



## Effect of extensive consumption of black pepper on intestinal glands of male rats

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### Abstract

The objectives of the present work was conducted to investigate the effect of oral administration of water extract of black pepper (*Piper nigrum*) on intestinal glands of male rats. Rats were divided randomly into two groups, Group I: control group, Group II: black pepper group, treated with 500 mg / kg black pepper for 12 weeks. The microscopic examination of the black pepper treated animals revealed damage and irregular arrangement of duodenal glands, decomposition of duodenal glands inflammatory cells infiltrated, vacuolations with necrotic nuclei. Transmission electron photographs of the duodenal glands showing deformed nuclei and dilated of intercellular spaces, necrotic glandular cells with deformed shape nuclei. Lysis of cell organelles with karyorrhexis nuclei, fragmented with dilatation parts of rough endoplasmic reticulum and mitochondrial destructed also were seen.

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## Introduction

There are several reports elucidated that different spice principles form an important role in free radicals scavengers. In the past three decades, it has been experimentally documented that several common spices can exert health beneficial physiological effects (Srinivasan, 2005). The black pepper, *Piper nigrum* L. (Piperaceae), genus Piper has been used traditionally as spice and preservatives. The black pepper contains small quantities of different compounds such as piperine, capsaicin, carotene and tannic acid. *Piper nigrum* fruits stimulate the appetite by the aroma. It is a spice used to improve the flavour of foods, preservation of meals and as an essential ingredient in pepper soup. Piperine is a pungent alkaloid present in black and white pepper (*Piper nigrum*), long used as a spice and preservative. (Rauscher *et al.*, 2000). Many authors reported that Piperine has been demonstrated to protect against oxidative damage by inhibiting free radicals and Reactive oxygen species (ROS) in vitro experiments (Mittal and Gupta, 2000). Oral daily intake of aqueous extract of black pepper seeds play an important role in controlling the level of glucose and lipids and also useful in strengthening the antioxidant potential in diabetic rats induced by alloxan (Kaleem *et al.*, 2005). Red pepper is the mostly used spicy for food throughout the world, especially in Central America, Latin America, Africa and Asia (Nopaintaya and Nye, 1974; Chukwu, 2006). Red pepper is known to be harmful if consumed in excess (Nwaopara *et al.*, 2004; Nwaopara *et al.*, 2007). Red pepper possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing tissue damage (Govindarajan and Sathyanarayana, 1991). Limited research has been reported into the effect of black pepper on the histopathology of the intestine. So it makes interesting to study the effect of consumption of black pepper on the histopathology of the intestine. The aim of this study is to evaluate the effect of extensive consumption of Black Pepper On the intestinal glands of male rats.

## Materials and methods

### *Animals and experimental design*

Twenty male albino rats (180-220gm) were obtained from the animal house, King Abdulaziz University were used in the present work. Animals were divided randomly into two groups, 10 animal for each and each group was putted in a plastic cage. The cages were kept in a room temperature. The animals were fed with a suitable quantity of water and complete diet. Group I: control group, treated with 0.1ml of normal saline. Group II: black pepper group, treated with 500 mg/ kg black pepper daily for 12 weeks.

Animals from control and treated groups were dissected and small pieces of the intestine were quickly removed, then fixed in 10% buffered formaldehyde solution. Following fixation, specimens were dehydrated, embedded, and were cut five microns thickness. Sections were stained with general stain, Haematoxylin and Eosin for histological examinations.

### *Transmission electron microscopy*

For Transmission electron microscopy, small pieces of intestine were rapidly fixed with 2.5 percent glutaraldehyde with 0.1 M sodium phosphate buffer at 4°C for 3 h, washed in the same buffer at 4°C for 1 h and postfixed in 1 percent osmium tetroxide. The tissues were then dehydrated in graded series of ethyl alcohol (50%-100%). The tissue specimens were embedded in Araldite. Semi-thin and Ultrathin sections were stained with Mg-uranyl acetate and lead citrate for transmission electron microscope (TEM) evaluation.

## Results

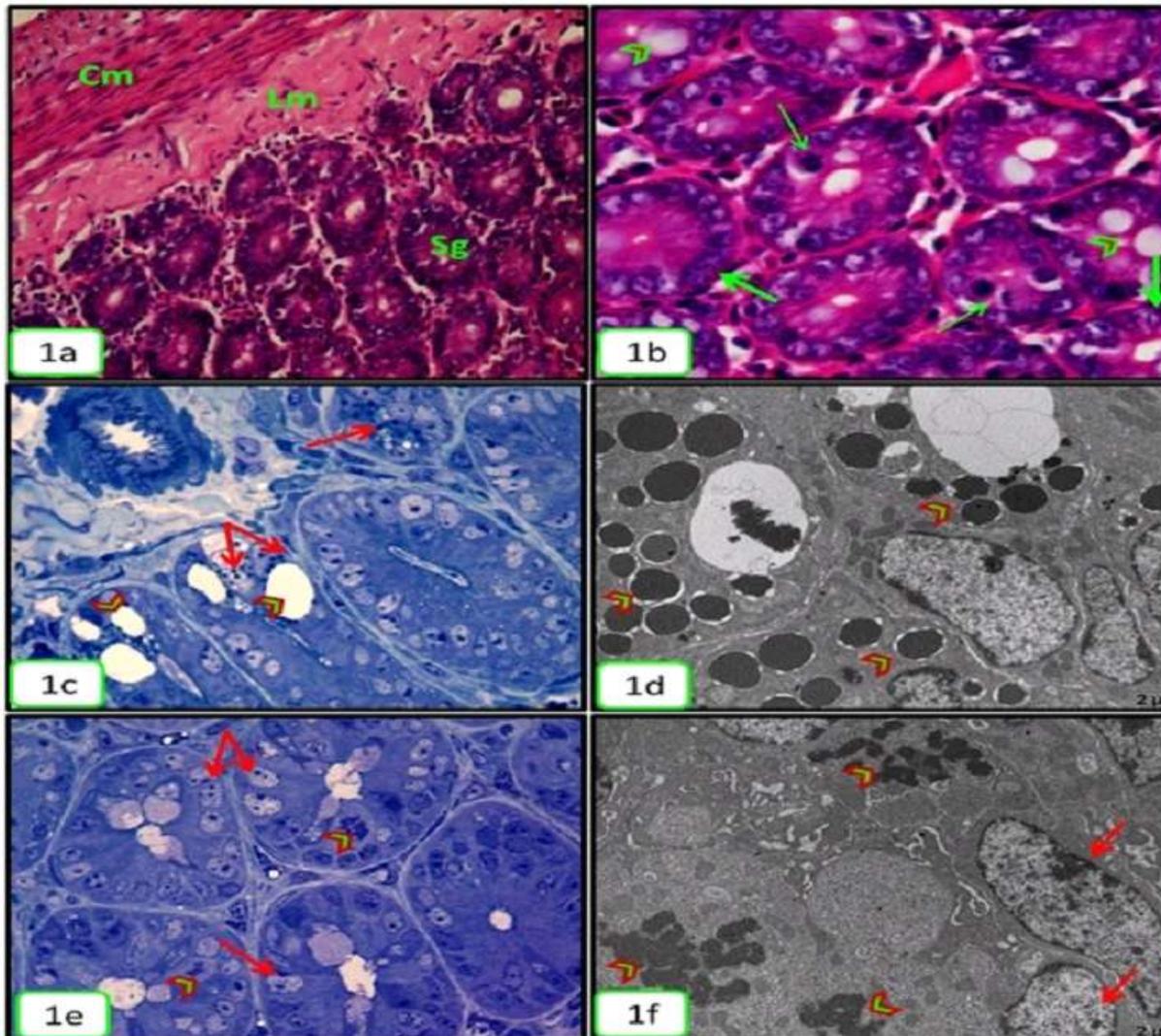
### *Control group*

#### *Histological structure*

The microscopic investigation of the duodenal sections of the control animals (Figs. 1a&b) showed their characteristic layers; serosa, muscularis, submucosa and mucosa. The mucosal layer is the most important absorptive layer, it exhibit numerous evaginations (villi) into the lumen of the gut. The characteristic feature of the duodenum is the Brunner's glands which embedded in the submucosa,

are compound tubular submucosal glands. The glands are covered by epithelium which contains multiple types of cells: enterocytes, goblet cells which are oval or round, with flattened basal nuclei, endocrine cells,

Paneth cells at the base of the gland, which are flask-shaped. Brunner's glands secrete mucous secret containing bicarbonate which serves to neutralize the gastric acid.



**Fig. 1. (a-f).** Sections of normal duodenal glands. **(a,b):** transverse sections of Brunner's glands **(a)** showing submucosal mucous glands (Sg), circular muscles (Cm), longitudinal muscles (Lm), (H&E; X400). **(b,c)** goblet cells filled with mucus (arrowheads), mitotic cells (thin arrows), Paneth cells (thick arrows), (H&E; X1000); (TB; X1000). **(d)** part of submucosal gland showing Paneth's cells filled with secretive granules (arrowheads) consist of protein of high electronic density surrounded by a halo of low electronic density of polysaccharide. EM, X5600. **(e,f)** mitotic cells (arrowheads) in division stages and endocrine cells (arrows) with electronic low density of mitochondria, (TB; X1000; EM, X 5000.).

#### *Transmission electron microscopy*

Transmission electron microscope (Figs.1d&f) revealed that submucosal glands showing Paneth's cells filled with secretive granules consist of protein of high electronic density surrounded by a halo of low electronic density of polysaccharide. Mitotic cells in

division stages and endocrine cells with electronic density low of mitochondria.

#### *Treated group*

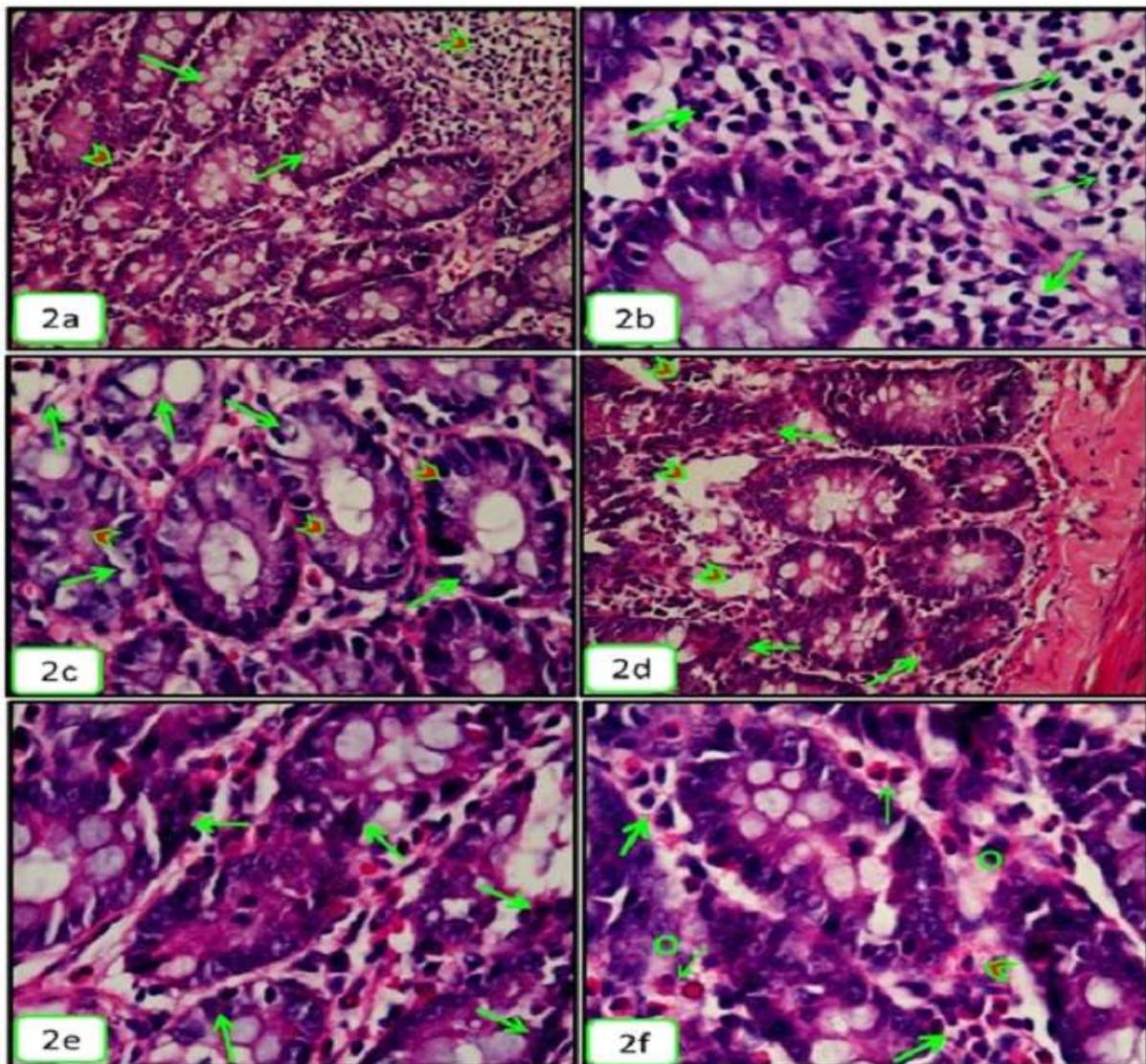
The microscopic examination (Figs.2a-f) of the treated animals with black pepper revealed duodenal

glands lysis due to dissociation and necrosis of glandular cells, damage and irregular arrangement of duodenal glands, inflammatory cells infiltrated, macrophages aggregates between the duodenal glands, glandular cells vacuolations with necrotic nuclei, increased paneth cells granules, duodenal pyknosis with aggregated of glandular cells nuclei.

#### *Transmission electron microscopy*

Electron photographs (Figs. 3a-d) of the duodenal

glands tissue of black pepper treated rats showing goblet cells proliferation filled with homogenous granules of mucus, deformed nuclei and dilated of intercellular spaces, necrotic glandular cells with deformed shape nuclei and lysis of cell organelles, pyknosis with karyorrhexis nuclei. Fragmented with dilatation parts of rough endoplasmic reticulum and mitochondrial destructed. proliferation of paneth's cells secretory granules were seen.

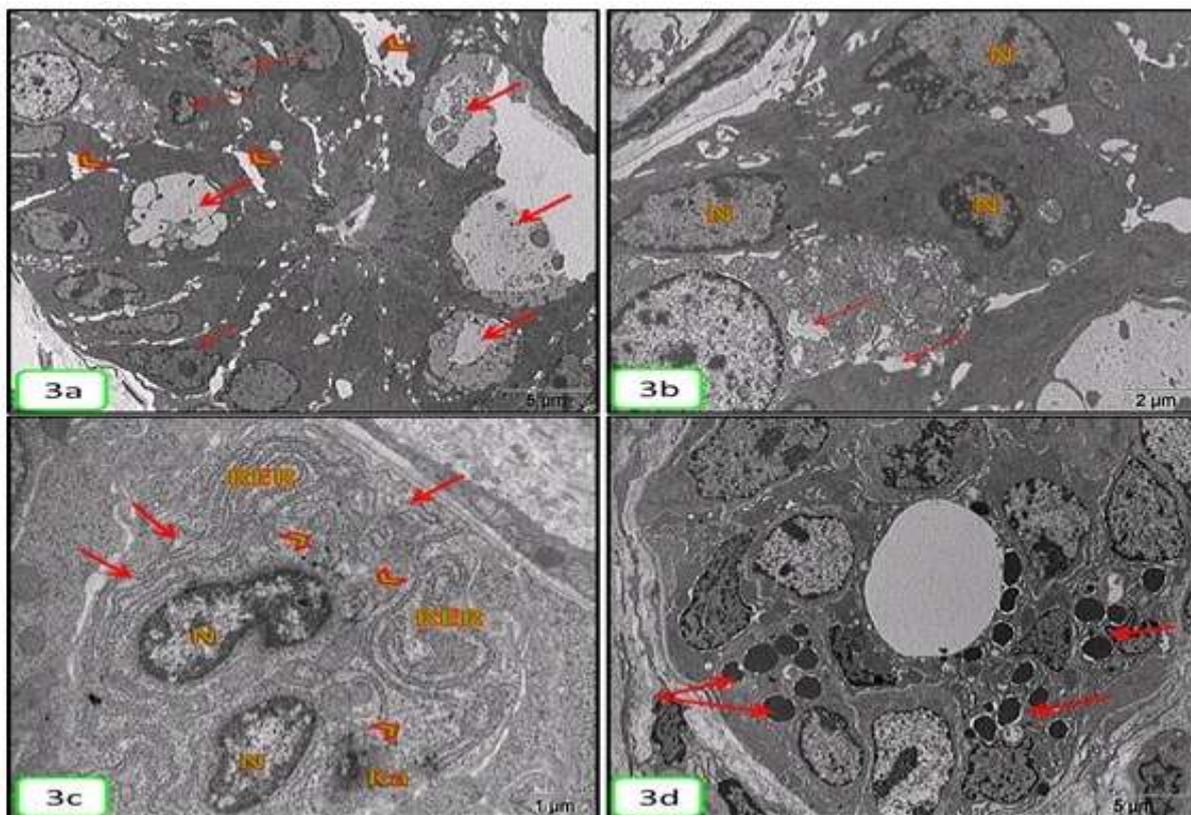


**Fig. 2. (a-f).** Transverse sections of duodenal glands treated with 500 mg/ kg black pepper (H&E); **(a)** glandular goblet cells proliferation (arrows), inflammatory cells infiltrated (arrowheads) X400. **(b)** higher magnification of (Fig. a) macrophages aggregates (thick arrows) and lymphocytes (thin arrows) X1000. **(c)** glandular cells vacuolations with necrotic nuclei (arrows), increased Paneth cells granules (head arrows) X1000. **(d)** duodenal glands necrosis (arrows) lead to the glands loss (head arrows) X1000. **(e)** pyknosis with aggregated of glandular cells nuclei (arrows) X1000. **(f)** oedematous (O) with neutrophils (thin arrows), mast cells (head arrow), lymphocytes and macrophages infiltrated (thick arrows) between the duodenal glands X1000.

### Discussion

Several studies have illustrated that various essential spice form have antioxidants activities. The present study indicated that extensive consumption of black pepper. Fruits can cause damage to the intestinal epithelium. This damage includes irregular arrangement of duodenal glands, inflammatory cells infiltrated, glandular cells vacuolations with necrotic nuclei, increased Paneth cells, pyknosis with aggregated of glandular cells nuclei. Researches which has been reported into the effect of red pepper on the histopathology of the intestine are rare but

some researches were done on other organs such as liver, kidney, testis and salivary glands (Durham *et al.*, 1990; Samaranayake *et al.*, 2000; Nwaopara *et al.*, 2007; Chaiyasit *et al.*, 2009). Shahverdi *et al.*, 2013 reported that use of red and black pepper powder on broilers diets cause increase total diameter of small intestinal parts. Use of red and black pepper significantly increased the absorption surface of the duodenum and the ileum. Galib *et al.*, 2011 stated that black pepper has effective role in increasing digestive enzymes acts, weighs gain and chicks dietary by increasing the absorption power.



**Figs. 3. (a-d).** Electron photographs of the duodenal glands tissue of 500 mg/ kg treated rats showing; **(a)** goblet cells proliferation, filled with homogenous granules of mucus (thick arrows), deformed nuclei (thin arrows), and dilated of intercellular spaces (arrowheads); X 2800. **(b)** necrotic glandular cells with deformed shape nuclei (N), and cell organelles lysed (thin arrows); X5600. **(c)** pyknotic (N), with karyorrhexis (Ka) nuclei of two mitotic cells, fragmented with dilatation (arrows) of parts of rough endoplasmic reticulum (RER), and mitochondrial destructed (arrowheads); X11000. **(d)** proliferation of paneth's cells secretory granules (arrows); X3500.

They also found that when the broiler chicks fed supplemented diets with hot pepper, this can improve feed ratio. This may be due to its effect on digestion, absorption and anti-microbial properties.

Oral supplementation with piperine can reduce DNA damage. It can induce apoptosis in B(a) p-induced lung carcinogenesis in Swiss albino mice (Selvendiran, *et al.*, 2005). Vijayakumar, *et al.*, 2004

studied the effect of supplementation of lower doses of black pepper on tissue lipid peroxidation and antioxidant enzymes in rats and they stated that these spices can reduce oxidative stress resulting from high-fat diet-induced. Khajuria, *et al.*, 2002 demonstrated that piperine with high doses affected obvious changes in the ultra-structure of intestinal epithelium such as increasing the length of the microvilli.

#### Conclusion

It can be concluded that while black pepper may be useful as a spice and in treatment of several diseases, it can be toxic in high doses.

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