

*Full Length Research Paper*

# Histo-pathological alteration in the intestinal tissues of Swiss albino mice *mus musculus* fed with contaminated bread: An un-noticed serious health hazard

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Accepted 30 July, 2011

Microbiological analysis is the useful way to assess the safety and quality of food. Substantial number of micro-organisms suggest a general lack of cleanliness in handling and improper storage practices. Although it is generally agreed that bakery products are microbiologically safe food but post baking processes, packaging and wrong handling cannot be denied. Bakery products have been an important part of diet. Due to substandard handling operations, mould spoilage has become a serious threat for bakery industries. Further large mass of people like students, children, daily workers, tourists, all prefer bread preparations. Local bread preparations from road side vendors form a major part of their food. Improper handling and storage facilities lead to microbial contaminations as the higher water activity can lead to certain pathogenic anaerobic microbial growth at ambient temperature causing serious unnoticed health hazards. Hence, an attempt was made to collect the local bread samples from densely populated areas of the city for their microbial contaminations. Maximum count was  $256.83 \pm 2.59 \times 10^5$  cfu / gm of colonies for yeast and mould against permissible count of  $120 \times 10^4$  in recommended media. This food was given to mammalian model Swiss albino mice, *Mus musculus* for 45 consecutive days to see the gradual effect of microbes on different intestinal tissues. T.S of intestine, when observed revealed severely damaged villi with degenerated columnar epithelial cells and goblet cells. Damage in villi structure is an indication of impairment in the normal process of absorption. As the days advanced i.e. 30 to 45 days, the villi started atrophying with thin and short out growth like structure from sub mucosal layer. At 45 days villi along with mucosal and sub mucosal layers were completely damaged with thin atrophied cellular structures. This is an alarming situation and warns against the contaminated food. It may pose a serious threat to health if not taken seriously.

**Key words:** Food contaminations, Bread, Intestine, Villi, Goblet cells, Sub mucosal layers

## INTRODUCTION

Adulteration is a term used to designate that a product is not what it should be, from biochemical, cleanliness and hygienic point of view. Adulteration of food stuff is a common practice in India by the trade. Since, we have a large mass inhabiting under poverty line and in an unhygienic conditions, the consumers want to get maximum quantity for as low price as possible resulting poor quality and adulteration. (Swaminathan, 1987). Adulteration in the food is the main cause of food

borne illness. It is an ever present threat that can be prevented with proper care and handling of food products.

With the increasing awareness of the environmental problems, contamination and hazards of edible food items have become one of the important issues amongst the scientific world. We all are aware of the fact that toxicity in the edible items is creating panic in the entire world. Every day we come across various reports of food poisoning due to contamination of pesticides, spoilage due to microorganisms and adulteration with various toxic and hazardous materials. Microorganisms grow on a wide range of substrates which may serve as vehicles for

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their spread to various other environments, including the human body (Ayanbime *et al.*, 2007). Food borne illness is estimated to affect 10% of the population annually. Estimates have placed the annual treatment and work hours lost was more than \$3 billion (Bohdan *et al.*, 2003).

Bacteria and fungi are the most common cause of food poisoning and food borne diseases. (Bloomfield *et al.*, 1990). These microorganisms are ubiquitous and are found in a variety of habitats, either as transient or permanent dwellers. There is an increasing knowledge and understanding of the role played by molds in the food spoilage. Especially the discovery of mycotoxins production in food has highlighted the importance of molds food spoilage. The recent agreement in taxonomy has led to the discovery that a specific, very limited fungus (*Mycobola*) is associated for food spoilage. (Borg *et al.* 1996) molds are filamentous (fuzzy or dusty appearing) fungi that occur commonly in feed stuffs, including roughage and concentrates. Similarly, the effect of poisoning by mycotoxins from fungus is through food consumption. Mycotoxins prominently affect human and animal health. An outbreak which occurred in UK in 1960 caused the death of 100,000 turkeys which had consumed aflatoxin contaminated peanut meal and the death of 5000 human lives by alimentary toxic aleukia (ALA) in USSR in world War ( Michael., 2007).. Mycotoxins induce various biological effects, such as liver diseases, alteration in growth rates, change in the mechanism of immunogenesis and carcinogenic and mutagenic effects in different animal species (Pier, 1973).. Various vital organs such as elementary canal, liver, kidney and nervous tissues are badly affected after ingesting spoiled food and the raw food such as meat, fish, milk, and vegetables grown in sewage that are likely to be contaminated. Recent studies have shown that food grains, legumes and oil seeds when stored in humid atmosphere are infected by pathogenic microbes which can cause serious illness. The pathogenic microorganisms of contaminated food are responsible for causing serious illness and this contaminated food also affects different systems and organs of human body.

South Rajasthan (India), especially Udaipur city of India is a blend of tourists, tribal's and desert culture,. Bread is type of bakery product that is used by all sectors in one form or the other. At some places PAV (local bread or bada pav) are used as regular food by many people .Being a tourist city, and Gujarati culture people consume many local bread preparations and these are sold by vendors of road side with many unhygienic conditions..

Hence, it was thought to collect local bread samples for contamination studies and see its effect on mammalian intestine by taking Swiss Albino mice *Mus musculus* as model and comparing it with normal tissues. This study unfolded many issues of health hazards caused by such contaminated food

## MATERIALS AND METHODS

Three types of bread samples were selected for the studies. Three samples of all three varieties were collected from highly populated areas of city i.e. Surajpole(heart of city), Udaipole(bus stand and hotels with maximum tourists)and Chetek circle ( general hospital). Samples were brought from market in sterilized containers to the laboratory .Serial dilutions of the samples were made and cultured for yeast and mould on Potato Dextrose Agar (Ronald,2006) .Highly contaminated samples ( where colony count was much higher than recommended limit)with a maximum count (  $256.83 \pm 2.59 \times 10^5$  cfu / gm of colonies for yeast and mould against permissible count of  $120 \times 10^4$  ) were given to a group of six mice of 30-50 days old weighing 30-40gms each.. Another group of same number of mice were fed on normal bread (where colony count was within permissible limit) to run as control . Animals were kept in plastic cages under controlled conditions. Both treated and control groups were fed for 15,30 and 45 consecutive days. Mice were observed for their behavioral and morphological changes to correlate the symptoms with architectural changes in corresponding tissues. Animals were then sacrificed for histopathological observations .All standard protocol of culture and histopathology were adopted for experimentations.8  $\mu$  thick sections were cut and stained with haematoxiline and eosin by adopting standard protocols .For experimentation permission from Indian Ethical Committee was taken.(No.CS/Res/07/759).

## RESULTS

### Morphological abnormalities due to feeding of contaminated food

Gradual effect of contaminated food in the morphology of Swiss Albino mice were observed during the experimental period. Gradual loss of hair with skin exposed (Plate 1 a, b), deflected feet (b), change in the structure of snout (Plate 1 b, c) excessive loss of body hairs and body weight (Plate 1 c, a), change in the posture of tail, abnormalities in feet (hind feet) and over all body contour (Plate 1 c, d) were observed during the experiment period in the mice fed with Yeast and Mold contaminated food. Gradual weight loss in the body weight after 15 days , 30 days , and 45 days were also observed.

### Normal Histoarchitecture of T. S. of intestine of Swiss Albino mice *Mus musculus*.(Plate 2,a and b)

Villi were well developed with proper orientation of various parts (Plate 2,a).Brush border (BB), columnar epithelial cells (CEC), goblet cells (GC) lamina propria (LP) and lumen of the villi were well evident with normal structure. Intact nature of two villi with densely packed columnar epithelial cells , brush border (BB) and lamina propria (LP) were observed.. Globlet cells (GC) were

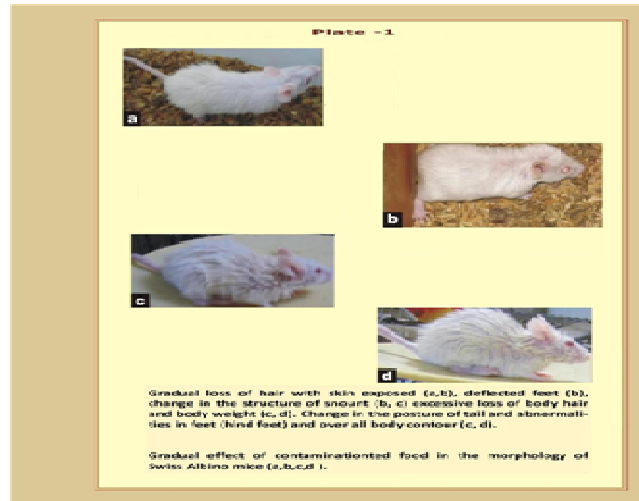


Plate 1

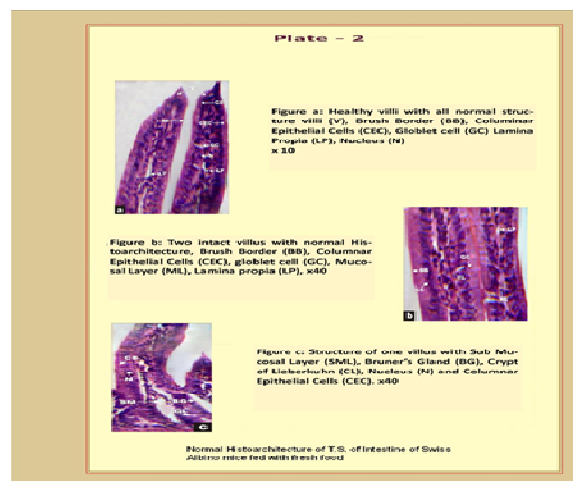


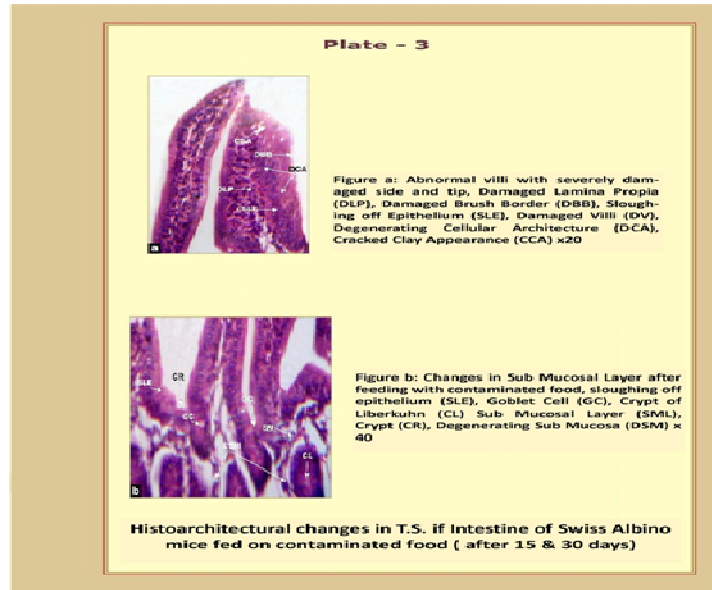
Plate 2

equally spaced and normally developed.(Plate-2,b) .

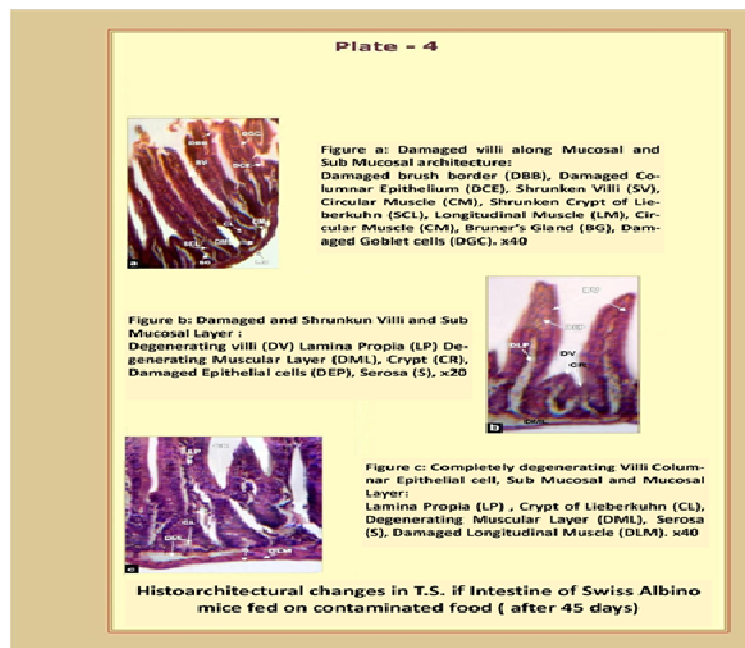
#### Histoarchitectural alterations in the intestinal tissues of Swiss Albino mice ,*Mus musculus* fed with contaminated food:(after 15,30 and 45 days)(Plate-3 and 4)

Severe pathological alterations were observed in the intestinal tissues of mice fed with mould contaminated bread .The thickness of the intestinal wall was greatly reduced after 45 days The Villi were atrophied with damaged brush border all along the villi border. (Plate 4, 45

days).The columnar epithelial cells along with the microvillus were seen sloughing off at the tip region of the villi .Intestinal mucosa was almost destroyed and dislocations of epithelial cells were observed prominently. The mucosal epithelium was eroded and became necrotic 15 days and 30 days .(Plate-3,fig-a and b) .At some places villi were greatly reduced and damaged 15 days and 30 and 45 days .Some parts of villi were damaged so severely that it gave wavy appearance with undistinguished cellular structure.(Plate-3,fig-c) .Mucosal layer from some areas became totally damaged. Circular and longitudinal muscles were completely atrophied and reduced considerably leaving a thin layer after 45



**Plate 3**



**Plate 4**

days.(Plate-4) .Spacing could be clearly seen between sub mucosal layers and muscular layers after 45 days .

Lamina propria were entirely damaged after 45 days .Hypertrophied sub mucosal cells and Crypts of Liberkuhn could also be seen distended with large empty spaces with poorly developed cellular components. And

after 45 days this Crypt of Liberkuhn became very small and was shrunken (Plate-4,fig-c). Large space formation due to disturbance in general cellular arrangements were quiet prominent. Histoarchitecture at the sub mucosal level became apparent after 30 days with considerable destruction of villi and loss of villous structure were seen

at the end of 45 days.

## DISCUSSION

Overpopulation and procurement of healthy food for all is a burning problem of today's world especially for undeveloped and developing countries.. World Health Organization's Food and Agriculture Panel considers that current and emerging capabilities for the production and preservation of food should ensure an adequate supply of safe and nutritious food up to or beyond 2010 when the world's population is projected to raise more than 7 billion. Despite overall sufficiency, it is recognized that a large proportion of the population is malnourished. Any long term solution to this must lie in improving the economic status .One way of initiating this is to reduce the substantial pre and post harvest losses which occur particularly in developing countries where the problem is more acute. In absolute terms, the US National Academy of Science has estimated the losses in cereals and legumes in developing countries as 100 million tones, enough to feed 300 million people.

Food borne illness usually arises from improper handling, packaging and storage of food. Good hygiene practices before, during and after food preparation can reduce the chances of contacting an illness, so the action of monitoring for fresh food is very important.

After feeding the mice with contaminated food, various morphological changes were observed. These morphological and behavioral changes included hair loss, body weight loss, change in snout structure, general limping of limbs were some of the prominent changes that could be observed. The overall sluggishness in mice activities were also noticed .Different behavioral and morph metric changes in the animals may be attributed to the effect of mycotoxins present in the contaminated bread sample. Okud *et al.*, (2004) observed effect of Chromoblastomycosis (a disease caused by fungus) both morph metrically and ultra structurally in two patients. They found coetaneous lesions in the dermis. Most of the fungal elements appeared as sclerotic cells and their wall showed an irregular leaf like appearance.

Effect of Aflatoxin (mycotoxins secreted by fungus) has also been observed in other animals. When the fishes were treated with toxin, they became darker in color with unbalanced swimming behavior and ultimately they died (Mahrons *et. al.* 2006). Fumitremorgin A (FTA), a neurotropic mycotoxin induced dose-dependent abnormal behaviors, including tremor, colonic convulsion, kangaroo posture and tonic extensor convulsion in the mouse (Suzuki *et.al.*, 1984).

Haematoxylin and Eosin stained sections revealed a massive damage in almost all tissues of the intestine. The damages were quite gradual revealing the continuous and gradual effect of toxins on the physiology and histopathology of the animal.

The digestive tract is a tube lined by specialized epithelial cells continuous with epithelial lining of the skin thus the tract is open to the external environment and is also a potential site for the exposure to toxic substances that are introduced through ingestion.

In the present study, the epithelial layer of the villi was seriously damaged (plate 3,4) giving the appearance of waves. It was sloughed off along with brush border from outside and lamina propria and mucosa from inner side. This damage might have caused direct entry of toxins in the body as the epithelial cells forms a semi permeable membrane that selectively allows passage of fluid, electrolytes and dissolved nutrients. It also acts as a physical barrier against foreign particles entering directly into blood stream and giving access to viscera (Arquinzia, 1997). The intestinal tract is the first barrier to ingested mycotoxins and also the first line of defense against intestinal infection. Ingestion of mycotoxin in yeast and mold contaminated bread by the animal might have increased the mucosal susceptibility by infecting mucosa. This infected mucosa further spread the infection to epithelial layer causing completely degraded cells and shrunken villi (30 days and 45 days). Our results are in agreement with Catherine (2006) and Diekman, (1992) who reported degeneration and infection in mucosal layer of mycotoxin treated tissues. Since villi are lined by epithelial cells and differentiated according to location on the villi to absorb fluids and nutrients (tip), secrete electrolytes and fluid (crypt and side) and replace damaged cells (crypt), (Bloom & Fawcet., 1993). The gradual loss in villus architecture from 15 to 30 to 45 days (Plate,3 and 4 )was a clear indication of hampering all the above processes in mice fed with contaminated food. Ramos *et al.*, (1996) reported harmful effect on intestinal epithelial by absorption of mycotoxins.

Further, it was observed that as the time of exposure to the fungus contaminated food was advanced, the degeneration and alteration in various layers also progressed accordingly. In 30 and 45 days old mice, the villi showed shortening of length with small epithelial cells and abnormal goblet cells. The sub mucosal layer showed extensive changes. The entire sub mucosal area became sparse and vacuolated with sunken poorly developed crypts of Liberkuhn and Bruner's gland. . Further the muscular layers (circular and longitudinal) started contracting and became very thin with almost disappeared after 45 days (Plate 4 ). The treated animals almost stopped feeding and became dull. Some of them also died. These symptoms further got confirmation by the intestinal histoarchitecture that revealed severely damaged thin small villi with almost no distinction of epithelial cells and brush border leaving no space between mucosa and blood vessels .Further sub mucosal and peritoneum layer became very thin and indistinguishable (Plate 4 ).

The protective effect of mucous is further evidenced by increased mucosal surface and goblet cells hypertrophy

in response to toxins. Mucous is the barrier of fungal and bacterial invasion. Belquees & Parneen, (1996), also observed the loss of mucosa and sub mucosa in parasite infected *Nesokia indica* (short tailed Indian mole rat). Chnicharn and Bullock (1967), also observed detached epithelium and connective tissues.

In 30 hours, the adenomatous stages of villi mucosa were observed that might be an indication of first hand counter attack by body to kill the pathogen. Khatooni (2004) observed similar observations in short tailed Indian rodent *Nesokia indica*. The term alimentary mycotoxicosis poisoning by mycotoxin through food consumption and mucromycosis are the common name given to different disease caused by fungus. Mucoraceae is the most common intestinal mold infection (Marques *et al.*, 2003).

The secretory glands of intestine, goblet cells and crypts of Lieberkuhn further revealed general dystrophy after 30 days (Plate 4) indicating the effect of toxins on these structures too. Crypts of Lieberkuhn secrete the intestinal juice, Lysozyme (paneth cells) and serotoxin (Peristaltic stimulator), secretin (Pancreas) and cholecystokeninis secreted by gall bladder (Bloom & Fawcett 1993). The goblet cells secrete mucous. (Bloom and Fawcett 1993). The increase in number of cells at the outer surface after 30 days might be an outcome of the cells in defensive mood to fungal invasion but loss of villi architecture and there by the goblet cells at 45 days indicated the dystrophic and damaging effect of fungus on the secretory cells as well. Our results further got support by the study of Llewellyn *et al.*, (1981) who observed that exposure of intestinal mucosa to *vibrio cholerae* enter toxin resulted in excess mucous secretion from goblet cells.

Hence, to sum up the present study is the need of the hour. Since bread is a very popular food for tourists, general people, students, children and even labor class. Improper handling, unhygienic conditions lack of awareness can bring heavy contamination to bread food if consumed regularly may pose a severe damage to intestinal tissues that ultimately bring a heavy toll to life. Since the results are slow and gradual, cause of damage generally go unnoticed and people suffering from such health risk might not be able to point out the reasons. Hence, strict rules against adulteration and proper training and awareness for shopkeepers and vendors are of utmost importance.

## ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Zoology for providing necessary facilities to conduct work and Dairy Science College for their help in microbiological experimentations.

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