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Toxic Effect of Green Leaf Color as Food Dye on Liver, Kidney, and Intestinal Tissues in Animal Mice Model

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ABSTRACT

Food dye is a synthetic chemical that restores natural color that is lost during processing and enhances the color, flavor, and taste of food. It mostly affects youngsters and produces a variety of health issues. This investigation was conducted to examine the deleterious effects of the unclassified dye "green leaf color" on the liver, kidney, and intestine tissues of a mouse model. Fifteen adult mice were separated into two groups: control and treatment. The treatment groups were fed standard poultry feed with dye in 20µl and 10µl (1.0mg/kg/bw and 2.0mg/kg/bw, respectively). At the conclusion of the 90 days experiment, animals were euthanized and sacrificed, and pieces of liver, kidneys, and intestine were collected and histologically processed for visualization under a light microscope. Feeding of green leaf color induced a range of histological changes in all treated mice compared with their control group. Results from the histopathological examination showed mainly vacuolization of renal tubules, raising space between the walls of Bowman's capsule, shrinkage of glomeruli and glomerular necrosis, congestion of tubules, inflammatory cellular infiltration, dilation of tubular lumen, and hemorrhage in renal tissues. In the case of the liver, congestion of the central vein, vacuolization of hepatic cells, pyknotic nuclei, karyorrhexis, karyolysis, and inflammatory cellular infiltration were observed. Similarly, significant histopathological alterations were identified in the intestinal section including disrupted brush border, necrosis, vacuolization, swelling, uncontrolled cell proliferation, disrupted mucosa, and submucosa. Therefore, we strongly recommend here the ban of unclassified dye green leaf color to use as a color additive.

Keywords: Green leaf color, Histopathology, Kidney, Food dye, Liver, Intestine, and Mice.

INTRODUCTION:

Color is an important feature and factor in choosing food. Due to their accessibility, affordability, and tinctorial strength (Sahar SA and Manal MEM, 2012), natural and synthetic color additives found widespread application in the food, pharmaceuticals, and cosmetic industries (Hallagan *et al*., 1995). Color additives, often known as dyes, pigments, or chemicals, are used to increase the aesthetic appeal of meals and medications (Macioszek and Kononowicz, 2004). The history of food coloring may be traced to the early Egyptian and Roman civilizations, when people colored their food with saffron, different flowers, carrots, mulberries, beets, and other ingredients (Dwivedi and Kumar, 2015), perhaps indicating that coloring agents have been utilized since ancient times.

Though, people started using manufactured colors instead of natural colors around the middle of the nineteenth century (Dwivedi and Kumar, 2015). Since then, despite their legal restriction, widespread usage of synthetic food azo dyes has increased owing to the popularity of canned and fast food. Additionally, these colors are not preservatives, have no nutritional value, and provide no health advantages (Martin *et al*., 2013). They only make food appealing to satisfy new customer demands, since end consumers are thought to pick items mostly based on how they look (Assad and Buraydah, 2011). According to Hallagan *et al*. (Hallagan *et al*., 1995), dyes are often water soluble and utilized in a wide variety of food preparations, including pickles, jams, jellies, sweets, candies, and ice cream. For coloring and preservation, a large variety of food additives - more than 2,500 different substances - are utilized (Toledo, 1999; Shahen *et al*., 2019).

Some artificial coloring additives have a detrimental effect on consumer health, such as tartrazine E102 and chocolate brown, which Comet assays shown to have damaged DNA in the liver and kidneys (Hassan, 2010). Synthetic colorants, such as chocolate colorants, cause rats' body weight, blood cholesterol, and HDL cholesterol to drop while their liver enzyme levels rise (Aboel-Zahab *et al*., 1997; Mohamed *et al*., 2022). In vitro, testing on human lymphocytes revealed that food colorings (amaranth, erythrosine, and tartrazine) might be harmful, and it seems that they bind to DNA directly (Mpountoukas *et al*., 2010). Various physiological changes are observed when the color additives are added at different concentrations and high doses of color additives affect children mostly (Hashem *et al*., 2010). When the food colorants bind directly to DNA they expressed severe harmful consequences on human lymphocytes in vitro (Mpountoukas *et al*., 2010). Bangladesh is an extremely overpopulated and developing country in the world. A large number of populations need more food for their survival. In contrast, to meet up the food requirement, a large number of food industries were made here. Today, the use of different food colorants additives is spreading throughout the world along with industrial progress and they are often used to simulate the presence of healthful, colorful fruits and vegetables. Maximum food industries do not follow the food safety rule for children and human health. All industries produce a large number of foods with different colors for inviting and appealing people, especially to the children. They use various types of unclassified food dyes that are not approved by the FDA. The food's green leaf color is one of them. Histopathology is considered an essential tool to determine the potential dangers posed by foods, medications, chemicals, biologicals, and medical devices. It is important that the way histopathological examinations are done to meet regulatory requirements for fair observations while making it easy to evaluate large amounts of microscopic information in a sensitive and efficient way (Crissman *et al*., 2004). Histopathological research is a method that is both sensitive and repetitive. Typically, it is used to identify changes in animal tissues to determine the harmful effects of toxic chemicals on various tissues and organs, as well as to measure the toxicity and risk assessment of toxic chemicals (Adams, 2002; Bernet *et al*., 1999; Crissman *et al*., 2004; Mela *et al*., 2007).

For the adverse effects of food additives on humans and animals, in this study, we investigated whether the unclassified food dye green leaf color is harmful to health or not. This research aimed to examine the histopathological effects of green leaf color on mice liver, renal, and intestine after 90 days of treatments.

MATERIALS AND METHODS:

Experimental Animals

Fifteen albino mice weighing between 22 and 25 grams were obtained from Dept. of Pharmacy Jahangirnagar University, Dhaka, Bangladesh. For 15 days, mice were acclimated to the trial room environment, which consisted of a constant temperature of 25±2°, in plastic mouse cages. Sufficient water and standard poultry feed were provided for the animals. All mice were handled in accordance with the typical guide for the care and use of laboratory animals (under the license of Institutional animal, medical ethics, bio-safety and bio-security committee (IAMEBBC) for experimentations on animal, human, microbes, and living natural sources No. 33/320/IAMEBBC/IBSC).

Test Material

Green leaf color is an unclassified food dye. So, its molecular structure, chemical composition, and purity are unknown. The experimental dye was collected

from a local bakery of Bhedarganj market, Shariatpur district, Bangladesh.

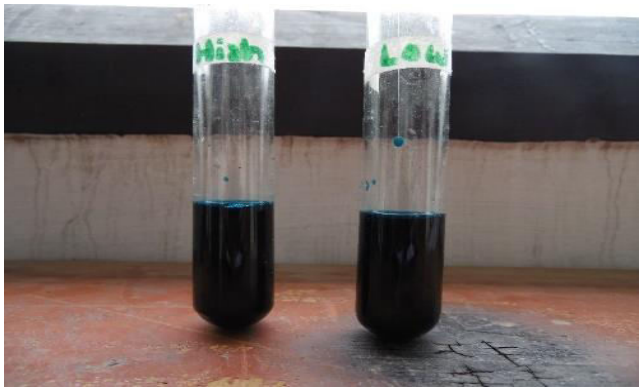

Fig. 1: Liquid solution of test dye green leaf color.

Experimental design

Fifteen adult Swiss albino mice were randomly assigned to one of two groups: control or treatment.

Control group

The animals in the control group received a diet of 10 g/mouse (a total of 50 g for five mice) without any food additives. Using a feeding bottle, plenty of water was given.

Treatment groups

Two treatment groups, each with five mice, were used in the study;

- Group 1: Over the course of 90 days, experimental animals were given dye orally at a concentration of 01 mg/kg/bw
- Group 2: Over the course of 90 days, experimental animals were given dye orally at a concentration of 02 mg/kg/bw

Preparation of histological slide

Histopathological analysis of three organs from both groups of animals were conducted based on the technique previously described by Carleton *et al*. (Carleton *et al*., 1967). Animals were given chloroform anesthesia and killed by cervical dislocation after the treatment period was over. All traces of the chemical were washed away by rinsing the collected liver, kidney, and intestine under running tap water for an hour after being fixed with Bouin's solution (fixative) for 16-18 hours. After being washed, the tissues were dehydrated by being submerged in alcohol at progressively higher concentrations (50 %, 70%, 80%, 95%, and 100% alcohol) before being embedded in paraffin wax to form blocks. The wax on the block had to be scraped away to reveal the tissue below. Tissue sections were cut using a microtome (Optical. Co LTD Tokyo, Japan under the brand name SHI-BUYA). Tissues of about 6 µm in thickness were sliced using a microtome that had been preprogrammed. Blocks Tissue slices were deparaffinized with xylene solution and put on a microscope slide using warm distill water with a few drops of Mayer's albumen. The tissue was stained with hematoxylin and alcoholic eosin solution for the permanent slide. Under a light microscope (Optika, Italy), histopathological alterations were studied and the photographs were obtained at 20x and 40x magnification.

RESULTS:

Effect of green leaf color on mice liver

Microtome slices from control mice in our experiment revealed a regular and compact architecture of the liver, including healthy hepatic cells, sinusoids, vasculature, and arteries (**Fig. 2a**). However, histological examinations of liver tissue from treated mice revealed serious changes. Hepatic cell vacuolization, pyknosis (the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis), karyorrhexis (the destructive fragmentation of the nucleus of a dying cell), and karyolysis (the disintergration and dissolution of the nucleus of a necrotic cell) were observed in case of treatment group1 (**Fig. 2b**). As well as congestion in the central vein, vacuolization, and inflammatory cellular infiltration were seen in mice liver from the second group (**Fig. 2c**).

Effect of green leaf color on mice kidney

Similarly, the microtome sections of kidney tissues of control mice showed regular structure with well-distributed glomerulus and related tubules (**Fig. 3a & b**). However, in the case of green leaf color-treated kidneys, gross changes in the histology were observed. Glomeruli were found to be shrieked and ruptured, tubules were disrupted and vacuolized, and space between the walls of Bowman’s capsule was raised (**Fig. 3c**) in the cortex part of treated group 1. The slides from the same treatment group for the medullary part showed vacuolization of tubular cells, congestion of tubules, infiltration of leucocytes, and hemorrhage (**Fig. 3d**). Histological sections from treatment group 2 highlighted shrinkage of glomeruli, glomerular necro-

sis, degeneration of tubular epithelium cells, and dilation of tubular lumen were observed in cortex region and medullary regions (**Fig. 3e & f**).

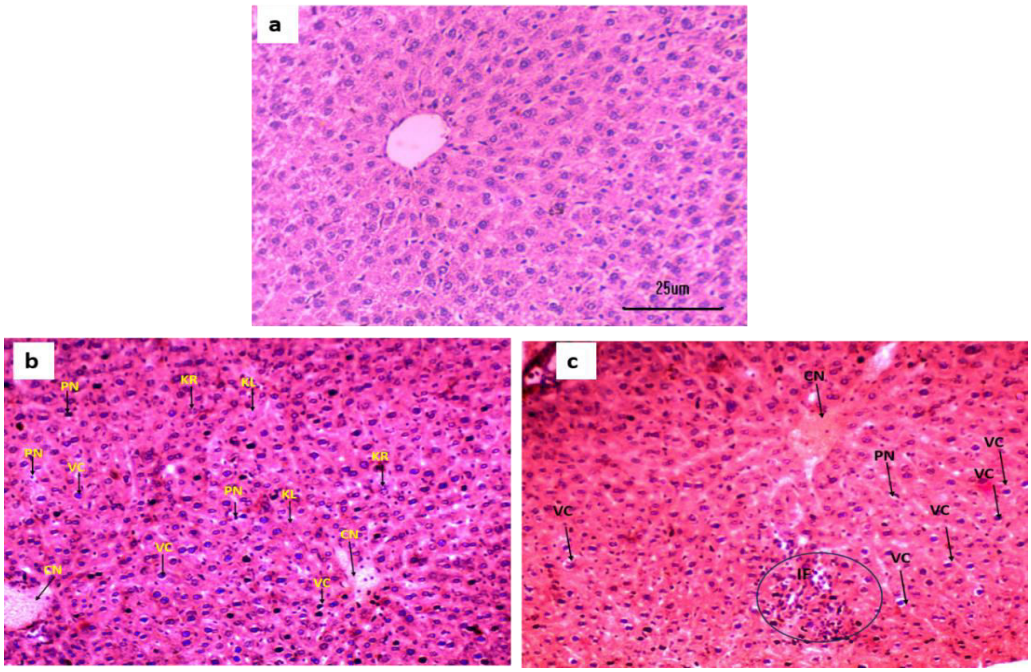

Fig. 2: Light microscopy photographic sections of mice liver stained by eosin and hematoxylin (200X H and E). (a) The liver of control mice showed the normal histological structure of hepatic cells. (b) Cross section of high-dose treated mice liver showed congestion of the central vein (CN), vacuolization of hepatic cells (VC), pyknotic nuclei (PN), karyorrhexis (KR), and karyolysis (KL). (c) Histological section from low-dose treated mice liver indicated congestion of the central vein (CN), vacuolization of hepatic cells (VC), and inflammatory cellular infiltration (IF).

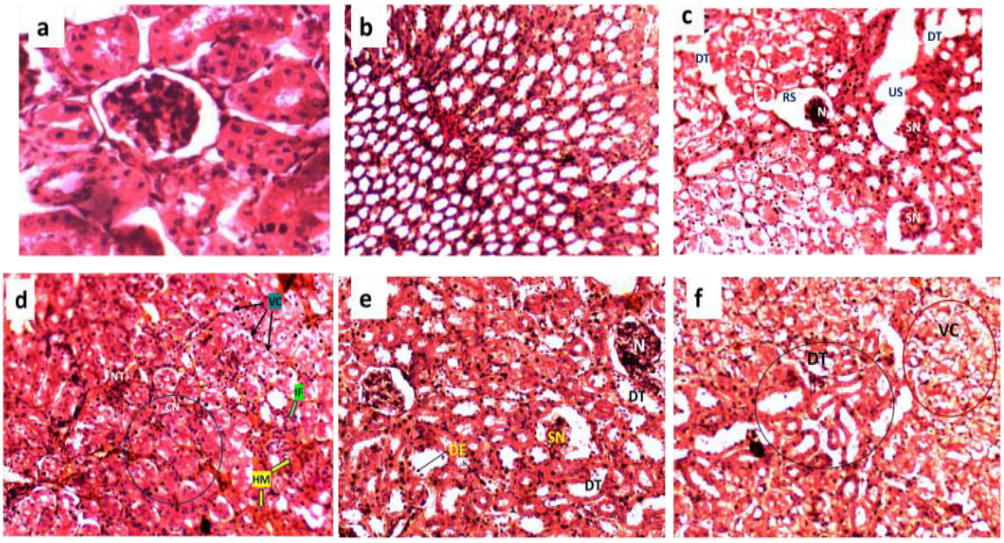

Fig. 3: Light microscopy photographic sections of mice kidneys stained by eosin and hematoxylin (400X H & E). Cross sections of (a) cortical and (b) medullary regions of the kidney of control mice showed compact glomerular and tubular structures. (c) Histological section from high dose treated renal cortex region indicated degeneration/disrupted and vacuolization of tubules (DT), raising space between the walls of Bowman's capsule (RS), shrinkage of glomeruli (SN), and glomerular necrosis (N). (d) The medullary part of high-dose treated mice showed vacuolization of tubular cells (VC), tubular necrosis (NT), congestion of tubules (CN), infiltration of

blood cells (IF), and hemorrhage (HM). (e) Low-dose treated mice highlighted shrinkage of glomeruli (SN), glomerular necrosis (N), degeneration of tubular epithelium cells (DE), and widened tubular lumen (DT) in the cortex region. (f) Vacuolization of tubular cells (VC) and irregular widening of the tubular lumen (DT) were observed in the medullary part of low-dose treated mice.

Effect of green leaf color on mice intestine

It was observed that the intestinal tissue from control mice was normally compact and regular structure of villus, mucosa, and submucosa (**Fig. 4a**). However, cross-sections of treated mice showed some severe histological alterations. Degenerated and disrupted brush border, necrosis in the intestinal crypt, vacuolization of cells, uncontrolled cell proliferation, swelling, disrupted mucosa and submucosa, and irregular villi were observed in the case of the high-dose treatment group (**Fig. 4b & c**). Histology from the low-dose group exhibited degenerated and disrupted villi (**Fig. 4d**).

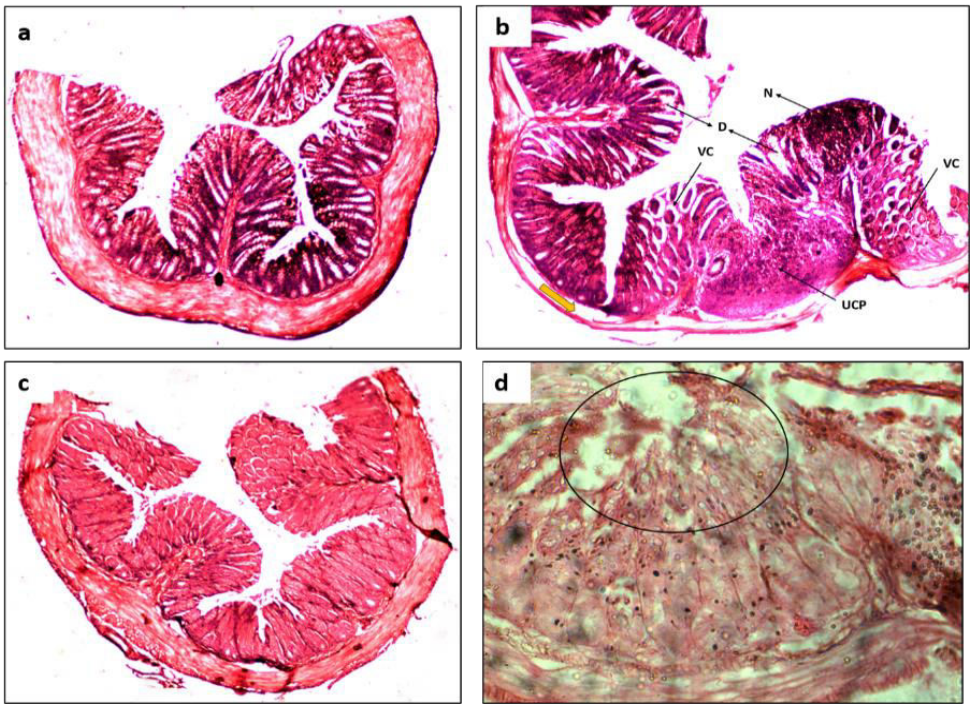

Fig. 4: Light microscopy photographic sections of mice liver stained by eosin and hematoxylin (200X H and E). (a) The intestine of control mice showed a normal histological structure. (b) The photographic section of the intestine at high dose treated mice indicated degenerated and disrupted brush border(D), necrosis in intestinal crypt (N), vacuolization of cells (VC), uncontrolled cell proliferation (UCP), and degenerated and disrupted mucosa and submucosa (yellow arrow). (c) Another section of high dose exhibited swelling of different parts of the intestine with irregular villi. (d) Intestine at low dose treated food dye showed degenerated and disrupted villi.

DISCUSSION:

In the current study, the introduction of green leaf color in mice resulted in hepatic necrosis and vacuolation, and these findings are consistent with those of a previous researcher, Mahmoud (Mahmoud, 2006), who discovered that a synthetic food dye called brilliant blue indicated histological modification in the liver of the rats. Hepatocyte necrosis, infiltration, and vacuolation are some of the changes that he found in his study. According to Soltan (Sahar SA and Manal MEM, 2012), lymphocytic infiltration around central veins is caused by the food color. Commercial fruit drinks can cause hepatocellular necrosis in mice as observed by Eissa (Eissa *et al*., 2014). The brown pigment deposition in the Kupffer cells and vacuolar degeneration of the liver in the treated group obtained in this study are in accordance with results recorded by Aboel-Zahab. (Aboel-Zahab *et al.,* 1997) who observed brown pig-

ment deposition in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. The liver sections from the treated mice showed evidence of congestion and bleeding. Our findings are consistent with those of Rus *et al.* (Rus *et al*., 2010), who studied the effects of tartrazine on the livers of guinea pigs by administering the chemical to the animals in drinking water at 1, 2, and 3% concentrations for three weeks. At low-dose treatment, the liver showed hepatic cell breakdown and cellular damage with empty vacuole cells. The vacuole in hepatocytes will eventually take up the whole of their cytoplasm, pushing the nucleus aside and giving the hepatocytes a signet ring shape, which is consistent with the previous results (Rus *et al*., 2010).

In this study, kidney slices from treated mice showed degeneration of the tubules, dilation, and vacuolation of the glomerular capillaries, hardening of the spaces between the capillaries, and glomerular necrosis. These modifications are in line with the findings of Rus *et al*. (Rus *et al*., 2010), who documented the effects of tartrazine in drinking water at concentrations of 1, 2, and 3% for three weeks when it is given to guinea pigs. The results of this study showed that being exposed to green leaf color caused tube, renal, and interstitial tissue changes that got worse over time. Previous research (Al-Majed *et al*., 2002; Ali *et al*., 2011; Can *et al*., 2000; Dehghani *et al*., 2011; Ekor *et al*., 2006; Kumar *et al*., 2000; Nitha and Janardhanan, 2008; Saleemi *et al*., 2009) also found tubular necrosis and degenerative changes in kidneys due to dye treatment. The current study demonstrated that proximal convoluted tubules of the intestine were more severely damaged than distal tubules. These results are consistent with those of previous researchers (Mingeot-Leclercq and Tulkens, 1999). The small intestine plays a key role in the body by completing the digestive process, absorbing nutrients, and secreting endocrines.

In the small intestine, epithelial cells absorb the nutrients that have been broken down by the digestive process. The histological changes in the ileum in the present study were similar to the observations recorded by Amend *et al*. (Amend *et al*., 1976) who reported hyperplastic mucosa with sub-mucosal accumulation of inflammatory cells. The short-term exposure to lactose in his study led to histology changes that were similar to those seen in rats that were fed lactose for 14 days in a study by Galvez *et al*. (Galvez J *et al*, 1995).

In that study, rats that were fed lactose had intestinal mucosal hypertrophy and smaller average cell sizes. But the cytopathological changes that the authors found could not be predicted in this study. This could be because the length of time the cells were exposed to lactose varied. The thickening of the gut in this study was similar to the findings of Tellez *et al*. (Tellez *et al*., 1993) who reported a marked reduction in lamina propria thickness and subjective epithelial cell proliferation in chicks following either 14 or 19 days of lactose administration. In addition, inflammatory cell infiltration, necrosis in the intestinal crypt, vacuolization of cells, uncontrolled cell proliferation, and irregular villi were observed.

CONCLUSION:

It can be concluded that exposure to the tested dye causes serious histopathological alterations in liver, renal and intestinal tissues including shrinkage of glomeruli, glomerular necrosis, congestion of tubules, dilation of the tubular lumen, and hemorrhage in the renal tissues, congestion of the central vein, vacuolization of hepatic cells, pyknosis, karyorrhexis, karyolysis, and inflammatory cellular infiltration in the liver tissues, and brush border degeneration, necrosis, swelling, uncontrolled cell proliferation, disruption of the mucosa, and submucosa in the intestinal tissues. Further investigations need to be carried out for more toxicological evaluation using the Polymerase Chain Reaction (PCR) technique to examine the expression level of cancer-related genes.

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CONFLICTS OF INTEREST:

The authors declare there are no conflicts of interest.

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