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# Vitamin A downregulating Bcl-2 and TGF-α expression during colon cancer in AFB1-induced female rats

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**Abstract**

Aflatoxin B1 (AFB1) is the most toxic and is usually predominant. Aflatoxins are not only contaminate our food stuffs, but also are found in edible tissues, milk and eggs. The present study designated to clarify the immunomodulatory effect of vitamin A and if it could ameliorate the cancerous effects of AFB1 on histopathological, ultrastucture and immunohistochemical changes of rat colon. Group (I): Animals of this group was normal control. Group (II): Rats of this group were orally administered vehicle 50% dimethylsulfoxide. While, animals in Group (III) were administered 132 IU Vitamin A, and rats in Group (IV) were administered 0.05 µg/kg AFB1 dissolved in 50% dimethylsulfoxide for 14 weeks. Group (V): Animals of this group were administered AFB1 with Vitamin A.

AFB1 administration caused colon damage characterized by aberrant crypt foci, which authenticated with the increase in mucous production, Bcl-2 and TGF-α expression. The immunological effect of vitamin A appeared in the improved histological picture of the colon tissue and the decrease in Bcl-2 and TGF-α expression. This is the first study to report the immunomodulatory effect of vitamin A on Bcl-2 and TGF-α in AFB1-induced colon cancer.

**Keywords:** Colon cancer - Aflatoxin B1- Vitamin A- Bcl-2 - TGF-α - young female rats

## 1. Introduction

Colon cancer is one of the major causes of cancer death. There is a wide geographic variation in incidence, with a 20-fold variance worldwide [1]. Aflatoxins are secondary metabolites produced by *Aspergillus* a widely occurring genus of mold fungi. Food and feed products infested by mold fungi can be contaminated with aflatoxins [2]. This is a serious hazard to animal and human health, as aflatoxins are mutagenic and carcinogenic. Aflatoxin $B_1$ ($AfB_1$) is the most hazardous mycotoxin in this group, and there is no threshold concentration for its toxic effect. AFB1 is classified as a group-1 carcinogen. Since the ingestion of aflatoxins-contaminated food is associated with several liver diseases [3-5], but is also a colon carcinogen [6]. Several factors may enhance the occurrence of mycotoxin in the human diet in developing countries. These include eating habits, existing marketing problems which encourage long storage periods; the pre- and post-harvest practices that encourage accumulation of moisture and thus mold growth, ignorance, and poverty. This is aggravated by the fact that there are no strict regulations that impose limits on the concentration of mycotoxins in crops that are marketed in these countries as well as lack of relevant technology required in monitoring fungi and mycotoxins in grains [7]. The biological response to AFB1 in terms of genotoxicity and cytotoxicity depends on the metabolic formation of AFB1-8,9-epoxide [8], which can covalently bind to nucleic acids or proteins, provoking cell membrane damage, necrosis and mutagenesis in the affected cells [9].

Apoptosis is the programmed death of cells. It is central to the development and homeostasis of multicellular organisms [10], and it is the route by which unwanted or harmful cells are eliminated from the organism. Bcl-2 is a family of genes and proteins that govern the MMP. Bcl-2 derives its name from B-cell lymphoma 2 which came from being the second member of a range of proteins initially described as a reciprocal gene translocation in chromosomes 14 and 18 in follicular lymphomas. The genes and proteins can be either pro-apoptotic (Bax, BAD, Bak and Bok) or anti-apoptotic (Bcl-2, Bcl-xL, and Bcl-w). These genes interact with the Bcl-2 protein structure, which result in either a pro- or anti-apoptosis function. Bcl-2 is involved in a number of cancers. These include melanoma, breast, prostate, and lung carcinomas, as reported by Reed et al. [11].

Transforming growth factor alpha (TGF-α) is a polypeptide, which binds to the epidermal growth factor receptor to carry out its function related to cell proliferation and differentiation [12]. TGF-α is synthesised as a larger precursor of 159 or 160 amino acids, which is an integral membrane glycoprotein (pro TGF-α). The mature form is released by proteolytic cleavage of pro TGF-α [13]. Different forms of pro TGF-α have been shown to accumulate in the membranes of growth factor producing cell lines.

Vitamin A (vit.A) is significantly prevents aflatoxin-induced damages in the tissue such as liver, kidney and gizzard of chicks. Many reports delineated that vit.A is anti-mutagenic, both *in vivo* and *in vitro* to prevent aflatoxin induced liver damage. Gradelet et al. [14] reported that carotenoids exert their protective effect through the deviation of AFB1 metabolism towards detoxication pathways. Carotenoids are also effective in reducing DNA damage but less effective than vit. A. However, no data are currently available on the ability of vit. A to prevent aflatoxin toxicity in rat colon. Young animals are more susceptible, with the sex and mode of administration of the toxin affecting the response. Therefore, this study was designed to evaluate the immunomodulatory mechanism of vitamin A to reduce toxicity of aflatoxin B1 in colon of young female rat.

## 2. Material and Methods

### 2.1 Animals and experimental doses:

Young female albino Wistar rats weighing 80-100 g, were used in this study. Animals were purchased from Animal House, Faculty of Medicine, Ain Shams University. The rats were housed in a room maintained at constant humidity (60±5%), temperature (23±1°C), and a 12-h light/dark cycle. The standard diet and tap water were available *ad libitum* throughout the study. After one-week acclimatization period, the animals were subsequently divided into five groups of 5 rats each.

**Group (I):** Rats of this group kept as normal without any treatment and considered as controls.

**Group (II):** Rats belonging to this group were orally administered vehicle 50% dimethylsulfoxide (DMSO).

**Group (III):** Animals of this group were orally administered vehicle with Vitamin A (132IU), which is the double human therapeutic dose.

**Group (IV):** Animals of this group were orally administered 0.05µg/kg AFB1 dissolved in 50% DMSO.

**Group (V):** Animals of this group were orally administered 0.05µg/kg AFB1+Vitamin A.

The study duration lasted for 14 weeks, the animals were anaesthetized under ether vapor and dissected 24 hours post treatment.

### 2.2 Chemicals:

Aflatoxin-B1 were obtained from Sigma Chemical Company (St. Louis, USA). It was dissolved in sun oil and orally given at dose 0.05 µg/kg bw/day. Vitamin A is available in market as capsules contain 50000 1U, as described by Sinha and Dharmshila [15].The therapeutic dose for rat was calculated according the weigh of rats as tabulated by Paget and Barnes [16].

### 2.3 Histological and Electron Microscope Preparations:

Pieces of colon were immediately removed after sacrifice, fixed in 10% formalin solution, dehydrated in ascending series of alcohol, cleared in xylene and finally embedded in paraffin wax. Sections of 4 mm thickness were cut using rotary microtome and mounted on clean slides, for histological examination sections were stained with haematoxylin and eosin according to [17]. For electron microscopic studies, fresh small pieces of colon were fixed in 3% glutaraldhyde-formalhyde for 5 h. then in (0.2 M) Na cacodylate for 2h. at 4°C, then washed in phosphate buffer pH. 7.2 for 30 min. and post fixed in 1% osmic acid (2% $OsO_4$+ 0.3 M of Na cacodylate) for 2 h. at 4°C. Then tissue pieces washed in phosphate buffer (pH 7.2) for 30 min. at 4°C. Samples were dehydrated through ascending grades of ethanol and embedded in epoxy resin in an oven at 60°C for 14 h. to produce a firm block. Ultrathin sections about 80 nm thickness with ultramicrotome, stained with uranyl acetate and lead citrate were finally examined by Transmission E.M. JOEL 1200 EX II at the Central Lab., Faculty of Science, Ain Shams University.

### 2.4 Histochemical Investigations:

Colon tissue slides were stained with Alcian blue P.A.S technique [18] for distinguishing between acid and neutral mucins. The rationale of the method is that acid mucins stain first with alcian blue and are unable to react with the subsequent P.A.S. Following on with P.A.S., only neutral mucins and carbohydrates such as glycogen will stain red.

### 2.5 Immunohistochemistry Detection:

Immunohistochemistry is the process of localizing proteins in tissues by exploiting the principle of antibodies binding specifically to antigens. The visualization of the antibody is commonly accomplished by

conjugating an enzyme to the antibody. This can produce a color changing reaction. The advantage of this method is the ability to show exactly where a given protein is located. The expression of Bcl-2 and TGF-α in colon tissue was determined immunohistochemically in formalin-fixed, paraffin-embedded tissue blocks were cut into 4 mm thick sections mounted on glass slides, and then kept in an oven at 4°C overnight. Sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 20 min. To improve the quality of staining, microwave oven-based antigen retrieval was performed. Slides were probed with either anti-Bcl-2 (1:100, mouse mAb) or anti-TGF-α (1:100, mouse mAb). Sections were washed with PBS for 10 min each and incubated with biotin-labeled anti-mouse IgG for 1 h at room temperature. After washing, sections were stained with a streptavidin-peroxidase detection system.

## 3. Results

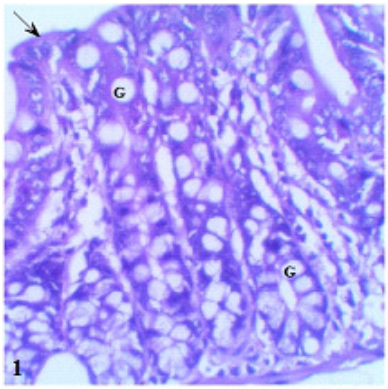
### 3.1 Histopathological Observations:

Sections of colon of control rat revealed the luminal surface is lined by simple columnar cells with basal oval nuclei and apical brush border. Goblet cells predominate in the glands characterized by mucous granules occupying the apical two thirds of the cell. The nucleus is located in the basal region of the cell. The bases of the crypts are lined by columnar cells with basal and oval vesicular nuclei (Figure 1). Colon sections of rats treated with vehicle and from rats treated with Vit. A showed normal crypt lined with columnar cells with basal nuclei, few goblet cells and normal arrangement of fibrils of muscularis mucosa (Figures 2 & 3).

Colonic sections from rats treated with AFB1 showing many deleterious effect such as well differentiated clusters of abnormal tube-like glands in the lining of the colon (called tubular adenocarcinoma) and there is a strong relationship between the number of aberrant foci crypt and increased goblet cells (Figure 4), larger aberrant crypts consisted of five crypts stained more darkly with thicker epithelial lining than normal crypts and each lumen was compressed or not distinct (Figure 5). Section of colon showed also differentiated adenocarcinoma and increased mitotic activity among lymphocytes in the lamina propria (Figure 6), abnormal foci consisted of ten crypts with numerous goblet cells and lymphocytes infiltration as appeared in Figure (7).

Colonic sections of rats treated daily with AFB1+Vit. A showed the luminal surface lined by simple columnar cells with brush borders and basal oval nuclei. The bases of the crypts lined by columnar cells with basal and goblet cells and the muscularis mucosa showed dettachment (Figure 8), near to normal arrangement of the colonic crypts (Figure 9), closely packed simple tubular colonic crypts extending down to muscularis mucosa (Figure 10), more or less crypt normal, lymphocytes infiltration in the lamina propria (Figure 11).



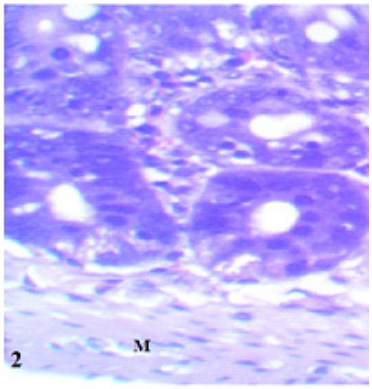




Figure 1: Photomicrograph of colon cross section of control rat showing the luminal surface is lined by simple columnar cells (arrow) with basal oval nuclei and apical brush border. Goblet cells (G) predominate in the glands characterized by mucous granules occupying the apical two thirds of the cell. The nucleus is located in the basal region of the cell. The bases of the crypts are lined by columnar cells with basal and oval vesicular nuclei (H-E, X400).

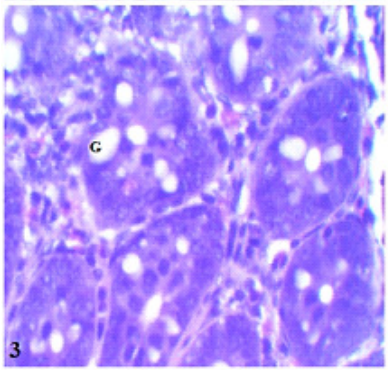
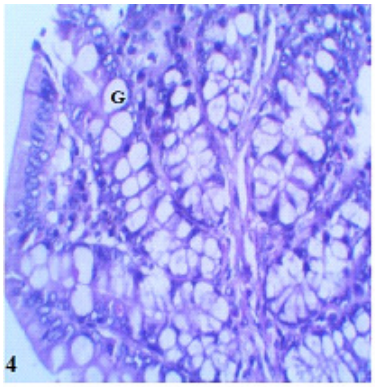



Figure 2: Photomicrograph from cross section of colon from rat treated with DSMO normal crypt lined with columnar cells with basal nuclei and normal arrangement of fibrils of muscularis mucosa (M) (H-E, X400).

Figure 3: Photomicrograph of colon cross section from rat treated with vit. A showing normal histological structure of the colonic crypt with few goblet cells (G) (H-E, X400).



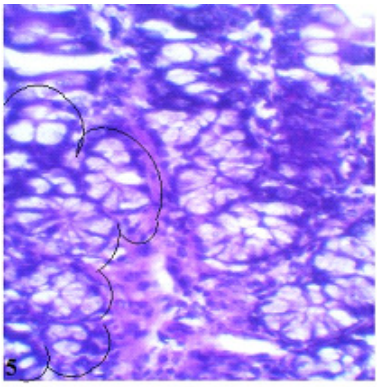
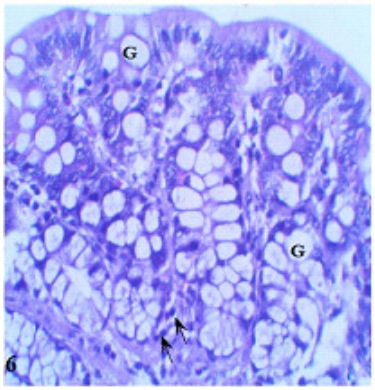




Figure 4: Photomicrograph from cross section of colon from AFB1 treated rat showing well differentiated tubular adenocarcinoma and there is a strong relationship between the number of AFC and increased goblet cells (G). (H-E, X 400).

Figure 5: Photomicrograph from cross section of colon from AFB1 treated rat showing larger aberrant crypts consisting of 5 crypts (surrounded by line) stained more darkly with thicker epithelial lining than normal crypts and each lumen was compressed or not distinct. (H-E, X400).



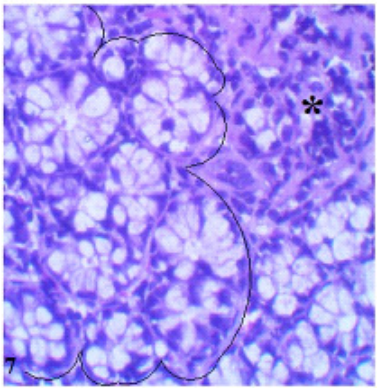




Figure 6: Photomicrograph from cross section of colon AFB1 treated rat showing differentiated adenocarcinoma, goblet cell (G) hyperplasia and increased mitotic activity (arrows) among lymphocytes in the lamina propria. (H-E, X400).

Figure 7: Photomicrograph from cross section of colon AFB1 treated rat showing abnormal foci consisted of 10 crypts (surrounded by line) with numerous goblet cells (G), lymphocytes infiltration (*). (H-E, X 400).

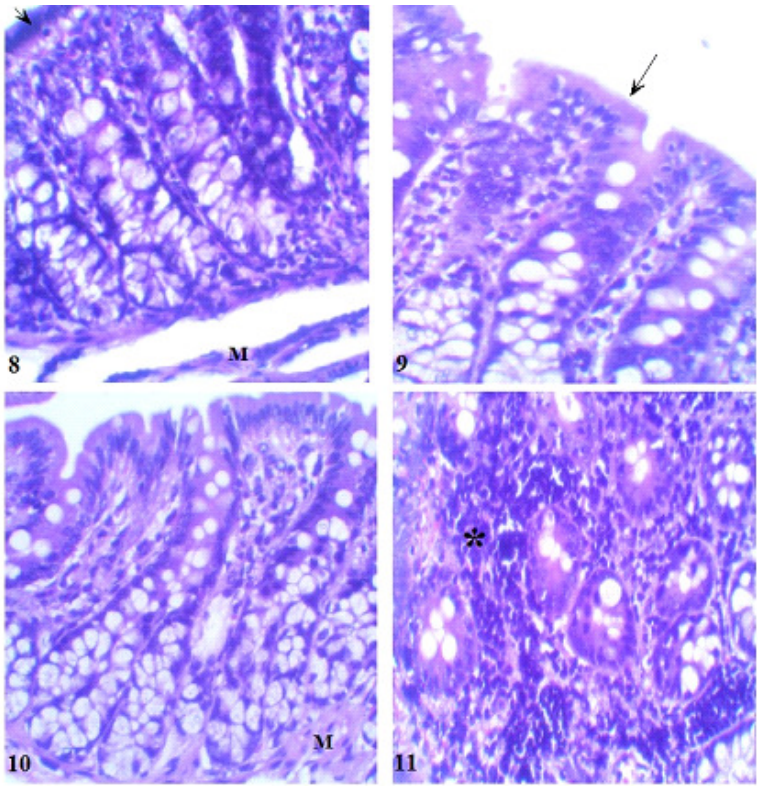



Figure 8: Photomicrograph from colon section from rat treated with AFB1+Vit.A showing the luminal surface is lined by simple columnar cells with apical brush borders and basal oval nuclei (arrow). The bases of the crypts are lined by columnar cells with basal and goblet cells and detached muscularis mucosa (M). (H-E, X400).

Figure 9: Photomicrograph from colon section from rat treated with AFB1+Vit.A showing near to normal arrangement of the colonic crypts. (H-E, X400).

Figure 10: photomicrograph from colon section from rat treated with AFB1+Vit.A showing closely packed simple tubular colonic crypts extending down to muscularis mucosa (M). (H-E, X400).

Figure 11: photomicrograph from colon section from rat treated with AFB1+Vit.A showing more or less crypt normal and lymphocytes infiltration (*) in the lamina propria. (H-E, X400).

### 3.2 Ultrastructure Studies:

#### *3.2.1 Semithin Sections:*

Histological investigation of the semithin colon sections from control and Vit. A treated rats showed normal histological pattern including simple columnar epithelium lining the colon with goblet cells (Figures12 & 13). Colon sections from rat treated with AFB1 showing goblet cell hyperplasia and abnormal colonic crypt (Figure 14), distorted columnar cells and colonic foci consists of sixteen crypts (Figure 15). While colon tissue following the administration of Vit. A to AFB1 treated rats showing improvement in the histological picture delineating in the well defined columnar cells and decrease in goblet cell numbers as compared with AFB1 treated rats (Figure 16).

#### *3.2.2 Ultrastructure Examination:*

The ultrastructure examination of the normal rat colon, illustrated normal columnar cells with basal nuclei, showed abundant microvilli projecting from the apical plasma membrane (Figure 17). Membrane bound vesicles were commonly seen and abundant mitochondria were seen in the supranuclear region. Both smooth and rough endoplasmic reticulum could also be seen through the cytoplasm. The nucleus was located in the basal third of the cell. Electron micrographs of the colon of rats treated with DSMO and Vit.A showed no apparent ultrastructure changes as compared with normal control. Transmission electron micrograph from rat colon treated with DSMO showing normal columnar cells with their basal nuclei and microvilli, mitochondria and rough endoplasmic reticulum also appeared (Figure 18) and normal colonic crypt arrangement in colon section from Vit.A treated rat (Figure 19).

Fourteen weeks after the administration of AFB1to rats, colon sections showed many histopathological disturbances such as goblet cell with degenerated nucleus, the nucleus of the columnar cells appeared in an apoptotic state, and other cells appeared with dense lysosomal vesicles (Figure 20), abnormal crypt with increased number of goblet cells loaded with mucus. The nuclei of columnar epithelium appeared apoptotic (Figure 21), aberrant crypt foci visualized with increased number of mucus secreting cells. Cryptic nuclei appeared in an apoptotic status (Figure 22), columnar cells nuclei with different apoptotic degrees and increased number of mitochondria in goblet cells as appeared in Figures (23a & b).

Whereas, with Vit.A treatment in AFB1-induced rats, colonic tissue appear to be normal and all columnar cells which were damaged due to AFB1 administration appeared to be regenerated. Electron micrograph of colon section from AFB1+Vit.A treated rat showed regained normal structure of the nuclei of the

columnar cells and increased numbers of mitochondria in the supranuclear region (Figure 24), with preserved nuclei and normal chromatin distribution (Figure 25), improved picture of the cryptic columnar cells with increased number of mitochondria in the suprenuclear region and decrease in goblet cell number as compared with AFB1 treated rats (Figure 26).

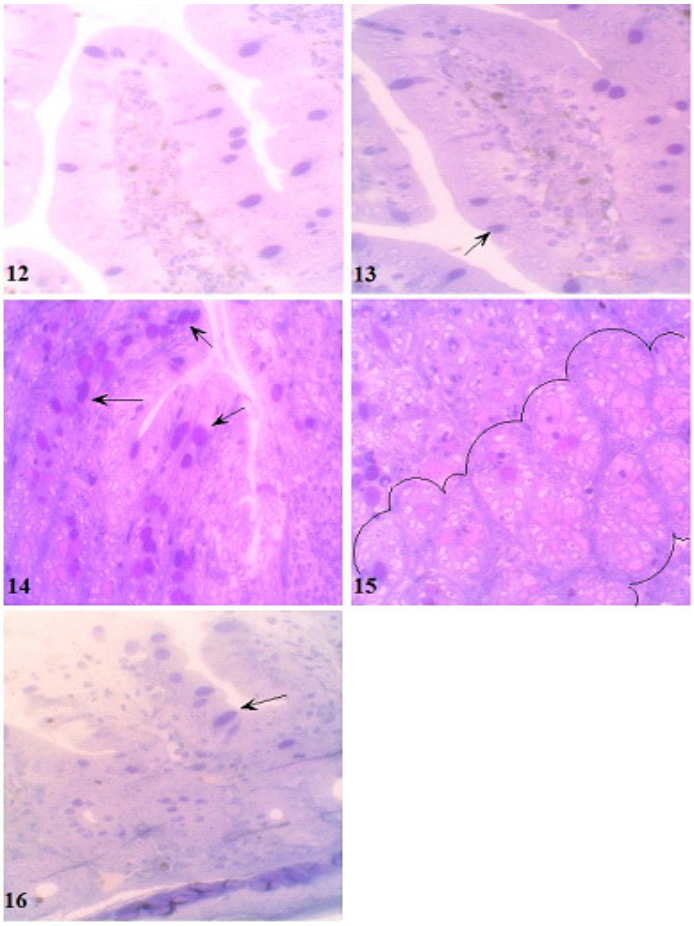



Figure 12: Photomicrograph from control rat showing simple columnar epithelium lining the colon with goblet cells (arrow). (Toluidine blue, X400).

Figure 13: Photomicrograph from colon rat treated wit Vit. A showing the normal structure of the colonic tissue. (Toluidine blue, X400).

Figure 14: Photomicrograph from colon rat treated with AFB1 for fourteen weeks showing goblet cell hyperplasia (arrows) and abnormal colonic crypt. (Toluidine blue, X400).

Figure 15: Photomicrograph of colon section from rat treated with AFB1 showing distorted columnar cells and colonic foci consists of at least sixteen crypts (surrounded by line). (Toluidine blue, X 400).

Figure 16: Photomicrograph of colon tissue from rat treated with AFB1+Vit. A showing improvement in the histological picture delineating in the well defined columnar cells and decrease in goblet cell (arrow) numbers as compared with AFB1 treated rats. (Toluidine blue, X 400).

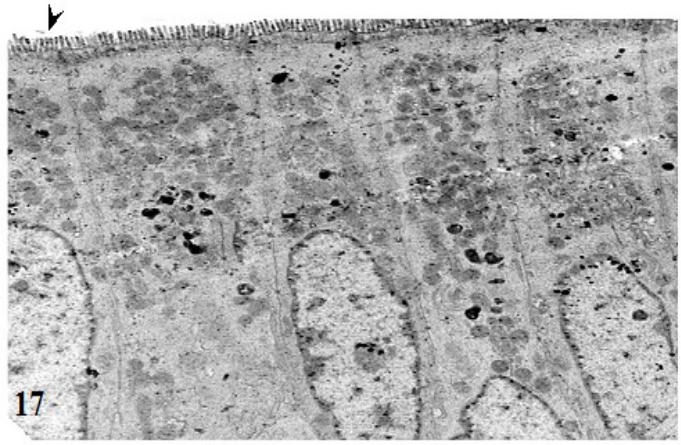



Figure 17: Ultrastructure micrograph from control rat colon showing normal columnar epithelium with basal nuclei and microvilli on the free surface (head arrow). Cells rich in mitochondria in the apical part of the cell. (EM, X 6000).

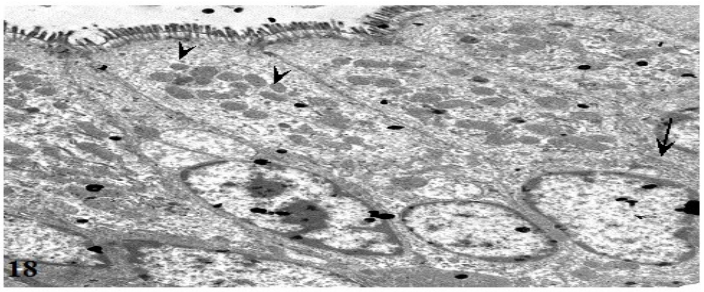



Figure 18: Photmicrograph from rat colon treated with DSMO showing normal columnar cells with their basal nuclei and microvilli, mitochondria (head arrows) and rough endoplasmic reticulum (arrow) also appeared. (EM, X 6000).

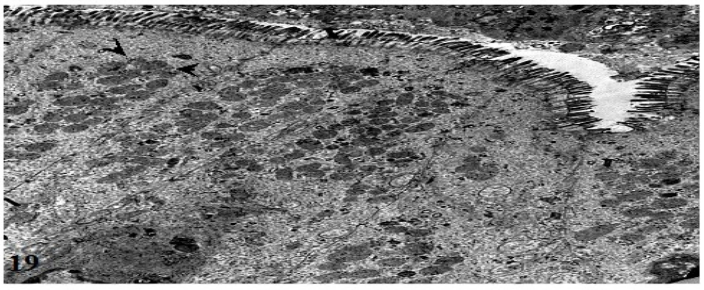



Figure 19: Transmission electron micrograph of colon tissue from rat treated with Vit. A showing normal colonic crypt arrangement with large number of mitochondria (head arrows) in the supranuclear region. (EM, X8000).

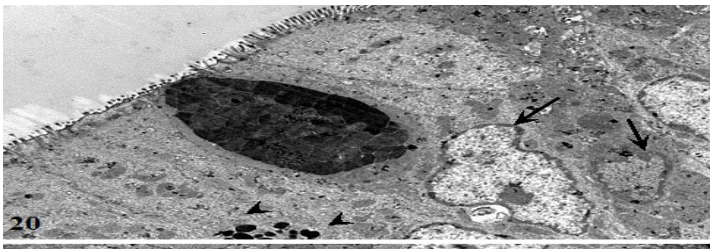



Figure 20: Electron microscopic micrograph from colon rat treated with AFB1 showing goblet cell with degenerated nucleus (thin arrow), and the nucleus of the columnar cells appeared in an apoptotic state (thick arrow), another cell with dense lysosomal vesicles (head arrows). (EM, X5000)

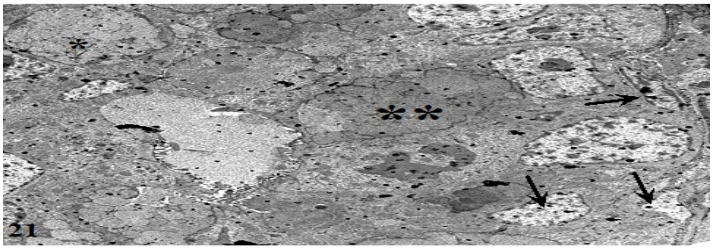



Figure 21: An electron micrograph of colon section from rat treated with AFB1 showing abnormal crypt with increased number of goblet cells loaded with mucus (*) and its lumen is obstructed. The nuclei of columnar epithelium appeared apoptotic (arrows). (EM, X 4000).

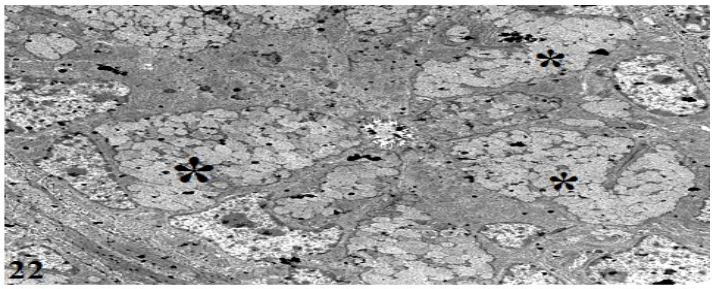



Figure 22: Electron micrograph of colon section of rats treated with AFB1 showing aberrant crypt foci with increased number of mucus secreting cells. Cryptic nuclei appeared in an apoptotic (arrows) status with incommodious lumen. (EM, X 4000).

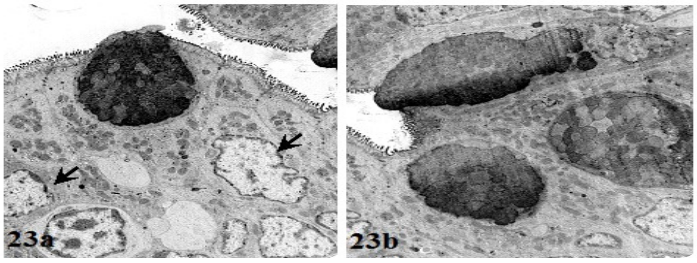



Figure 23a & b: Electron micrographs of colon section of rats treated with AFB1 showing columnar cells nuclei with different apoptotic degrees (arrows) and increase numbers of mitochondria in Goblet cells respectively. (EM, X 6000).

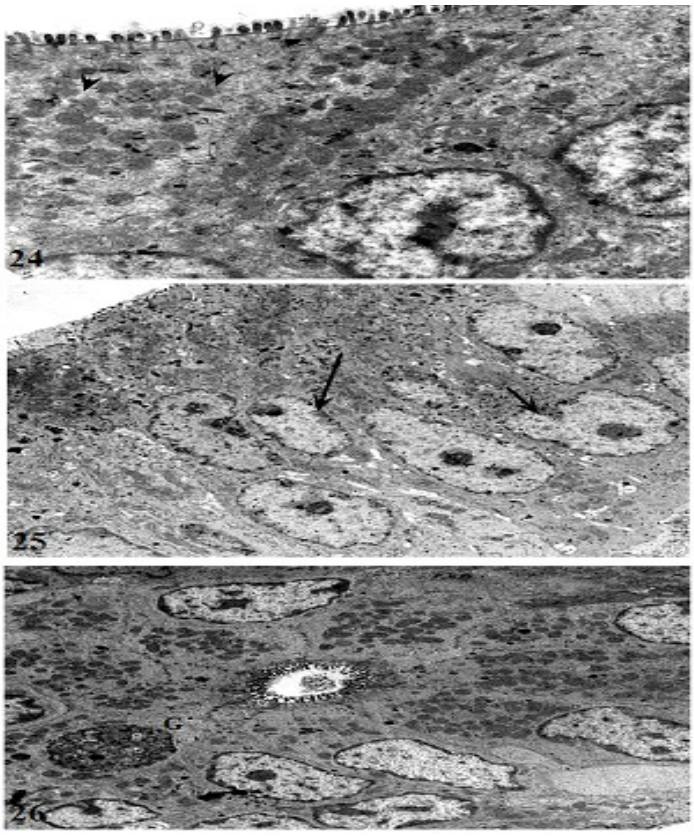



Figure 24: Electron micrograph of colon section from AFB1+Vit.A treated rat showing regained normal nuclei structure of the columnar epithelium, increased numbers of mitochondria (head arrows) in the supranuclear region. (X 12000).

Figure 25: An electron micrograph of colon tissue from rat treated with AFB1+vit. A showing improved histological picture of the columnar cells with preserved nuclei (arrows) and normal chromatin distribution. (X4000).

Figure 26: Transmission electron micrograph of colon tissue from rat treated with AFB1+Vit.A showing improved picture of the cryptic columnar cells with increased number of mitochondria in the supranuclear region and decrease in goblet cell number (G) as compared with AFB1 treated rats (X 5000).

### 3.3 Histochemical Studies:

Colon sections from control, DSMO and Vit.A treated rats showed the normal distribution of mucous granules (Figures 27& 28). Colon sections from AFB1 treated rats revealed an increase in mucous positive material scattered in the lining cells (Figure 29). On the other hand, colon sections from AFB1+Vit. A showed decrease in mucous granule distribution (Figure 30) as compared with colon sections from AFB1 administered rats.

### 3.4 Immunohistochemical Investigations:

#### 1) Bcl-2

Sections of colon of control and Vit A treated rats stained immunohistochemically for Bcl-2 delineated the normal distribution of Bcl-2 (Figure31 &32). On the other hand section in colon from rats received AFB1, showing increase in Bcl-2 distribution in colonic tissue (Figure33). Colon sections of rats treated with AFB1+Vit A showed normal structure of (Figure 34).

#### 2) Transforming growth factor-α:

Sections of colon of control and DSMO treated rats stained for TGF-α revealed normal distribution in columnar cells of colon (Figure 35 & 36). Augmentation of positive staining with the anti-TGF-α a antibody was seen in the columnar and goblet cells of the colon from rats received AFB1and the strongest reaction confined to the cytoplasm (Figure 37). Sections in rat colon treated with AFB1+Vit.A revealed near to normal distribution in TGF-α in colonic cells (Figure 38).

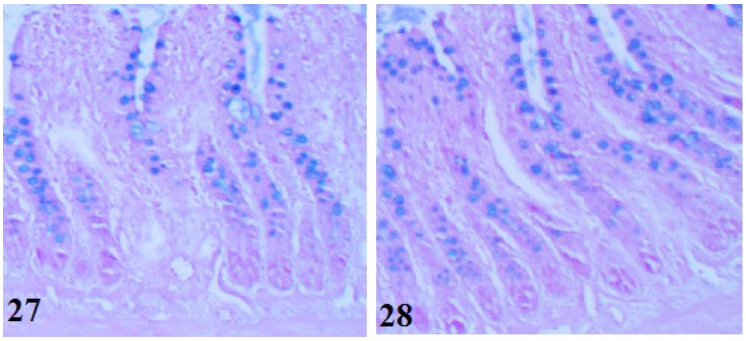



Figure 27: Photomicrograph of colon section of control rat showing the normal distribution of mucous in the colon tissue.

Figure 28: Photomicrograph of colon section of DSMO administered rat showing the normal distribution of mucous granules.

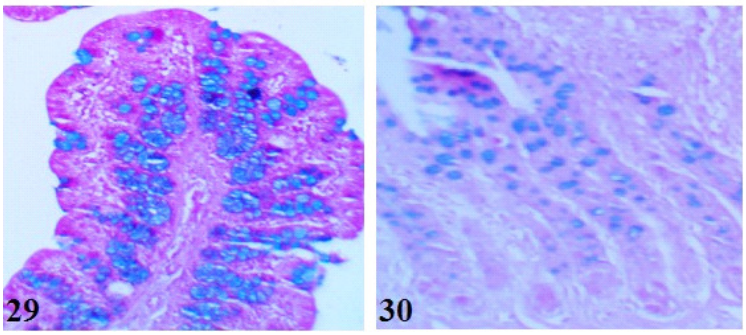



Figure 29: Photomicrograph of colon section from rat treated with AFB1 for 14 weeks showing augmentation in mucous granules in the goblet cells, which may be as a result of the hyperplasia of goblet cells and due to the increased rate of production of mucous.

Figure 30: Photomicrograph of colon section from rat after the administration of AFB1+Vit.A showing decrease in mucin granules as compared with AFB1 administered rat (Alcian blue P.A.S., X400).

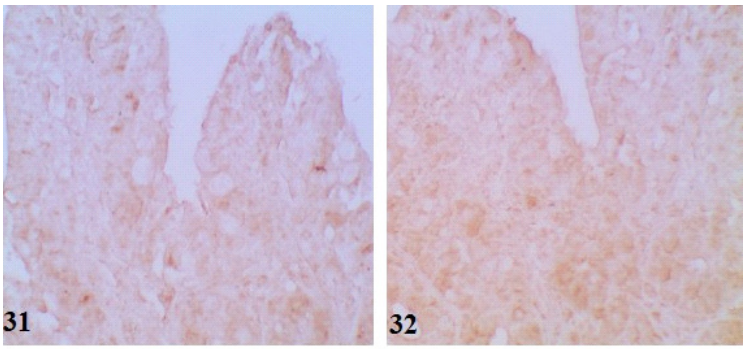



Figure 31: Photomicrograph of colon section from control rat showing the normal levels of Bcl-2 positive columnar cells in brown (Immunohistochemical stain, X200).

Figure 32: Photomicrograph of colon section from rat treated with Vit A showing the normal levels of Bcl-2 positive cells (Immunohistochemical stain, X200).

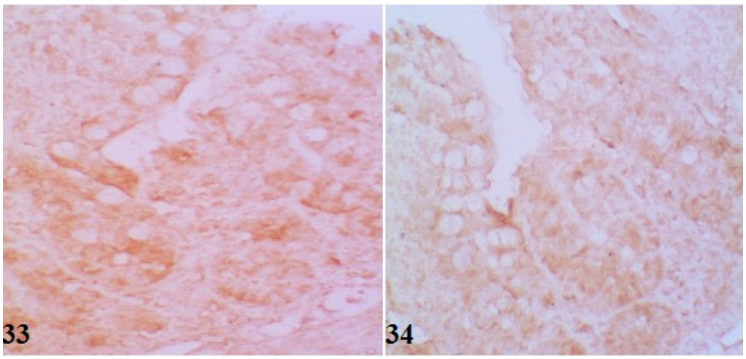



Figure 33: Photomicrograph of colon section from rat treated with AFB1 for 14 weeks showing increase in Bcl-2apoptotic activity in columnar cells (Immunohistochemical stain, X200).

Figure 34: Photomicrograph of colon section from rat treated with AFB1+Vit A showing decrease in Bcl-2 positive columnar cells as compared with AFB1 treated rat (Immunohistochemical stain, X200).

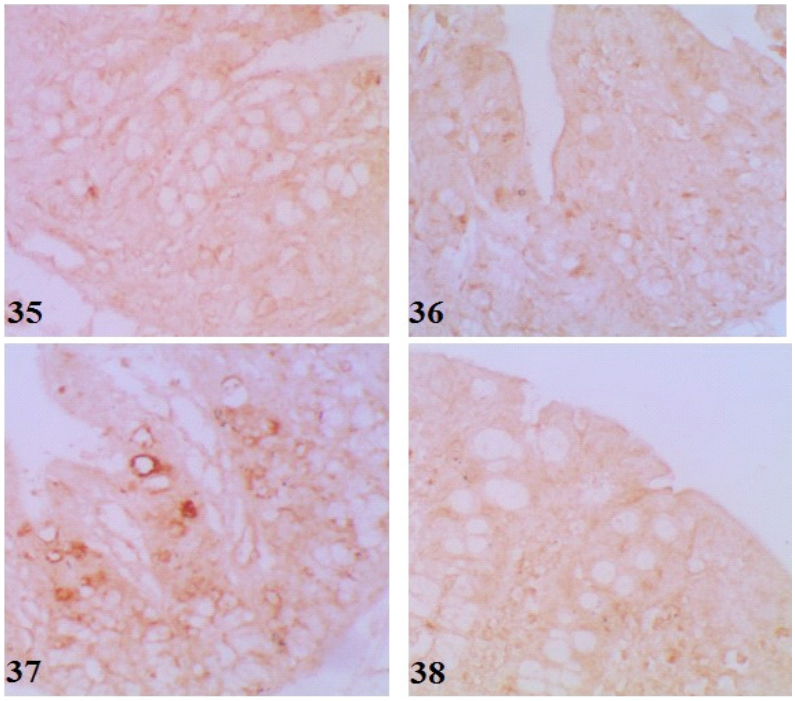



Figure 35: Photomicrograph of colon sections from control rat showing the normal cytoplasmic expression of TGF-α in the columnar cells (Immunohistochemical stain, X200).

Figure 36: Photomicrograph of colon sections from rat treated with DSMO showing the normal cytoplasmic expression of TGF-α in the colonic tissue (Immunohistochemical stain, X200).

Figure 37: Photomicrograph of colon sections from treated with AFB1 for 14 weeks showing increase in the expression of TGF-α in the cytoplasm of columnar and goblet cells (Immunohistochemical stain, X200).

Figure 38: Photomicrograph from rat colon section treated with AFB1+Vit A showing slight decrease in TGF-α expression in the columnar cells as compared with AFB1 treated rat (Immunohistochemical stain, X200).

## 4. Discussion

Colon cancer is thought to develop by multistep process in which normal crypts are initiated to form aberrant crypt foci (ACF) that proliferate by crypt fission to form microadenoma (MA). The MA enlarge to give macroscopic adenoma, adenomatous polyps, and finally adenocarcinoma [19]. The main target of AFB1 is liver and it undergoes transformations in hepatocytes: biotransformation to active AFB1-8,9-epoxide, which gets bound with DNA; irreversible hydroxylation, forming metabolites $M_1$, $P_1$, and $Q_1$; reversible hydroxylation, forming aflatoxicol [20-22]. Aflatoxins that come into animal and human gastrointestinal systems with contaminated food can be mitigated by various enterosorbents [23,24]. The toxico-pathological spectrum of AFB1 (in a broad spectrum of vertebrates) is very wide encompassing acute toxicological effects, carcinogenicity, teratogenicity, genotoxicity, immunotoxicity and sometimes death [25]. The fungal metabolites namely mycotoxins represent the most significant contaminants of food and feed [26]. Various members of myoctoxins were detected in animal sera, feed and food and produced severe dangerous changes in active organs [27].

In the present study, many histopathological changes were seen such as cell degeneration, pyknotic nuclei and apoptosis, which may lead to cancer. These results are abreast to those reported by Simonich et al. [28] postulated that AFB1 administration increasing putative pre-neoplastic foci in the colon. Chemically induced ACF in rodents have been used extensively to test chemicals and diets that might prevent colorectal cancer, and reported the colon of albino rats after treatment with aflatoxin including presence of aberrant crypt foci (ACF) and increase in mucous production, which lead to adenocarcinoma. This come in accordance with Takayama et al. [29] who reported that ACF form before colorectal polyps and are one of the earliest changes seen in the colon. According to the same line, Orner et al. [30] and Gursoy et al. [31] showed that aflatoxin B1 (AFB1)-induced preneoplastic foci of the rats liver and colon in animals and humans. The term "apoptosis" describes the change of morphology different from cell necrosis. Hallmarks of apoptosis include chromatin condensation, nuclear segmentation, cytoplasmic shrinkage, blebbing, and formation of apoptotic bodies as revealed by Sakai et al. [32] and Xu et al. [33].

Vitamin A is controlling the differentiation program of epithelial cells in the digestive tract and respiratory system, skin, bone, nervous system, and immune system; and for hematopoiesis [34]. Some studies have shown that a vitamin A deficiency in the diets of coccidiosis-challenged broilers resulted in compromised immune defenses as reflected in lymphocyte profiles, oocyst shedding, and interferon- levels [35]. Hamzawy et al. [2] and Alpsoy et al. [36] showed that AFB1 significantly decreased the level of GSH and the activities of superoxide dismutase and GPx and increased level of malondialdehyde. Simultaneous supplementation with vit.A, C, and E restored these parameters to that of normal range. Webster et al. [37] reported that vit.A thus may control carcinogenesis by manipulating molecular events at the initiation stage. Enzyme concentrations in

intestinal and colon mucosa, and in intestinal and colon contents suggested that AFB1 may have different metabolites and that there may be differing susceptibilities of colon mucosa to carcinogenesis. These results suggest that the effect of vitamin A on the metabolism of the carcinogen, particularly on binding of AFB1 to cellular macromolecules, may be the mechanism by which vitamin A modifies aflatoxin's carcinogenic potential, influenced in part through enzymatic mechanisms [38]. Ayub and Sachan [39] delineated that Vitamin A supplemention in rats inhibited AFB1-DNA binding [40]. The protective effects of retinoids such as retinol, retinal, retinoic acid, and retinal esters on AFB1 carcinogenicity were due to inhibition of AFB1-DNA adduct formation by affecting the CYP45O systems resulting in less epoxide being formed [41]. Retinal had the same inhibitory effect on the formation of AFB1 -protein adducts [40]. Vitamin A has been shown to induce the activity of glutathione S-transferase, thereby enhancing the detoxification of AFB1-epoxide. On the other hand, vitamin A deficiency decreased glutathione S-transferase activity. Kurt et al. [42] showed that lycopene and vit.E administration to AFB1-induced rats were found to be protective against the damage on gastric mucosa. Chlorophyllin (CHL) reduced the mean number of aberrant crypt foci per colon by 63%. These results show CHL provide potent chemoprotection against early biochemical and late pathophysiological biomarkers of AFB1 carcinogenesis in the rat colon as reported by Simonich et al. [28].

The epithelium of the intestinal tract is covered by a layer of mucus composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells [43]. The mucus layer acts as a medium for protection, lubrication, and transport between the luminal contents and the epithelial cells [44]. Goblet cells, epithelial cells, macrophages, and dendritic cells are the major cellular constituents of the innate defense system, and the mucus layer containing mucins represents the front line of this system [45]. Mucus is secreted by goblet cells throughout the gastrointestinal tract and forms a gel adherent to the mucosal surface [46]. This layer acts as a barrier between the luminal contents and the absorptive system of the intestine and protects the mucosal surface from exogenous or endogenous luminal irritants such as laxatives [47]. Changes in the properties of this barrier could affect the absorption of both dietary and endogenous macromolecules and ions [48].

Mucins are complex glycoproteins that provide the viscoelastic properties of mucus that are essential for protection of the alimentary canal [42]. Mucins are classified into neutral and acidic subtypes; the latter are further distinguished by sulfated (sulfomucins) or nonsulfated (sialomucins) groups [49]. Neutral mucins appear to be the predominant subtype expressed in gastric mucosa. Acidic mucins are expressed throughout the intestinal epithelium and dominate in the large intestine. Administration of AFB1 for 14 weeks caused augmentation of mucous secretion in colon tissue. Archer et al. [19] found that there is a strong relationship between ACF and the increased production of mucous, while, Kurt et al. [42] delineated the increase in mucin secretion in stomach as a result of AFB1 administration. Consistent changes in mucus-related indexes in a variety of intestinal and nutritional disorders, including enteric infections, inflammatory bowel disease, colon cancer [50]. Mucin is the predominant secreted glycoprotein in the colon and is elaborated by most colon cancers. The mucin extracted from colon cancer is immunologically distinct from that in the normal colon [51] but the exact biochemical nature of this difference has remained elusive. Attention has also been drawn to the goblet cell mucin of the epithelium immediately adjacent to colon cancer in which the histochemical staining characteristics differ from those seen in the normal colon. However, the histological appearance of the tissue falls short of the criteria for malignancy, and therefore it has been termed “transitional mucosa”. Jarry et al. [52] showed that the proinflammatory cytokine interleukin 1 (IL-1) stimulates rapid mucin release from mucin-secreting cell line (HT29-Cl.16E cells) in a dose-dependent manner. The ability of IL-1 to trigger mucin release and to up-regulate mucin expression was later confirmed in studies of perfused rat colons [53] and the colonic LS180 cell line [54]. Enhanced mucin release appears to be a common mechanism for the intestinal clearance of gut parasites [55] and is routinely mediated by cytokines produced by the TH2 subset of CD4+ T cells, which subsequently stimulate immunoglobulin E (IgE) production [56]. Lake et al. [57] showed that IgE mediated mast cell discharge of histamine enhanced the release of goblet cell mucus into the rat duodenum.

As vit. A administered ti AFB1-induced rats, colonic tissue showed decrease in mucous production as compared with AFB1 treated animals, this may be related to its antioxidant activity. Kurt et al., [42] reported that lycopene and vit. E administration reverse the increase in mucin secretion in gastric mucosa in AFB1 administered rats.

A considerable body of evidence exists suggested that $AFB_1$ suppresses immune function by affecting T-cell dependent immunity in various animal species. Studies with laboratory test species such as the mouse [58,59], rat [60], and rabbit [61] reinforce these findings. Immunosuppression by a toxicant can result from various mechanisms such as decreased protein and/or DNA synthesis, changes or loss in enzymatic activity, and changes in metabolism or cell cycles, which may result in apoptosis or necrosis. Immune mechanisms affected

by AFB1, in addition to T-cell dependent immunity, include reduced production of complement by the liver and decreased phagocytosis by neutrophils and macrophage activities [62,63]. Toxic effects on T-lymphocytes [63] and/or other lymphoid cells such as the cytotoxic T-cells and natural killer cells [64], which impair the function of direct or indirect killing of tumor cells, can have pronounced effects on tumorigenesis. Immunosuppression can result in a greater rate of tumor progression [60]. Moreover, cellular components of the immune system are known to produce various cytokines, which play a key role in host resistance and protection against tumor progression. These same cytokines, however, are involved directly in the inflammatory mechanisms that are initiated when various organs have been damaged by toxic assault [65].

Apoptosis is a specialized process of cell death that is part of the normal development of organs and tissue maintenance, but may also occur as a response to various environmental stimuli, indicating toxicity [66]. Since apoptosis can play a critical role in the development of cancer, the ability of toxins to induce apoptosis appears to be related to their toxicological effects [10,67,68]. Bcl-2 is a suppressor gene of apoptosis, which was found from follicular B cell lymphoma with *t*(14,18) chromosome malposition [33,96]. Orientating as 18q21, Bax, and Bcl-2 are homologous proteins, and Bax is an induction gene of apoptosis. Bcl-2 and Bax can exist in the form of homodimer and form heterodimer too. When the expression of Bax increases, the homodimer of Bax-Bax can induce apoptosis. When the expression of Bcl-2 increases, Bax can combine with Bcl-2 to form more stable heterodimers which can inhibit apoptosis. The ratio of Bcl-2/Bax can regulate apoptosis [70].

The present study revealed that increase in Bcl-2 expression associated with decreased apoptosis and increased cell proliferation in colon tissue. Proteins in the Bcl-2 family are central regulators of programmed cell death, and members that inhibit apoptosis, such as Bcl-X(L) and Bcl-2, are overexpressed in many cancers and contribute to tumour initiation, progression and resistance to therapy [71]. Bcl-2 protein blocks a distal step in an evolutionarily conserved pathway for programmed cell death and apoptosis [11]. Wu et al., [72] suggested that long-term dietary corn oil promotes AOM-induced colon cancer development partly by inhibiting the tumor suppressor gene p53 mediated mitochondria-dependent apoptosis. Which leading to upregulation of Bcl-2.

Neoplasia of the normal colonic epithelium goes through ordered stages, first to adenomatous then to malignant change. Epidermal growth factor/ TGF-α receptor and TGF-α production have been individually detected in colon carcinomas and in numerous colon cancer cell lines [73,74]. Coexpression of TGF-α and epidermal growth factor receptor and growth stimulation by TGF-α has also been shown in multiple colon cancer cell lines, and TGF-α has been proposed as an autocrine growth factor in colon cancer.

AFB1 administration caused increase in TGF-α expression in rat colon tissues. TGF-α, a stimulatory growth factor inhibit apoptosis and member of the epidermal growth factor family, is a mediator of malignant progression in colorectal carcinogenesis. TGF-α expression in the rectum was higher in patients compared with controls and statistically significantly associated with accepted risk factors for colorectal neoplasms [75-77]. These findings support the potential for TGF-α as a modifiable biomarker of risk for colorectal cancer. Basic science evidence strongly supports a progrowth and hyperproliferative mechanism in colon carcinogenesis with elevated TGF-α expression in colon adenoma and adenocarcinomas [78]. Many studies suggested that TGF-α may be a potential marker of colorectal cancer risk [78,79].

Administration of vit. A, resulted in inhibition of Bcl-2 and TGF-α expressions in rat colon. This come in accordance with a research from Hong et al. [80] who has shown that dietary fish oil increases apoptosis by down regulating Bcl-2 expression in rat colon. Again, Kauntz et al. [81] investigated the potential of silibinin (a flavonolignan substance) as a chemopreventive agent in rat colon carcinogenesis. Mechanisms involved in silibinin-induced apoptosis included the downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein Bax, inverting the Bcl-2/Bax ratio.

## 5. Conclusion:

To our knowledge, this is the first prospective investigation, which has assessed the association between vitamin A and Bcl-2 and TGF-α expression in colon cancer. Present data concluded that the immunomodulatory mechanism of action of vitamin A in enhancing cell apoptosis, which is inhibited by Bcl-2 and TGF-α in AFB1 induced rats, and as a result enhancing cancerous cell death. These results may open up a new way for the treatment of colon cancer, by adding it to our daily diets.

The present investigation delineated that histopathological, ultrastructure, histochemical and immunohistochemical damage is attributed to the environmental pollution of food and feeds by fungi and their

toxins. Therefore, sanitary care must be conducted during all steps of feed and food processing and production. Farmers and food manufacturers, whose work related to contact with animals must provide them with awareness through workshops and health bulletins to prevent such pollution. This will lead to maintain human health and keep livestock sources safe.

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