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Effects of Lycopene and Vitamine E Administration Over Gastric Mucosal Damage Induced by Aflatoxin B_1

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Abstract: In the present study, we aimed to determine, the changes induced by Aflatoxin B_1 (AFB_1) administration on rat gastric mucosal barrier and gastric mucins alongside revealing the protective effects of lycopene and Vit E. Thirty-five Wistar-Albino male rats weighing 180-220 g, were divided into 7 groups as to include 5 rats in each group: control, lycopene (10 mg/kg/day lycopene for 15 days (Lycopene 10% FS), AFB_1 (single dose of 2.5 mg AFB_1 kg^{-1} on the 12th day of the study), Lycopene + AFB_1 (10 mg lycopene/kg/day for 15 days and single dose of 2.5 mg AFB_1 kg^{-1} on the 12th day of the study) and Vitamin E + AFB_1 (10 mg/kg/day Vitamin E for 15 days and single dose 2.5 mg AFB_1 kg^{-1} on 12th day). Following the sacrifice of study subjects on the 15th day, gastric mucus and phospholipid levels were determined and their stomachs were examined histopathologically. Examination of mucus and phospholipid levels revealed a significant reduction in group 3-5, in which AFB_1 has been applied (respectively, $p<0.001$, $p<0.001$). When lycopene and vitamine E groups are compared with the AFB_1 group, a significant elevation was detected in mucus and phospholipid levels (respectively, $p<0.001$, $p<0.001$). Whereas, histopathological examination of gastric mucosas of the aflatoxin group showed degenerative changes, gastric mucosas of the control group and the remaining study groups were normal. Histochemically, while neutral mucins were predominant in general structure of stomach, mixed and sialomucins were observed, as well. Particularly acid mucins with suplhate and periodate reactive acid mucins were found to be more predominant in the aflatoxin group compared to control and other groups. Histochemical features of mucins were observed to be consistent with specific functions of the different regions of stomach. Lycopene and vitamine E administrations were found to be protective against the damage induced by aflatoxin on gastric mucosa.

Key words: Aflatoxin B_1, gastric barrier, Lycopene, mucin, rat

INTRODUCTION

Because mucosas of digestive, respiratory and urogenital system have direct relation with exterior environment, they are covered with a mucous substance called mucus, which functions as a physicochemical barrier. This substance not only acts as a barrier, but also provides hydration to mucosa, blocks bacterial adhesion and prevents penetration and colonization of pathologenic microorganisms. Moreover, it protects mucosa against physical and chemical injuries (Mckee and Mckee, 2003; Reid and Haris, 1998). An investigator named Hollander (1954) has proposed barrier theory in, which he presented this barrier as a structure composed of 2 components and having the ability to regenerate itself. According to this hypothesis, gastric mucosal barrier is made up of 2 main layers. While, first layer is of mucous and adhesive character and functional in protection, second layer is the surface epithelium composed of columnar and cubic cells. Werther (2000) explained the details of this theory in light of current knowledge on mucus and mucosal epithelium.

Many investigators believe that the excessive HCI secretion in stomach may be the main cause leading to gastric ulcer formation. Moreover, mucus, which forms the gastric mucosal barrier and the factors causing an increase in destruction and a decrase in production of phospholipids, are known to weaken the barrier and facilitate ulcer formation even in presence of low levels of HCL (Kwiecien *et al*., 2002).

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Mucus is made of glycoproteins called as mucins. Mucins are grouped in two as neutral and acid mucins. Neutral mucins do not contain reactive radical acid, however, bear free hexose groups. Neutral mucins are seen in stomach, prostate and goblet cells. Acid mucins are examined in 2 groups as mucins with sulphates (sulphomucin) and mucins with carboxyl (sialomucin). Sulphomucins are classified in 2 categories as strong and weak sulphomucins. Strong sulphomucins include glucuronic acid containing sulphate and react with cationic stains (Alcian Blue) in low pH levels. Generally, those display a PAS (-) character. On the other hand, weak sulphomucins are of epithelial origin and react with cationic stains in low pH levels. Those can be encountered in goblet cells localized in colon). Sialomucins are categorized in 2 groups, as well (Bancroft and Stevens, 1990). N-acetyl sialomucin (labile sialomucin): They include one sialic acid molecule and bind with cationic stains at pH levels >2. They can be identified by sialidase enzyme. Display PAS (+) characteristic. N-acetyl-O-acetylsialomucin (resistance sialomucin): Those include sialomucins, which are completely resistant against sialidase extraction. Display PAS (-) characteristic. Recent studies have shown that different types of mucin, differing in their carbohydrates and core protein structure, are expressed in different regions of the gastrointestinal tract. In the stomach, the corpus mucin differs from the antral mucin and in each region, the surface-type mucins (surface mucous cell-type mucins) differ from the gland-type mucins, synthesized in deeper layers of the gastric mucosa (Corfield *et al*., 2000). Histochemical studies revealed that surface-type mucins have different carbohydrate chains from gland-type mucins in the stomach. For instance while, surface-type mucins are stained by Galactose Oxidase Cold Thionine Schiff (GOCTS) staining, glandular mucins are stained by Paradoxical Concanavalin a Staining (PCS) (Ota *et al*., 1991; Ota and Katsuyama, 1992). Because gastric mucin has an important role in protecting the mucosa from gastric acid, pepsin and pathogens, the biochemical characterization of individual mucin molecules, is important to understand their functions and specific tools to recognize particular mucin species are essential (Corfield *et al*., 2000). Mucus glycoproteins (mucins) are the most important structural components of the mucus, which are synthesized by highly polarized specialized cells (Dekker *et al*., 1991).

Aflatoxins are the toxic metabolism products of molds. Aflatoxin B_1 (AFB_1) has been announced as a natural carcinogen agent by The International Agency for Research on Cancer (IARC, 1993). Aflatoxins containing foods and animal diets, pose a great threat over human and animal health. Animal diets contaminated by aflatoxin cause huge reductions in overall yields and by passing into the foods consumed by humans, they put human health under risk, as well. *In vivo* and *in vitro* studies have revealed that AFB_1 leads to elevation of free radicals depending on the dose (Towner *et al*., 2002). As a result of aflatoxin applications, increases in malondialdehyde levels and drops in several antioxidant agents such as glutathion, associated with oxidative stress, have been found in tissues.

Carotenoids are thought to diminish the incidence of certain degenerative diseases, but the mechanisms involved in their intestinal absorption are poorly understood (Tyssandier *et al*., 2003). Recently, lycopene has received particular attention as a result of studies indicating that it bears highly efficient antioxidant and free radical scavenging capacity (Atessahin *et al*., 2006a, b). Several researchers believe that lycopene may be valuable in preventing and slowing the growth of prostate, lung and stomach cancers (Velmurugan *et al*., 2002). These scientists describe lycopene as a powerful antioxidant, a compound that blocks the action of activated oxygen molecules known as free radicals, which can damage cells (Giovannucci, 1999; De Stefani *et al*., 2003).

Vitamine E is known to function as an *in vivo* free radical scavenger that inhibits lipid peroxidation in biological system (Guzel *et al*., 1998). The molecular mechanism of action of vitamin E in mammalian cells, remains to be elucidated (Cassand *et al*., 1993). Vitamine E is a fat soluble antioxidant vitamin, which helps to neutralize potentially damaging free radicals in our body (Obikoya, 2006). Vitamine E is considered to be the most important antioxidant at the membrane level, as it is lipid soluble (Phull *et al*., 1996).

The present study has been designed to histochemically show, the effects of lycopene and Vit E on mucus and phospholipid levels along with the aim to determine changes induced by them over gastric mucins.

MATERIALS AND METHODS

In the present study, 35 male Wistar-Albino rats with a mean weight of 180-220 g, obtained from Health Research Center of Dicle University (DUSAM) were used. Animals were divided into 5 groups as to include 7 rats in each: control, lycopene (10 mg/kg/day lycopene was applied for 15 days within corn oil via gastric gavage (Lycopene 10% FS), AFB_1 (single dose of 2.5 mg AFB_1 kg^{-1} was applied on the 12th day of the study via intraperitoneal route), Lycopene + AFB_1 (10 mg lycopene/kg/day for 15 days and single dose of 2.5 mg AFB_1 kg^{-1} on the 12th day of the study, were applied via intraperitoneal route), Vitamine E + AFB_1 (10 mg/kg/day Vitamine E for 15 days and single dose 2.5 mg AFB_1 kg^{-1} on the 12th day were applied via intraperitoneal route). Animals were sacrified on 15th day of the study and after their stomachs were removed and sampled to be examined

under light microscope, the stomachs were divided into 2 portions along the great curvature. Mucus amounts were determined in one half of the stomachs by UV spectrophotometer with the method of Corne *et al*. (1974). In the other halves of the stomachs, phospholipid amounts were measured by UV spectrophotometer with the method of Bauer *et al*. (1974).

Stomach tissue samples obtained for histological analysis were blocked with routine histologic technique after being fixed in formol-alcohol. Besides, Crossman's triple stain; staining methods such as Alcian Blue-Aldehyde fuchsin, PAS-Phenylhydrazine and PAS-Aldehyde fuchsin, which discriminate between sulphomucins and sialomucins, were used. Examination of preparations and the taking of their photos were carried out with a Nikon Eclisse-400 microscope.

Statistical analysis: The data obtained from the study were evaluated by SPSS (version 10.0) package programme with One-way Analysis of Variance (ANOVA) and post-hoc Tukey- HSD tests.

RESULTS

In terms of mucus and phospholipid levels, while a reduction in AFB_1-applied 3rd, 4th and 5th groups was detected, lycopene and vitamine E groups showed an increase compared to the solely AFB_1-applied group. Important changes were found to be belonging to Aflatoxin group. Affects of aflatoxin B_1 over gastric lesions and levels of mucus and phospholipids of gastric mucosal barrier in aflatoxin B_1 group, are shown in Table 1. In aflatoxin B_1 group, whereas mucus amount was measured to be 72.84±19.56 μg g^{-1} wet tissue, phospholipid amount was 2.20±0.63 mg g^{-1} wet tissue. Aflatoxin B_1 causes a statistically significant reduction in mucus and phospholipid levels of gastric mucosal barrier ($p<0.001$, $p<0.001$, respectively). The effects of lycopene were found to be protective against the acute damage lycopene group was 91.60±15.79 μg g^{-1} wet tissue, phospholipid level was 3.47±0.80 mg g^{-1} wet tissue. In inflicted by aflatoxin B_1. While, the mucus amount in vitamine E group, mucus amount was 77.34±15.33 μg g^{-1} wet tissue and phospholipid amount was 2.90±0.49 mg g^{-1} wet tissue. Mucus and phospholipid amounts in rats exposed to lycopene and vitamine E were determined to be elevated compared to those of control group exposed to aflatoxin B_1 group (mucus $p<0.001$, $p<0.001$; phospholipid $p<0.001$, $p<0.001$, respectively).

Regarding the histological sections of the control group, stomach was found to be having a normal structure and gastric surface epithelium was determined to be composed of simple columnar and mucus-secreting cells (Fig. 1). In aflatoxin group, acidophilia on gastric surface, significant hemorrhagic foci in plica gastricas and disruptions in patches in parietal cells, structural degenerations and luminal dilatation in glands, were detected (Fig. 2). While, lycopene group was similar with control group, there was diffuse acidophilic staining due to lycopene deposition. Results of aflatoxin+lycopene and aflatoxin+vitamine E were similar with those of the control group.

Histochemical analysis showed glandular epithelium cells as containing both neutral and acid mucins in all the

Table 1: The effect of lycopene and vitamine E usage against damages occuring in components of gastric mucosal barrier as a result of aflatoxin B_1 toxicity

Groups	n	Mucus (μg g^{-1} wet tissue)	Phospholipid (mg g^{-1} wet tissue)
Control	7	160.52±14.11^a	6.43±1.13^a
Lycopene	7	173.94±11.20^a	7.21±0.96^a
Aflatoxin B_1	7	72.84±19.56^{bc}	2.20±0.63^{bc}
Aflatoxin B_1 + lycopene	7	91.60±15.79^b	3.47±0.80^b
Aflatoxin B_1 + Vitamin E	7	77.34±15.33^b	2.90±0.49^c

No difference was found between values shown with the same letter in the same column ($p>0.05$); The comparison of 3rd-5th groups with lycopene and control groups, revealed a statistical significance between mucus and phospholipid levels ($p<0.001$, $p<0.001$, respectively)

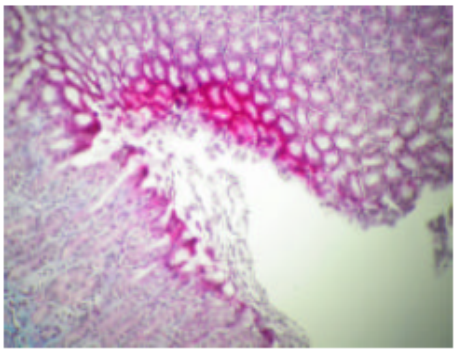

Fig. 1: Normal structure belonging to the control group, view of mucus on gastric surface (Crossman's Triplle ×20)

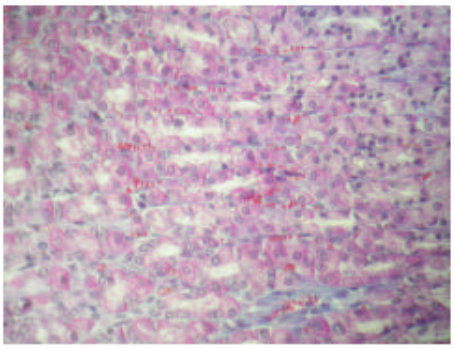

Fig. 2: Hemorrhagic foci formed due to aflatoxin use, view of dilatation in glands (Crossman's Triplle ×40)

groups. Acid mucins were found to be more predominant among glandular epithelium cells, however, there were remarkably significant differences between groups regarding their intensities. In PAS-alcian blue staining analysis, PAS (+) result was obtained at surface in all groups but in deeper tissues, mixed staining was more predominant. In control group, while cardia displayed PAS (+) and mixed staining, deeper portions manifested a mixed staining (Fig. 3). In aflatoxin group, while superficial areas and superficial glands exhibited more intense PAS (+) and mixed staining compared to those of control group, deeper tissues showed mixed stainingg (Fig. 4). Other groups exhibited similar appearances as in control group.

Aldehyde fuchsin and alcian blue combined staining revealed presence of sulphomucins on superficial portions starting from the region of gastric cardia and there were alcian blue (+) staining in patches (Fig. 5). Weak and strong sulphomucins are observed to be at higher levels in the area starting from the beginning of the gastric cardia compared to those of control group (Fig. 6). Other groups exhibited results similar to the control group.

PAS-Phenyl hydrazine staining applied for determining periodate reactive acid mucins, exhibited weak uptake over superficial areas in all groups (Fig. 7 and 8) and higher uptake in glandular epithelium cells,

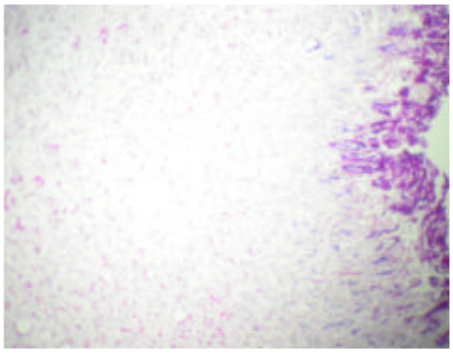

Fig. 3: View of PAS (+) and mixed staining in cardis and mixed staining in deeper tissues of the control group (view of mix staining in PAS+Alcian Blue ×20)

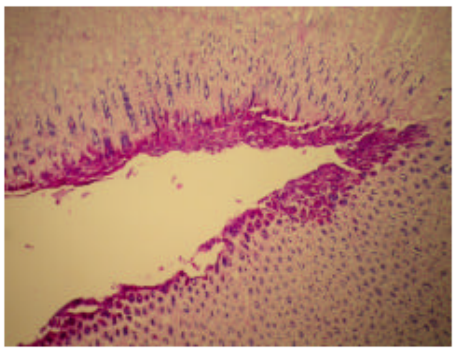

Fig. 4: View of PAS (+) on the surface and mixed staining in deeper tissues of aflatoxin group (PAS, Alcian Blue ×20)

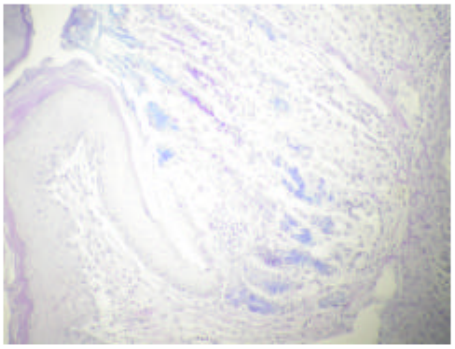

Fig. 5: View of Aldehyde Fuchsin (+) and Alcian Blue (+) in patches at surface starting from cardia in control group (Aldehyde Fuchsin, Alcian Blue ×20)

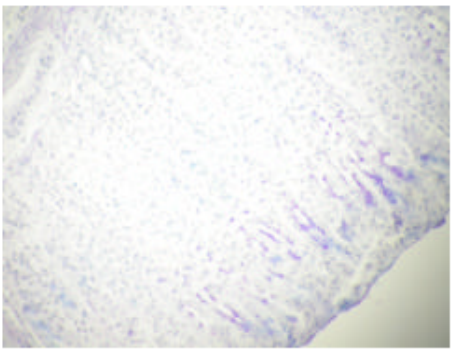

Fig. 6: View of weak and strong sulphomucins in cardia in aflatoxin group (aldehyde fuchsin-Alcian Blue ×20)

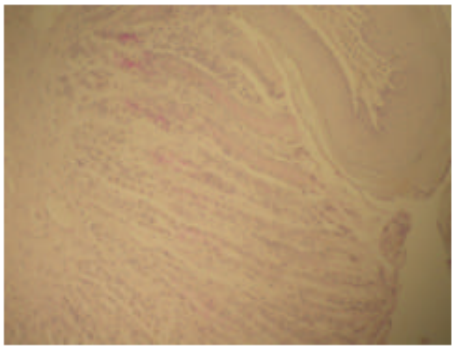

Fig. 7: Poor phenyl hydrazine staining on surface in control group (PAS-Phenyl hydrazine ×20)

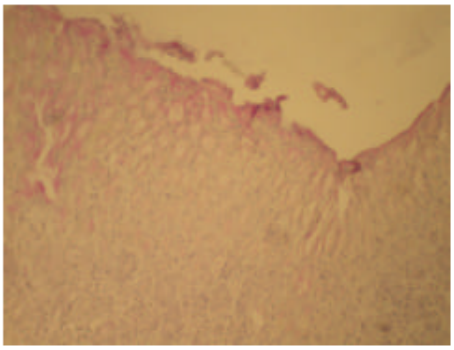

Fig. 8: Poor phenyl hydrazine staining on surface in aflatoxin group (PAS-phenyl hydrazine ×20)

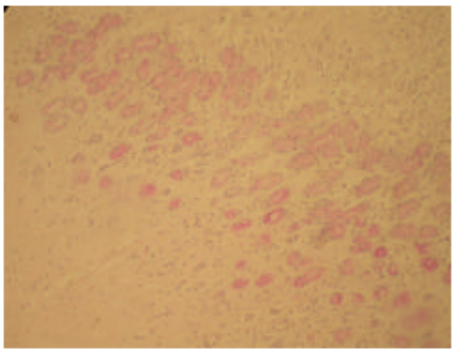

Fig. 9: Strong phenyl hydrazine staining on glandular epithelium of deep tissues in lycopene group (PAS-Phenyl Hydrazine ×20)

especially in lycopene group (Fig. 9). Other groups showed weak staining in deeper glands as observed in control group.

DISCUSSION

There are many toxic factors influencing gastric mucosal barrier. While, the pathogenesis of gastric mucosal barrier has not been understood completely yet, late studies have shown that imbalances between protective and destructive factors arising from various reasons could be the underlying cause (Spirt, 2004; Duerksen, 2003). Whereas acid, pepsin, bile, reperfusion damages and free oxygen radicals can be mentioned among main destructive factors, adequate mucosal blood circulation, mucus-bicarbonate layer, regeneration capability of epithelium cells and prostaglandins are regarded as protective factors (Andriulli *et al*., 2005; Daley *et al*., 2004).

Many studies have been conducted on mucins produced in gastric and glandular epithelium cells of different mammalian species. Submucosal glands in humans (Schulze *et al*., 2001; Henk *et al*., 1986) and dogs are thought to include neutral and acid mucins along with a low amount of sulphomucins. In the present study, mixed staining was higher in all of the groups exposed to PAS-Alcian Blue staining. This result indicated the likelihood of presence of strong and weak sulphomucins. The PAS (+) staining in those glands are underscored as indicators of the presence of neutral mucins, sulphomucins, sialomucins (Shibata *et al*., 1991) and visinal groups.

Considering features such as sulphomucins constituting majority in aflatoxin group exposed to aldehyde fuchsin and alcian blue, within framework of functions of sulphomucins (Srisai *et al*., 2002), sulphomucins can be thought to form a protective barrier.

The weak surface staining and strong deeper staining in all the groups exposed to PAS-phenyl hydrazine staining, has indicated presence of periodate reactive acid mucins, in other words, sialomucins.

There have been few experimental studies on the role of lycopene in preventing or treating cancer. One animal study found that lycopene treatment reduced the growth of brain tumors. Another animal study showed that chronic intake of lycopene considerably suppressed breast tumor growth (Brockhausen, 2003). Case-control studies have shown an inverse association between intake of tomatoes or lycopene and the risk of gastric cancer. To our knowledge, this is the first prospective study, which has assessed the association of lycopene intake and risk of gastric cancer (Giovannucci, 1999; De Stefani *et al*., 2003).

The histopathologic degenerations on gastric mucosa shown by an experimental study of Lakkawar *et al*. (2004), which included aflatoxin usage, showed parallel results with our study.

CONCLUSION

According to the results of our study, we can say that lycopene and vitamine E prevent reduction of mucus and phospholipid levels, which are known to be two of the important components of gastric mucosal barrier against gastric damage caused by AFB_1, however, the protective affect of lycopene can be mentioned as having a superior influence.

From a histochemical point of view, considering the high level of sulphomucins and their functions, we concluded that sulphomucins form a protective barrier on gastric mucosa against patogens.

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