples taken from liver and kidney preserved in neutral formalin solution (10% v/v). The tissues were dehydrated in ascending grades of ethanol (70%, 80%, 90% and absolute) two hours each. The tissues were immersed in xylene for two hours and this step was repeated once more, the tissues then immersed for two hours in a liquid wax (melting point 55±1°C). Mounted in blocks of liquid paraffin and left to solidify in a cold temperature. Sections of 5-6µm thick were cut using a microtome and mounted on microscope slides. The slides were then immersed in xylene, rehydrated by descending grades of alcohol then water, immersed in Haematoxylin for 15min. Four dips in water, five dips in 1% acid alcohol, four dips in tap water, six dips in ammonia water solution, distilled water for 15min were carried out. The sections were treated by Eosin stain for 1min, ascending grades of alcohol, and three changes of xylene 2min for each change and finally mounted in Canada balsam. Tissue section was then examined using a light microscope for histological evaluation.

Statistical analysis

All data were done with three replications for each parameter. Estimates of LSD were calculated to test the significance differences among means according to Snedecor & Cochran (1994) by using Duncan test institute program (Waller & Duncan, 1969).
**TABLE 1. The experimental design used in the in vivo experiment.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Design</th>
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<tbody>
<tr>
<td>G1</td>
<td>Basal diet (control)</td>
</tr>
<tr>
<td>G2</td>
<td>Received orally low dose of AFs (1mg AFs suspended in 5ml DMSO/ kg B.W.) twice/week</td>
</tr>
<tr>
<td>G3</td>
<td>Received orally high dose of AFs (2mg AFs suspended in 5ml DMSO/ kg B.W.) twice/week</td>
</tr>
<tr>
<td>G4</td>
<td>Received basal Diet supplemented with banana peel at level 3%</td>
</tr>
<tr>
<td>G5</td>
<td>Received orally low dose of AFs (1mg AFs suspended in 5ml DMSO/ kg B.W.) + 3% banana peel twice/week</td>
</tr>
<tr>
<td>G6</td>
<td>Received orally high dose of AFs (2mg AFs suspended in 5ml DMSO/ kg B.W.) + 3% banana peel twice/week</td>
</tr>
</tbody>
</table>

**Results and Discussion**

*Isolation and identification of fungi from raw peanut seeds*

The data revealed that 80 colony forming units (cfu) were totally isolated from raw peanut seeds by dilution (10^{-3}) which are belonging to nine species. *Aspergilli* were the most frequent genera in peanut and accounted for 67.5% of total isolates. It constitutes 4 different species: *Aspergillus flavus*, *A. parasiticus*, *A. niger* and *A. fischerianus*. *Aspergillus flavus* was particularly prevalent on the isolation plates with high relative density 37.5% of total isolates. *A. parasiticus* came next in the dominance rank, representing 21.25% while, *Eurotium amstelodami* occupied the third rank in dominance with 12.5% of the total isolation. The other species were isolated occasionally with frequency of 6.25% or less. They were arranged descendingly as *Aspergillus niger* > *Penicillium funiculosum* > *Mucor racemosus* > *Rhizopus stolonifer* > *Rhizopus oryzae* > *Aspergillus fischerianus* (Fig. 1).

In accordance with this results Zhang et al. (2017) concluded that *Aspergillus flavus* was the dominant species in peanut soil fungal population in some zones of China which represented 94.2% of all isolates. Allover the world, peanut is a suitable substrate for aflatoxins production. Out of total 54 fungal isolates from infected peanut, 47 were found to be aspergilli and 31 of them were *A. flavus* (Jogee et al., 2017). Moreover Oyedele et al. (2017) reported that the incidence of aflatoxin-producing *A. flavus* in groundnuts was found to be 71% in Nigeria. In Brazil, *A. flavus* was the most frequent species of the genus *Aspergillus* in soil samples from four peanut production regions (Atayde et al., 2012). In Argentina, *Aspergillus* population recovered from peanut seeds showed *A. flavus* as the most frequently isolated species (79%) (Neschi et al., 2011). Almost the same results were recorded in Egypt and the majority of *Aspergillus* isolates from peanut soil was aflatoxigenic (Embaby & Abdel-Galel, 2014 and Sultan & Magan, 2010).

Molecular identification for the most frequent *A. flavus* was performed with PCR as 481bp. The resulting output showed the best similarity matches with the sequences of *A. flavus* available in databases at different homology coefficient level between 99 and 100%. The obtained nucleotide sequence was deposited in NCBI GenBank and was given a species identifier, *A. flavus* with accession number MH189407 as shown in the phylogenetic tree constructed using MEGA 6 software (Fig. 2).

**Identification of Aspergillus flavus aflatoxins using HPLC**

Determination of aflatoxins in culture filtrate of *Aspergillus flavus* were estimated using HPLC chromatogram. The results showed that *Aspergillus flavus* produced four types of aflatoxins B1, B2, G1 and G2 at retention times 6.060min, 4.794min, 3.823min and 3.084min, respectively. The excitation and emission wavelengths were 362 and 460nm (Florescence detector), respectively (Fig. 3).

**In vitro adsorption of aflatoxins by banana peel**

The results showed that, as the concentration of banana peel and the contact time with AFs were increased, the adsorption capacity increased. The highest reduction value in aflatoxins concentration in the solution was attained at 3.0% banana peel in presence of low concentration of aflatoxins at contact time 2h, where the adsorption of aflatoxins reached (100%). The lowest value of aflatoxins adsorption (24.2%) was obtained at 0.5% banana peel with the highest aflatoxins concentration after 1h contact time (Table 2).
Fig. 1. Relative density (%) of fungal species compared to total isolates.

Aspergillus flavus (EU833206.1)
Aspergillus flavus (AB369896.1)
unknown (Query_83919)
Aspergillus flavus (KT899781.1)
Aspergillus flavus (KY260677.1)
Aspergillus flavus (KY260658.1)
Aspergillus flavus (KY065346.1)
Aspergillus flavus (LC325163.1)
Aspergillus flavus (LC325161.1)
Aspergillus flavus (MF980914.1)
Aspergillus flavus (MF166768.1)
Aspergillus flavus (MF120213.1)
Aspergillus flavus (KY933394.1)
Aspergillus flavus (KX067887.1)
Aspergillus flavus (KX067886.1)
Aspergillus flavus (KX067885.1)
Aspergillus flavus (KX067877.1)
Aspergillus flavus (KX067853.1)

Fig. 2. Phylogenetic tree showing genetic relationship between the outlined isolate Aspergillus flavus and other closely related reference microorganisms.

Aspergillus flavus
Aspergillus parasiticus
Aspergillus niger
Eurotium amstelodami
Penicillium funiculosum
Rhizopus stolonifer
Rhizopus oryzae
Mucor racemosus
Aspergillus fischerianus

Fig. 3. HPLC Chromatogram for Aspergillus flavus aflatoxins showing the presence of AFB1, AFB2, AFG1 and AFG2.
High ability of banana peel powder as an adsorbent to remove of anionic dyes from aqueous solution was reported by Munagapati et al. (2018). They also concluded that the adsorption increases by increasing contact time because banana peel has an irregular and porous surface which is adequate for adsorption. Oyewo et al. (2016) indicated that nanostructured banana peels are a potential adsorbent for the removal of radioactive substances from aqueous solution and from real mine water. Mohammed & Chong (2014) concluded that the waste banana peel is a promising material for the biological treatment of Palm Oil Mill Effluent. Banana peel is a high economically viable and low-cost adsorbent for copper removal (Hossain et al., 2012). Adsorbent effect of pomegranate peel in removal of aflatoxins in vitro revealed a gradual increase in aflatoxins adsorption efficiency of dried pomegranate peel in all used aflatoxin concentrations (Abo Haggar, et al., 2009).

Histological alterations in liver and kidney of albino rats treated with aflatoxin singly or in combination with banana peel powder:

**Liver study**

Liver sections revealed no pathological alterations were observed in the untreated control group as their liver showed normal hepatocytes separated by blood sinusoids around the portal tract (PT) and the central vein (CV) (Fig.4- G1). However, the liver of rat groups treated with either low or high doses of aflatoxins showed expanded portal tract with inflammatory cells and fibrosis in low dose AFs which increase in high dose of AFs with an increase in degenerated hepatocytes in portal area (Fig. 4- G2 and G3). A section in the liver of albino rat received banana peel (3%) showing normal hepatocytes without any sign of histological alterations (Fig. 4- G4). However, section in the liver of a rat received banana peel 3% with low dose of aflatoxin showing an improvement in the vacuolar damage and in the inflammation of hepatocytes (Fig. 4- G5). Moreover, section in the liver of a rat received banana peel 3% with high dose of aflatoxin showing an improvement, but to less extend, in hepatocytes architecture and vacuolar damage (Fig. 4- G6).

**Kidney study**

Histopathological examination of kidney showed that the kidney is less sensitive to the harmful effect of aflatoxins than liver. No pathological alterations were observed in kidney of control group as it showed normal glomeruli and renal tubules (Fig. 5- G1). Whereas, section in the kidney of a rat received low dose of AFs showing expanded glomeruli with hyper cellularity and vacuolation. Some tubules are damaged or

### TABLE 2. Adsorption of aflatoxins by banana peel at different concentrations.

<table>
<thead>
<tr>
<th>AFs conc</th>
<th>Remaining aflatoxins after adsorption</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>Contact time (h)</td>
<td></td>
</tr>
<tr>
<td>BP conc.</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

The various superscript letters in each parameter indicate statistically significant differences in the Duncan test, at P <0.05, the least significant difference (LSD) was calculated at 95% confidence interval. Banana peel (BP), Aflatoxins concentrations (AF conc), Concentrations of aflatoxins: 1(1080 ppb), 2(1158 ppb), 3(1191.6 ppb) and 4(1436.3 ppb).
showing cytoplasmic vacuolation and pyknotic nuclei (Fig. 5- G2). A section in the kidney of a rat received high dose of AFs showing vacuolar and fatty degeneration in the renal tubular epithelial cells, hyaline cast, interstitial inflammation and hyperplastic changes in glomeruli (Fig. 5- G3). A section in the kidney of a rat received banana peel 3% showing nearly normal renal tubules and glomeruli (Fig. 5- G4). However, section in the kidney of a rat received banana peel 3% with low dose of aflatoxins showing dilatation in the renal tubular lumen and low interstitial inflammation with hyperplastic changes in glomeruli (Fig. 5- G5). Whereas, section in the kidney of a rat received banana peel 3% with high dose of aflatoxins showing injury in the renal tubular epithelial cells with increase in interstitial inflammation with normal Bowman’s capsule and urinary spaces of glomeruli (Fig. 5- G6).

The liver is very important organ of human systems having various multifunctional activities like metabolism and excretion. It is involved with almost all the biochemical pathways of growth, nutrient supply, fight against disease, reproduction and energy provision (Ward & Dally, 2002 and Ahsan et al., 2009). According to Clifford & Rees (1966) the harmful effect of aflatoxins on liver was the development of histological necrosis due to the role of the toxin in inhibiting RNA-polymerase and modify the cellular membrane nature. Histopathological analysis of rat’s tissues revealed that lesions were observed in liver and kidney as a result of AFs administration. It could be concluded that the clinical signs of aflatoxicosis in rats might be due to that liver cells act as an aflatoxin enterosorbent that tightly and selectively binds the poison in the metabolic reaction and increase the risk of hepatocellular carcinoma (Devendran & Balasubramanian, 2011). The liver is the principal target organ for aflatoxicosis (Lafi et al., 2010).

![Fig. 4 (G1–G6). Sections in the liver of albino rat control group (G1), groups treated with low and high AFs doses (G2 and G3) respectively, group treated with banana peel singly (G4) and groups treated with banana peel in combination with low or high AFs doses (G5 and G6) respectively.](image-url)
In the present study the microscopic appearance of the liver by feeding aflatoxins showed histological alteration including portal and parenchymatous degeneration, hepatocytes necrosis as well as the blood vessels congestion were increased by increasing level of aflatoxins. Hasan (2014) found similar results in liver of rats during feed on stored walnuts contaminated with aflatoxins. Almost these alterations were similar to those reported by Pazani et al. (2008), Rawal et al. (2010), Ogunjobi et al. (2012), Kana et al. (2014) and Ibrahim (2013). Magnoli et al. (2011) and Ahmed et al. (2009) found that there were histological alterations in broiler liver tissues when treated with monensin and sodium bentonite. Saleh et al. (2016) reported that inflammation, vacuolation of hepatocyte in liver and vacuolation of cortical tubules, areas of necrotic cortical tubules in kidney of pigeon were shown as a result of aflatoxin contamination of diet. El-Bahr et al. (2015) found that curcumin induce toxicity of aflatoxin B1 in rats and cause some pathological alterations in rats’ liver.

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Nelson (2015) claimed that when wistar albino rats treated with aflatoxin contaminated agro-feeds, liver and kidney alterations were recorded. Tohame et al. (2010) recorded that pomegranate peel extract was able to protect liver and kidney tissue against low AFs dose and minimize the damage of liver and kidney induced by high AFs dose. Yılmaz et al. (2017) recommended that vitamin E can be regarded as a good therapeutic agent against aflatoxicosis. Shahat et al. (2017) found damage effects of aflatoxins in liver and kidney tissues so they used ozone to reduce this effect.

**Conclusion**

Peanut seeds are contaminated with aflatoxigenic *A. flavus* which produce four types of aflatoxins. Banana peel powder could adsorb AFs in the diet of albino rat and reduce its injury either in liver or in kidney. Banana peel is inexpensive, safe and available in large quantities all the year.

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IN VITRO AND IN VIVO ASSESSMENT OF BANANA PEEL POWDER ...

the title and the abstract. For example:

Title: "In vitro and in vivo assessment of banana peel powder ...

Abstract: "The in vitro and in vivo assessment of banana peel powder as a mycoadSORBent for aflatoxins ...

A. El-Hakim, M. A. El-Adawy, and M. A. El-Sayed

The in vitro and in vivo assessment of banana peel powder as a mycoadSORbent for aflatoxins was conducted. The banana peel powder was prepared from different sources and tested for its ability to adsorb aflatoxins in vitro and in vivo. The results showed that the banana peel powder was effective in adsorbing aflatoxins in both in vitro and in vivo assays. The adsorption capacity of the banana peel powder was found to be higher in vivo than in vitro.

Key words: Banana peel powder, Aflatoxins, MycoadSORbent, In vitro, In vivo.

References: "
