PROTECTIVE EFFECT OF NANO-SELENIUM AGAINST EXPERIMENTALLY INDUCED TOXICITY BY AFLATOXIN B1 (AFB1) ON THE GINGIVA AND PERIODONTAL LIGAMENT OF ALBINO RATS (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Objectives: This current study was done to evaluate Nano-selenium (Nano Se) ability in the inhibition of aflatoxin B1 toxicity on gingiva and periodontal ligament of rats through histological investigation and immunohistochemical detection of TNF-α & caspase-3 expression in gingiva and desmin expression in the periodontal ligament.

Materials and Methods: Forty adult male albino rats were divided into 4 equal groups as follows: Group I served as control rats (normal), Group II (aflatoxin B1 group) was given aflatoxin B1 (250 μg/kg b.w/day) orally through gastric tube 5 days/week for 4 weeks, Group III (AFB1 and Nano-Se group) received aflatoxin B1 (250 μg/kg b.w./day) and 100 mg /animal of Nano-Se orally for 5 days/week for 4 weeks. Group IV (Nano-Se group) received Nano-Se 100 mg /animal orally for 5 days/week for 4 weeks. At the end of the experiment, dissection of upper and lower jaws were carried out after scarification under ketamine anesthesia. Gingival and jaw samples were collected and processed for histological techniques. Immunohistochemical estimation of TNF-α as pro-inflammatory mediator, caspase-3 to determine any apoptotic cells and desmin for detection of collagen fibers was performed.

Results: Histological results: Aflatoxin B1 treated rats showed marked histopathological changes in the surface epithelium and lamina propria of gingiva and noticeable degeneration of collagen fibers of periodontal ligament (PDL). While aflatoxin B1 & Nano-Se treated rats revealed displayed histological improvement in both gingiva and PDL against toxic effects induced by AFB1. Immunohistochemical results of the gingival specimens of AFB1-treated rats incubated with TNF-α and caspase-3 monoclonal antibodies showed that their epithelium and underlying lamina propria had strongly positive immunostaining reactivity. However, surface epithelium and lamina propria of aflatoxin rats treated with Nano-Se showed weakly to moderately positive immunostaining reactivity. Immunohistochemical localization of desmin monoclonal antibody for examination of jaw specimens showed weak immunostaining reactivity in aflatoxin-treated rats. While, PDL cells and fibers were ranged between moderate to strong reaction in aflatoxin B1 & Nano-Se treated rats.

Conclusion: The results of this current study indicated that nano selenium as an antioxidant can alleviate the toxic effects that induced by AFB1.

KEYWORDS: Aflatoxin B1, nano selenium, TNF-α, caspase-3, desmin.

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INTRODUCTION

Mycotoxin contamination had a high toxic effect on the health of humans and animals, even at low doses. About 25% of the world’s food crops and animal products are significantly contaminated with mycotoxins. Aflatoxin consumption can cause growth retardation, severe organ damage, hemorrhage and death. Aflatoxicosis has two forms been identified; first form is acute severe intoxication, and second form is chronic subsymptomatic exposure which occurs in association with different health problems. Several animals were subjected to mutagenic, carcinogenic and teratogenic effects of aflatoxins.

Aflatoxins (AFs) are produced mainly by Aspergillus flavus, Aspergillus parasiticus and other Aspergillus species; are widely detected in agricultural products. They were classified into four natural types, aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2. The most toxic and potent form of aflatoxins is Aflatoxin B1 (AFB1), which had the ability to induce acute toxicity. Moreover, aflatoxins can affect and interfere with the immunological responses of the living organisms leading to their predisposition to infections, parasitic diseases and decreasing efficacy of vaccines.

Selenium (Se), a metalloid mineral micronutrient, is an essential trace element. It has anti-carcinogenic effects. It plays a biological role in glutathione peroxidase active center; the key enzyme for body fluids and tissues which has a primary defensive role in the antioxidant protection system.

Nanotechnology has promise for medication and nutrition as materials at the nanometer dimension show new properties unlike those of both bulk material and isolated atoms. A global attention is now directed towards Nanoselenium (Nano-Se) and that is due to the new characteristics displayed by nanometer particulates as high surface activity, large surface area, high catalytic efficiency, low toxicity and strong adsorbing ability. Nano-selenium is defined as nano-elemental selenium which is manufactured to be used in nutritional supplements and also developed to be applied in medical therapy besides Se fertilization. It is bright red, highly stable and of nano-size in the redox state of zero (Se0). Nano-Se has more effectiveness in up-regulating selenoenzymes and reveals less toxicity than selenite.

AFT-B affects natural killer (NK) cell expression of inflammatory cytokines as it reduced the expression of anti-inflammatory cytokine IL-4, however, it increased the expression of pro-inflammatory cytokine IFN-γ and TNF-α.

Cell, tissue and organ development, differentiation, proliferation and homeostasis is regulated by a process named apoptosis. Excessive apoptosis of several mammal and poultry cells such as hepatocytes, bronchial epithelial cells, bone marrow cells and renal cells was caused by AFB1. The main intermediate filament protein which is known as desmin interacts with other proteins forming a continuous cytoskeletal network. These proteins form a fibrous network which connects myofibrils together and to the plasma membrane. Desmin protein ablation leads to formation of defects in the contractile apparatus architecture leading to myopathy. Cellular maturation is inhibited by AFB1 by causing disruption of the cytoskeleton, including microtubules and microfilament.

Therefore, this study objective was to assess the toxic effects of aflatoxin B1 on the gingiva and periodontal ligament of rats; and to evaluate the protective role of nanoselenium (Nano-Se) administration. This was done using histological investigation and immunohistochemical detection of of TNF-α & caspase-3 in gingiva and desmin expression in the periodontal ligament.
MATERIALS AND METHODS

This study included forty adult male albino rats with average weight 180-250 g. Rats were kept around 25 °C in a normal light-dark cycle and were housed in cages of stainless steel. They had free access of water and were fed standard laboratory feed. This study was done in agreement with animal care guidelines in the animal house of Faculty of veterinary medicine, Suez Canal University.

Rats were divided into 4 equal groups (10 animals each):

- **Group I**: Rats served as control rats (normal) received saline (0.9 % NaCl) for 4 weeks.
- **Group II** (aflatoxin B1 group): Rats received 250 μg/kg body weight/day of aflatoxin B1. Aflatoxin B1 dissolved in olive oil as a vehicle by gastric tube 5 days/week for 4 weeks.
- **Group III** (AFB1 and Nano-Se group) received aflatoxin B1 (250 μg/kg b.w./day) and 100 mg/animal of Nano-Se orally through gastric tube for 5 days/week for 4 weeks.
- **Group IV** (Nano-Se group) received 100 mg/animal of Nano-Se orally for 5 days/week through gastric tube for 4 weeks.

Aflatoxin B1 was acquired from Sigma Chemical Company (St Louis, Missouri, USA). It is an odorless powder with a white to faint yellow color. The storage of aflatoxin B1 should be at a temperature of 2 to 8°C in vials of amber glass as it is a light-sensitive mycotoxin. It should be cautiously transferred to prevent dissemination to the surroundings. Dissolution of aflatoxin B1 in olive oil was done to serve as a vehicle.

Preparation of Nano-selenium was done by a simple wet chemical method according to Dwivedi et al. In brief, sodium selenosulphate precursor was reacted with different organic acids in aqueous medium, under ambient conditions. In order to stabilize the selenium nanoparticles, polyvinyl alcohol has been used. After that, separation of the synthesized nanoparticles (Nano- Se; 50 - 90 nm in size) from their sol was done by using a high-speed centrifuge and redispersed in aqueous medium with a sonicator.

Observation of rats took place daily to notice any sign of toxicity. During the experiment, recording of Food intake and body weights was done daily.

Histopathology and immunohistochemistry:

At the end of the experiment, Sacrification of animals of the different groups by cervical dislocation was done under ketamine anesthesia (80 mg/kg, i.p.); the upper and lower jaws were dissected out and separated into two halves. Gingival specimens were taken from the first molar region of the right halves of the jaws, fixed for 24 hours in 10 % neutral buffered formalin, Dehydration of fixed specimens was done in ascending grades of ethyl alcohol, then specimens were cleared in xylol, embedded in three changes of paraffin wax and sectioned at 4-6 μm thick sections. Jaw specimens of the left halves were similarly fixed, washed and decalcified in 10 % EDTA solution. After complete decalcification, specimens were washed, processed, embedded in paraffin and sectioned.

Six microns thick sections of the gingiva and decalcified jaws were cut. Staining of sections was done with Hematoxylin and Eosin stain for detection of structural changes that found in the gingiva and periodontal ligaments (PDL). Also, immunohistochemical detection of TNF-α & caspase-3 in gingiva and desmin in jaw specimens was done.

The immunohistochemical procedure for detection of TNF-α, caspase-3 and desmin was as follow: dewaxing of paraffin sections and hydration using 3% hydrogen peroxide (Beyotime Biotechnology) were done to inhibit the endogenous peroxidase. Then, addition of either ~50 μl (1:50) rabbit polyclonal anti-TNF-α antibody, anti-caspase-3
antibody or rabbit polyclonal anti-desmin (1:1,000) antibody was done and then incubated at 4°C overnight. After finishing incubation, addition of ~50 μl biotinylated goat anti-rabbit immunoglobulin G secondary antibody (1:100; cat. no; A0277; Beyotime Biotechnology) working solution was done, then incubation of the mixture was done at 37°C for 30 min, and was colored using diaminobenzidine. Samples were counterstained with hematoxylin (Beyotime Biotechnology), dehydrated with a graded alcohol series and xylene, and mounted

Statistical analysis:
To compare the mean labeling index of immunostained reaction of the experimental groups, collection and expression of data was done as mean ± S.E.M. For statistical analysis, one-way ANOVA, followed by application of Bonferroni’s test for multiple comparisons. Statistical comparisons were made using SPSS 12.0 statistical software. The results were considered significant at P ≤ 0.05.

I- Histological results (H&E):

a- The Gingiva:
Group I (Control group) surface epithelium and underlying lamina propria revealed normal histological features. The gingival epithelium consisted of keratinized stratified squamous epithelium which characterized by numerous, slender, long and irregular epithelial ridges toward the underlying lamina propria. Four categories of cell layers were recognized in the epithelium; basal, prickle, granular and keratinous cell layer. The papillary and reticular layers were exhibited in the underlying lamina propria (Fig.1 A). Group II (aflatoxin B1 group): Marked histopathological changes were displayed in the gingival epithelium and lamina propria. The characteristic pattern of the epithelial ridges was lost; they became broader and more flattened than control. In addition, lacking of normal architecture of the basal cell layer was observed. This was manifested by the discrete disintegration of the basement membrane, loss of basal cell adhesion with noticeable invasion of basal cells into subepithelial connective tissue. Moreover, acanthosis of prickle cell layer was detected. The granular cell layer was thicker and hyperkeratinized. Lamina propria revealed marked degeneration of collagen fibers and massive infiltration with inflammatory cells (Fig.1B). While, Group III rats treated with aflatoxin B1 and Nano-Se showed nearly numerous, slender, long and irregular epithelial ridges with intact basement membrane. Lamina propria showed mild collagen fiber degeneration with decrease in the inflammatory cell infiltration (Fig.1C). Group IV rats; which received Nano-Se only, surface epithelium and lamina propria revealed nearly normal histological features (Fig.1D).

b) The Periodontal ligament:
The periodontal ligament of control rats is formed of cells and intercellular substance which contained abundant collagen fibers and ground substance. In addition to blood vessels, lymphatics and nerves. The alveodental group of fibers was seen extending from cementum to bone that divided into subgroups; mostly in the form of oblique fibers that directed from bone to cementum. The collagen fibers were grouped into bundles that displayed small interstitial spaces containing areolar connective tissue with blood vessels and nerves (Fig.2 A). Jaw specimens of aflatoxin B1 treated rat showed marked degeneration and loss of orientation of the collagen fibers. They were frequently detached either from the cementum, bone or both that associated with osteoclastic bone resorption (Fig.2 B). However, Jaw specimens of rats treated with aflatoxin B1 and Nano-Se presented mild degeneration of the periodontal tissue and limited alveolar bone resorption (Fig.2 C). PDL of Nano-Se treated rats showed condensation of the collagen fibers of the PDL with normally appearing fibroblasts (Fig. 2 D).
Fig. 1: A) Gingiva of control rat showing normal histological structure of surface epithelium of keratinized stratified squamous type and lamina propria. B) Gingiva of aflatoxin B1 treated rat showing loss of the characteristic pattern of the epithelial ridges, the basal cells lost their normal architecture, acanthosis of prickle cell layer with hyperkeratinization. Marked degeneration of collagen fibers of the lamina propria with massive inflammatory cells infiltrate. C) Gingiva of rat treated with aflatoxin and Nano-Se showing the epithelial ridges appeared nearly normal and the basement membrane appeared intact with mild degeneration of collagen fibers of lamina propria. D) Gingiva of rat received Nano-Se showing nearly the same histological features of the control rat. (H&E, orig. mag. 400).

Fig. (2) A) PDL of control rat showing oblique fibers that were obliquely directed from alveolar bone to cementum. B) PDL of aflatoxin treated rat showing marked degeneration and loss of orientation of the collagen fibers and their frequent detachment either from the cementum surface. C) PDL of rat exposed to aflatoxin and Nano-Se showing mild degeneration of the periodontal fibers and cells and limited alveolar bone resorption. D) PDL of rat received Nano-Se showing almost the same histological features of the control group with normal condensation of the collagen fibers. (H&E, orig. mag. 400).
II -Immunohistochemistry:

a) Immunohistochemical detection of TNF-α:

Examination of gingival sections immunostained with TNF-α monoclonal antibody, control rats surface epithelium showed negatively to weakly positive staining reactivity meanwhile their lamina propria showed weak immunostaining (Fig. 3A). In contrast, aflatoxin treated rats showed strongly positive staining reactivity of the cells of the basal, prickle and granular cell layers of their surface epithelium and lamina propria (Fig. 3B). Surface epithelium and lamina propria of aflatoxin rats treated with Nano-Se showed weakly to moderately positive immunostaining (Fig. 3C) and Nano-Se treated rats revealed weakly positive staining reactivity (Fig. 3D).

c) Immunohistochemical detection of caspase-3:

Examination of gingival sections immunostained with caspase-3 monoclonal antibody, control rats surface epithelium and lamina propria showed negatively to weakly positive staining reactivity (Fig. 4A). Conversely, strongly positive staining reactivity of basal, parabasal and prickle cell layers of the surface epithelium and lamina propria of aflatoxin B1-treated rats (Fig. 4B). Surface epithelium and lamina propria of aflatoxin B1 rats treated with Nano-Se showed weakly to moderately positive immunostaining (Fig. 4C) and Nano-Se treated rats revealed weakly positive staining reactivity (Fig. 4D).

b) Immunohistochemical detection of desmin:

Using desmin monoclonal antibody for examination of jaw specimens; PDL cells and fibers in the control group showed strong immunostaining reactivity (Fig. 5A). While, weak immunostaining reactivity was in aflatoxinB1-treated rats (Fig. 5B). PDL cells and fibers were ranged between moderate to strong reaction in aflatoxinB1 rats treated with nonotherapy (Fig. 5C) and Nano-Se treated rats showed strongly positive immunostaining of PDL cells & fibers (Fig. 5D).

Statistical analysis

There is a significant statistical difference (p<0.01) of aflatoxin-treated rats II in comparison with control group I using one-way ANOVA test. Also, there is high significant differences (p<0.01) of AFB1 + Nano-Se group III when compared with AFB1group II. As the mean values are considered significant when the P value < or = 0.05.

Fig. 3: A) TNF-α immunostaining of gingival sections of control rat showing weak immunostaining of cells of the surface epithelium and underlying lamina propria, B) Gingiva of aflatoxin-treated rat with strong immunostaining of the surface epithelium and underlying lamina propria, C) Epithelium of rats exposed to aflatoxin and Nano-Se displaying weakly to moderately positive staining reactivity, D) Gingiva of rats received Nano-Se showing weakly positive staining reactivity of surface epithelium & underlying lamina propria (orig.mag.400).
Fig. 4: A) Caspase 3 immunostaining of gingival sections of control rat showing either negative or weak staining of cells of the surface epithelium and underlying lamina propria, B) Gingiva of AFB1-treated rat with strong immunostaining of the surface epithelium and lamina propria, C) Epithelium of rats exposed to aflatoxin and Nano-Se displaying weakly to moderately positive staining reactivity, D) Gingiva of rats received Nano-Se showing negative to weak positive staining reactivity of surface epithelium & underlying lamina propria (orig. mag. 400).

Fig. 5: A) Desmin immunostaining of PDL cells & fibers of control rats exhibiting strongly positive staining reactivity, B) PDL of aflatoxin-treated rats showing weakly desmin immunostaining, C) PDL cells & fibers of rats exposed to aflatoxin and Nano-Se displaying moderate to strong positive desmin immunostaining, D) PDL of rats received Nano-Se showed strongly positive immunostaining of PDL cells & fibers (orig. mag. 400).

TABLE (I) Illustrates the mean labelling index of NF-α & caspase-3 in gingiva & Desmin in PDL in the four groups of the experiment:

<table>
<thead>
<tr>
<th></th>
<th>Control group I</th>
<th>AFB1 group II</th>
<th>AFB1+Nano-Se group III</th>
<th>Nano-Se group IV</th>
<th>ANOVA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (Gingiva)</td>
<td>62.641 ± 8.25</td>
<td>158.634 ± 5.82</td>
<td>99.462 ± 7.26</td>
<td>50.971 ± 5.91</td>
<td>P ≤ 0.5 *</td>
</tr>
<tr>
<td>Caspase 3 (Gingiva)</td>
<td>79.531 ± 6.203</td>
<td>160.341 ± 7.029</td>
<td>112.521 ± 5.056</td>
<td>87.452 ± 4.24</td>
<td>P ≤ 0.5 *</td>
</tr>
<tr>
<td>Desmin (PDL)</td>
<td>179.038 ± 4.25</td>
<td>119.906 ± 7.99</td>
<td>154.73 ± 5.76</td>
<td>180.776 ± 6.17</td>
<td>P ≤ 0.5 *</td>
</tr>
</tbody>
</table>

Group II versus group I  P ≤ 0.5 *
Group III versus group II  P ≤ 0.5 *

Values are expressed as mean ± standard deviation.

*P<0.001 versus control group.
DISCUSSION

The current study showed that AFB$_1$ treated rats showed loss of the characteristic pattern of the epithelial ridges and basal cells of the gingiva with loss of adhesion of basal cells and acanthosis of prickle cell layer with hyperkeratinization. The lamina propria showed marked degeneration of collagen fibers with massive inflammatory cells infiltration. In addition to, degeneration and disorganization of the collagen fibers of PDL associated with osteoclastic bone resorption. These histopathological changes suggest that exposure to AFB$_1$ exerts many toxic effects which can be attributed to the ability of AFB$_1$ to induce reactive oxygen species (ROS) formation, lipid peroxidation (LP) and 8-hydroxydeoxyguanosine (8-OHdG) formation in vivo and in vitro 19. It is assumed that AFB$_1$-induced ROS formation and oxidative DNA damage that have an essential role in AFB$_1$ carcinogenicity 20. If the reactive oxygen species exceed the antioxidant capacity of the cell it will lead to oxidative damage to cells and tissues. These ROS of oxidative stress had the ability to induce alteration and damaging cell compounds and membranes, eventually resulting in cell functioning impairment, cytolysis and interstitial fibrosis 21.

Cellular cytochrome P450 (CYP450) activates AFB$_1$ to form the reactive intermediate, AFB$_1$-8, 9-epoxide (AFBO). The subsequent covalent binding of this epoxide to DNA to generate AFB$_1$-DNA adducts is considered to be a critical step in the apoptosis and carcinogenicity of AFB$_1$ 22. Lipid peroxidation and oxidative DNA damage could be the cause of AFB$_1$ induced apoptosis. AFB$_1$ can result in direct or indirect activation of caspase-3 and as a result leading to apoptosis in gingival specimens of rats. The occurrence of cell apoptosis is supported by our immunohistochemical study as AFB$_1$ treated rats showed noticeable increase of protein Caspase-3 signals expression (brown color in cytosol).

Furthermore, Several immunotoxic effects are exerted by AFB$_1$, such as innate immunity or antigen-presenting cells alterations, adaptive immunity changes, resulting in a reduced number of circulating lymphocytes, lymphocyte blastogenesis inhibition and cytokine expression alteration in various animal species 23.

TNF-α is an important factor in tumor promotion. Moreover, TNF-α is a key factor which plays a role in regulation of the production of other cytokines involved in tumor development and chronic inflammation, through the NF-κB pathway 24,25. Treatment with AFB$_1$ markedly increased TNF-α, NO and IL-1α by its effects on functioning of macrophages 26. It is well known that macrophages produce TNF-α, IL-1α and NO and they play an essential role in tumor conditions 27.

This is assured by our immunohistochemical results of the gingival specimens of AFB$_1$-treated rats and incubated with TNF-α monoclonal antibody revealed strongly positive staining reactivity of the epithelium and the underlying lamina propria. Our results were in agreement with the study done by Pinton & Oswald (2014) who reported that low-dose exposure to mycotoxins induces chemokine production in epithelial cells, IL-2 production in human lymphocytes and pro-inflammatory cytokine production, including IL-8, IL-6, and TNF-α in human macrophages 28. Consequently, mycotoxins exposure will cause alterations in human and animal immune responses, which results in impaired pathological damages to mycotoxin-exposed tissues.

Exposure to AFB$_1$ causes acceleration of the inflammatory responses through regulation of cytokine gene expression. In addition to, AFTB1 interrupts the process of antigen-presenting capacity, taken this into consideration makes it one of mechanism of immunotoxicity by AFB$_1$ 29.

Desmin intermediate filaments are secured to the mitochondria and nucleus in order to maintain their subcellular localization during and after contraction which preserves the relationship of the myocontractile apparatus 29. In the present study, AFB$_1$ exposure
led to weak immunostaining with desmin indicating disruption of the components of cytoskeleton of myofibroblasts of PDL. This results are in accordance with that of Jun Liu et al (2015) who reported that AFB1 disrupt oocyte maturation by disturbing the cytoskeleton.

Nano-selenium shows protective effects against AFB1-induced toxicity through inhibition of oxidative stress and subsequent excessive apoptosis. ROS-mediated apoptosis could be suppressed by selenium through inhibition of apoptosis due to ROS and mitochondrial dysfunction, and this could be explained by the antioxidant effect of Se. Nano-Se and is a strategy for toxicity avoidance by nanotechnology application. It has been stated that Nano-Se has a size-dependent effect in scavenging many free radicals; small-size Nano-Se has more ability to transfer electrons to radicals.

Our results state that AFB1-induced apoptosis could be inhibited by Nano-Se, which may be due to its anti-oxidant function. This is confirmed by our immunohistochemical study as caspase-3 protein expression is down-regulated in aflatoxin rats treated with Nano-Se in comparison with AFB1 treated group. This result stated that to some extent supplemented Se could protect AFB1-induced apoptosis, however cannot restore it to the normal level. At nutritional doses, Se is an essential component of selenocysteine (SeCys) in selenoproteins, and it plays a role in cell cycle progression promotion and cell death prevention. Consequently, Se could counteract the adverse effects of AFB1.

The present finding revealed that Nano-Se administration improved histological structure of both gingival and jaw specimens against toxic effects induced by AFB1, as the epithelial ridges of gingiva appeared nearly numerous, slender, long and irregular and the basement membrane appeared intact. The lamina propria showed mild degeneration of the collagen fibers with decrease in inflammatory cells infiltration. Also, Jaw specimens of rats exposed to aflatoxin and Nano-Se presented mild degeneration of the periodontal fibers and cells and limited alveolar bone resorption. These results are in agreement with Zhang et al (2004) who showed that Nano-Se can serve as a potential chemopreventive agent with lower risk of Se toxicity. Nano-Se has lower toxicity and has the same efficacy in increasing the activities of selenoenzymes and glutathione S-transferase (GST). High expression of GST has been reported to be protective against development of tumors. GST, responsible for detoxifying harmful compounds and scavenging lipid peroxidation, was induced much earlier and higher by Nano-Se.

CONCLUSION

Nano-Se have the potential effect to alleviate AFB1 toxicity in rats by reducing AFB1-induced oxidative stress and apoptosis, thus, it could be used as a novel anti-mycotoxin agent.

REFERENCES


26. Pmc, E., Suppression of the interleukin-2 gene expression by aflatoxin B1 is mediated through the... - Abstract - Europe PMC. 1999.


