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Concentration depending impact of Brown HT (E-155) color and biochemical and biophysical investigation of Long Evans rat

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ABSTRACT

The aim of this study was to investigate the impact as per concentration of Brown HT (chocolate color) on Long evans rat. This color is taken out from Kushtia local market as per given information from the nearby food processing plants of Kushtia, Meherpur and Jhenaidah district, Bangladesh. Three concentrations ingested to male rat experimental groups those were 200mg, 400mg and 600mg per kilogram flour with produced cake on group-2, group-3 and group-4, respectively. On the other hand control group also observed without Brown HT as a control group-1. Body weight loss was remarkably noticed on experimental group-4 in the end of six months. Biochemical reports of Uric acid, Creatinine, Bilirubin, Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) have revealed as elevated state at six month of experimental rat (group-2, group-3 and group-4) compare to control (group-1). Histopathological reports was also expressed the accumulation of Brown HT in liver and kidney in all case of group four.

Keywords: Brown HT, Biochemical, Biophysical, Histo-pathology

INTRODUCTION

Food colors/dyes are artificial chemicals, often added to foods or beverages during processing to maintain or enhance colors, to compensation of color loss due to the environmental elements or processing and to improve consistency thus attracting the consumers. Both industrial food processing and kitchen cooking make use of food coloring. Cosmetics, medications, house hold crafts, and medical gadgets are just a few of the many non-food uses for food colorants. (Lan P. Freeman, 2005). Food color can be natural and synthetic and used worldwide depending on the wish of the user. For a number of reasons, including their low cost, better aesthetics, and longer-lasting effects, synthetic food dyes are chosen over their natural counterparts. (Kobylewski & Jacobson, 2010). Mainly used to prevent food color loss caused by light, air, temperature, moisture, and storage conditions, it also makes food look and taste better. (Lan P. Freeman, 2005). Color is one of the most powerful flavor associations people have, and it may affect how we taste anything from sweets to wine (Delwiche, 2004). It is believed that Egyptian cities began adding food colorants as early as 1500 BC, when confectioners began using wine and natural extracts to enhance the appearance of their goods (Meggos, H., 1995). Basic food items that contain synthetic color include bread, cheese, milk, fried meat and fish, ice cream, juices, candies, sugar toys, jams, and jellies. (Tripathi et al., 2007; Madsen, 1997). According to the reporter, synthetic coloring compounds like chocolate brown (E155) can harm the liver and kidneys, which is just one example of how harmful most synthetic color additives are to consumers' overall health (Hassan, 2010). By the turn of the century, unregulated color additives had made their way across Europe and the United States in a wide variety of popular foods, such as ketchup, mustard, jellies, and wine (Walford, J., 1980 and Sharma, Vinita, et. al., 2011). The first synthetic color was created by Sir William Henry Perkin in 1856 (Nagendrappa, G., 2010). Synthetic color like chocolate color effect on the weight, serum cholesterol, and HDL cholesterol of rats and different liver enzyme (Aboel-Zahab and El-Khyat, 1997, Helal, *et. al*. 2000). In our country, local beverage and bakery industries are developed based on synthetic food colors, especially brown cake and biscuits. The qualities and characteristics of dyes determine which colors and dyes are most suitable for a given product (Anderson and Keiding, 1964). An estimated 8,000,000 metric tons of colorants are manufactured annually on a global scale (Revanker and Lele, 2007). The use of food dyes iscontroversial because they have only aesthetic role in the products. Furthermore, a number of them have been associated with health issues, particularly in youngsters, who are a particularly susceptible demographic (Hashem, et. al., 2010). As azo dyes are more stable and inexpensive than natural food dyes, they make up the bulk of the dyes used in the food and textile sectors, which is why Brown HT was chosen as the primary research subject (Azo dyes, 2022). Most people's perceptions are swayed by visually appealing items when making selections; this is especially true for children, who often give colored foods (such as chocolate cake, candy, etc.) higher priority when eating. Among all of the food color, chocolate is one of the most usable colors in the bakery and food industry being prioritized so we have selected this synthetic dye color for our research work. However, many study has been performing about brown HT but systematic study with baseline bakery and other confectionary field data has not been revealed, in this study produced cake was incorporated with brown HT, the primary objective of this study is to shed light on the effects of the actual quantity of brown HT used in cakes made at local bakeries in Bangladesh.

MATERIALS AND METHODS

In general, many people in Bangladesh are attracted to food colors. and they don't care of health. Most of the people like chocolate color and flavor especially kids. As a result our food industry and local bakery produce chocolate color cake and biscuits as well as ice cream and milk shake also. In this respect, more than twenty bakeries were visited in Meherpur, Kushtia and Jhenaidah region in Bangladesh and tried to collect information about the concentration of color that is being used in their Bakery and Dairy products. The powder form of this color was used for their less cost and easy. We were informed that they used approximate Brown HT color concentration which actual range about 200- 600mg/kg cake flour-mix was combined in separate places for color development and batter was made for preparing the chocolate color cake. In this research work, twenty male rats were taken at same age and about same weigh. One group served as a control, while the other three were assigned for experimental conditions. The cake was produced three types that contained around 200mg, 400mg and 600mg Brown HT per kilogram flour-mix was matched with color of the commercial one. In this respect, the control group were given normal cake (without Brown HT) & water and experimental rat (Group-2, Group-3 and Group-4) was fed on the produced-cake of 12-gram weight that contained 200mg, 400mg and 600mg Brown HT per kilogram of flour, respectively, along with required amount of foods were given per gm weight of rat every day and sufficient amount of water was supplied to the rats and observed their consumption rate, attraction to food etc.

STATISTICAL ANALYSIS

An analysis of variance (ANOVA) was conducted using IBM SPSS version 21 for Windows to examine the significant difference between the experimental group and control group.

RESULTS AND DISCUSSION

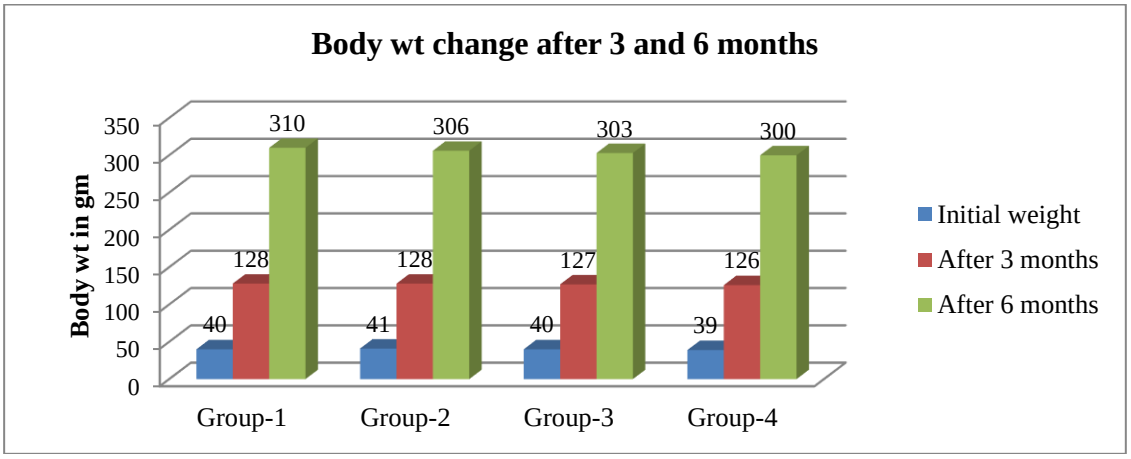
At the beginning of feeding time there were no significant changes in body weight were recorded. Rats were normal in behavior but with the increase of experiment time a slight behavioral change were found that the rats of group-3 and group-4 were restless and indifferent in taking their provided foods and water compare to control group and thus body weight of the experimental group was started to change. At the end of six months, the body weight gain was slightly reduced that was significant difference compare to control group. Each three months, the data on the rats' weight gain were summarized in table-1. After three months all of the rats weight gain was normal. The color has no effect on the rat's growth. After 6 months, the rat's weight of the experimental group were gradually decrease compare to the control group (Table-1).

Table-1: Weight gain data of rats at different duration.

Time	**Average weight gain(gm)**			
	Group-1	Group-2	Group-3	Group-4
Initial weight	40 ± 3	41 ± 4	40 ± 3	39 ± 3
After 3 months	128 ± 3	128 ± 3	127 ± 4	126 ± 3
After 6 months	310 ± 3	306 ± 4	303 ± 4	300 ± 4

The findings corroborate those of (Hashem et al. 2010), who found that food dyes such amaranth, sunset yellow, and curcumin had no effect on weight gain. The 12-hour the administration of colored fruit juice resulted in a 29.6 percent reduction in body weight gain. The same thing happened in rats; researchers found that rats given synthetic food colorant gained a lot of weight up until the fourth month, and then they started to lose a lot of weight. The works of (Brozelleca et al., 1989; Abouel-Zahab et al., 1997; and Osman et al. 1995). The primary purpose of utilizing a one-way analysis of variance (ANOVA) was to examine the disparity in means between the control group and the experimental group. A statistically significant difference was seen at

the p<0.05 level between the experimental group-4 and the control groups, as indicated by the F (3, 16) =49, p=0.00. The Tukey HSD post-hoc comparisons revealed that the mean score (M=10) for group-4 was significantly higher than that of the control group (group-1).



BIOCHEMICAL ANALYSIS

To investigate the accumulation of color component in the liver and kidney cell. From the rat, blood serum was obtained and examined for Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Uric Acid, Creatinine, and Bilirubin. The findings were then summarized in Table-2 and Table-3. The biochemical analysis indicated that the parameters were unchanged until three months (Table-2). May be the color had no effects on the rat within this duration. On the other hand, all the biochemical parameters that have been undertaken in this experiment were increased moderately compared to the control group after six months (Table-3). The value of the above parameters was increased sequentially from low to high dose after consuming chocolate color.

Table-2: Biochemical parameters of different groups of rat blood serum after three month from the starting of research work.

Groups	(After three months) Parameters				
	Uric acid(mg/dl)	Creatinine (U/L)	Bilirubin (U/L)	AST (U/L)	ALP (U/L)
Group-1	2.4 ± 0.2	0.65 ± 0.02	0.35 ± 0.03	36 ± 2	19 ± 1.5
Group-2	2.4 ± 0.3	0.66 ± 0.01	0.36 ± 0.02	37 ± 3	19 ± 2
Group-3	2.6 ± 0.2	0.69 ± 0.03	0.37 ± 0.01	39 ± 2	20 ± 3
Group-4	2.8 ± 0.1	0.71 ± 0.02	0.39 ± 0.02	40 ± 3	21 ± 4

Table-3: Biochemical parameters of different groups of rat blood serumafter six month from the starting of research work.

Groups	(After six months) Parameters				
	Uric acid(mg/dl)	Creatinine U/L)	Bilirubin (U/L)	AST (U/L)	ALP (U/L)
Group-1	2.4 ± 0.2	0.65 ± 0.02	0.36 ± 0.03	36 ± 2	19 ± 1.5
Group-2	2.7 ± 0.3	0.72 ± 0.01	0.40 ± 0.02	40 ± 3	22 ± 2
Group-3	2.8 ± 0.2	0.75 ± 0.03	0.42 ± 0.01	42 ± 2	23 ± 3
Group-4	3.0 ± 0.1	0.80 ± 0.02	0.45 ± 0.02	45 ± 3	24 ± 3

The formal reports supported the present data that indicated the rats were given a high dose of synthetic color

additives (Tartarzine, Carmoisin, sunset yellow, and quick green) exhibited a notable rise in serum ALT and AST levels in comparison to the control rats (Mekkawy et al., 1998; Amin et al., 2010). The current findings are consistent with the results reported by (Helal et al., 2000 and Amin et al., 2010), which demonstrated that rats consuming a high concentration of colored food (30%) and being administered color fruit juice for an extended period of time exhibited a significant increase in serum creatinine concentration compared to the control group. The level of creatinine may have dramatically increased, indicating a strong correlation with the decline in kidney function. Moreover, the current results align with the data presented by (Ashour and Abdulaziz ,2009), who noted a notable increase in the levels of blood creatinine and urea in rats that were administered Azo dye (fast green) orally for a duration of 35 days.

HISTOPATHOLOGICAL EXAMINATION

Analysis of Liver Histopathology

Liver and kidney cells from both the control and experimental groups of rats were taken and examined under a microscope. The following observations were made:

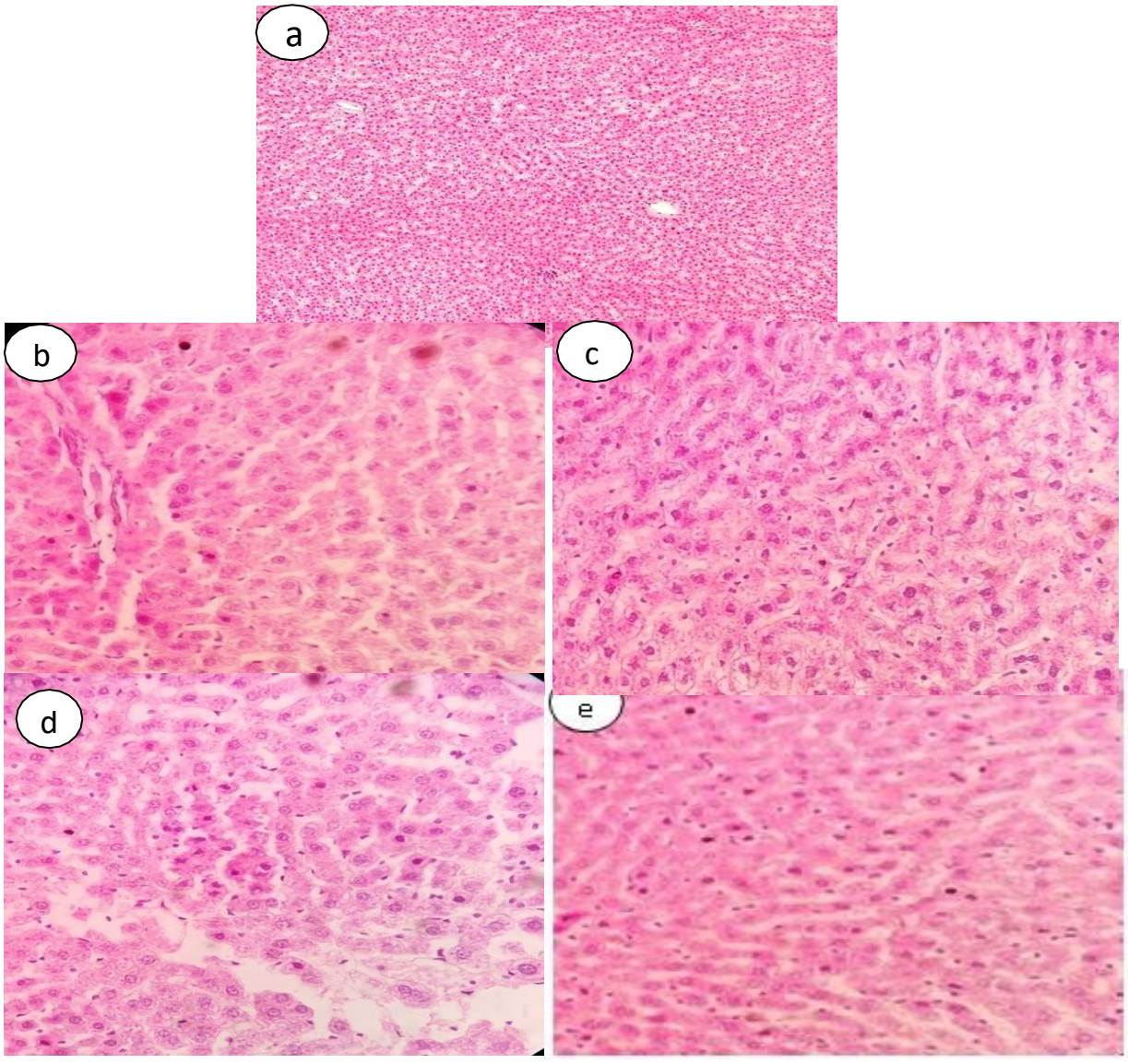


Figure-1: Histopathological view of group-4 (b-e) liver compare to group-1 (a) control

Hepatochromatic nuclei

A hepatocyte is a fundamental cell of the primary functional tissue of the liver. Hepatocytes constitute 70-85% of the liver's total mass. The functions of these cells include protein synthesis, protein storage, carbohydrate transformation, cholesterol synthesis, bile salts and phospholipids synthesis, detoxification, modification, and excretion of exogenous and endogenous substances, as well as initiation of bile formation and secretion (Celton-Morizur, S, et. al., 2010). The liver cell of the rats was divided into sections and compared to the control group (group-1) with experimental group (Figure:1-a & b, respectively) by feeding them cake having a chocolate color for a period of six months. The control group that was clearly shown but there was a number of spot of chromatin in the liver cells of the experimental rats group-4 (Figure:1-b) this result was partially supported by (Mahmoud; 2006) who noticed that treated with brilliant blue for 15, 30 and 45 days, respectively displayed necrobiotic changes in the Pyknotic nuclei of the liver. These results corroborate with the findings and observed that the dietary additive caused ballooning degeneration and the aggregation of chromatin material around the periphery of the nuclear envelope (Safer and al-Nughamish, 1999). The result indicated that may be consuming color was accumulated in the liver cell made such type of chromatin spot which one was absent in the control rat.

Peripheral hepatic congestion

Congestive hepatopathy, often referred to as nutmeg liver and chronic passive congestion of the liver, is a condition characterized by impaired liver function resulting from venous congestion, typically caused by congestive heart failure. Congestive hepatopathy of the liver cell was seen in the following (Figure-1-c). Liver tissues of group-4 was shown in the figure:1-c, whereas it contains many tiny dark spots, on the other hand there was no such type of spot in the normal rat’s tissues (figure:1-a). This finding aligns with the research conducted by (Klastskin and Oconn;1993), who concluded that the congestion of hepatic arteries and sinusoids is caused by the direct toxic impact of food color toxins, resulting in damage to the hepatocytes. These findings also were consistent with (Mahmoud's,2006) research, which shown that the artificial food coloring brilliant blue caused histological changes in the liver of rats. (El-Malky et. al., 2014) observed congestion in the central vein of rats that were administered an excessive amount of a commercial yellow dye. If the rat consumes the color for long time, may be the tiny spot to form the dark spot that is one of the causes for liver disease. We compare the control rat's liver cells with the experimental group (consume of chocolate color) and found it may have developed a congestive hepatopathy, if this types of color are consuming a long time.

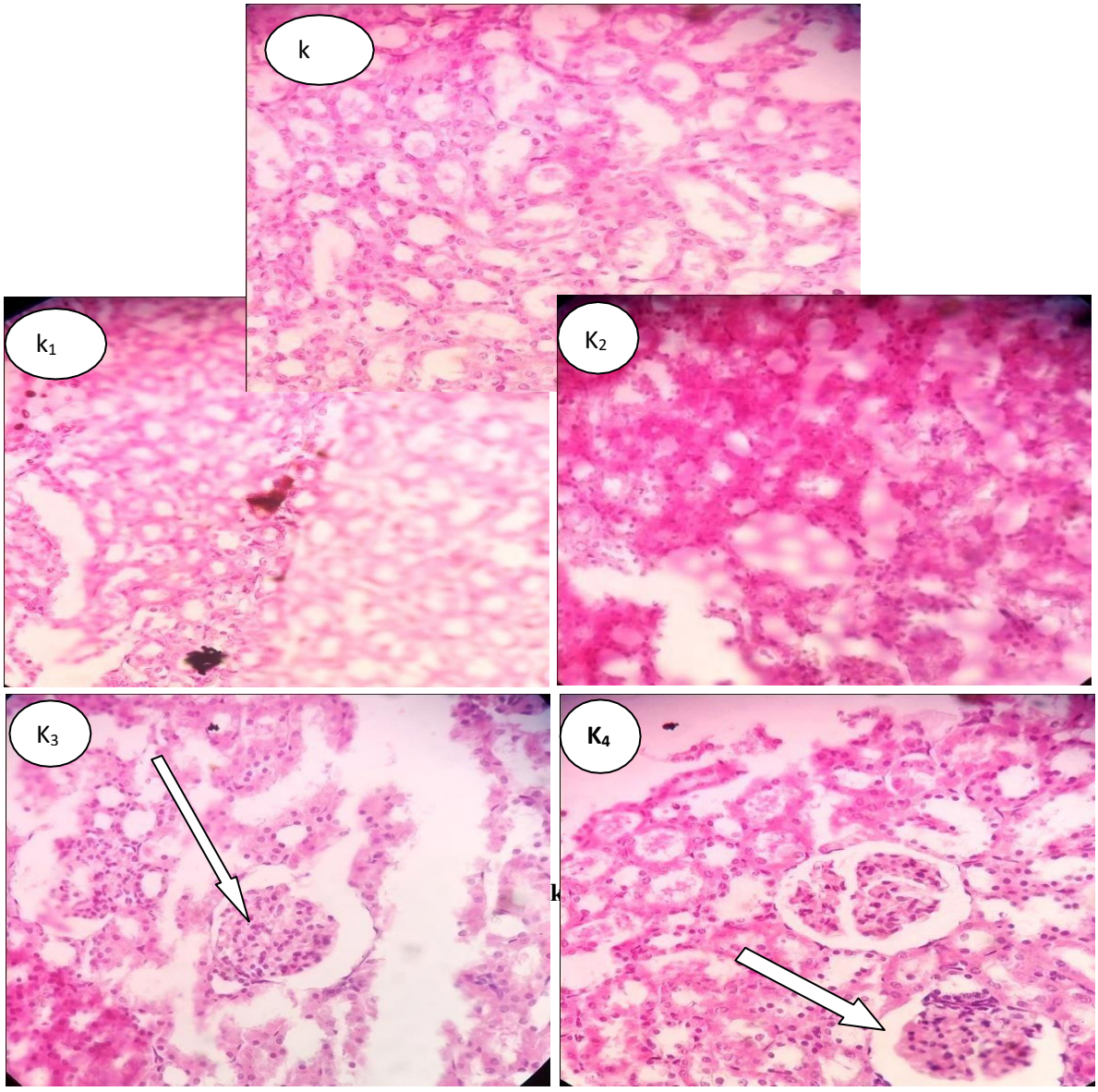
Fatty changes

Fatty liver is a disorder that can be reversed, characterized by the accumulation of huge vacuoles of triglyceride fat in liver cells through the process of steatosis, which refers to the aberrant retention of lipids within a cell. The liver exhibits a noticeable centrilobular macro vesicular steatosis, characterized by white or clear round/oval gaps, along with modest fibrosis seen by green coloration. Macro vesicular steatosis refers to the excessive accumulation of lipids in a cell, to the extent that it causes distortion of the cell's nucleus

(Reddy and Rao, 2006). Rat liver cell of group-1 and group-4 was shown in the figure-1 (a & d),respectively. Compare to the group-1 and group-4, white vacuole space was clearly seen in the liver cell of group-4. May be fat cells were decomposed in the liver cell that is resembled to the fatty liver cell that was seen. From this comparison, it can be suggested that fat may accumulate in the liver cell of color consumed rat. So, artificial chocolate color may create fatty liver disease.

Sinusoidal dilatation of Liver

A sinusoid is a form of capillary that resembles a fenestrated endothelium and is characterized by being a tiny blood artery. Sinusoids are categorized as open pore capillaries, which differ from continuous and fenestrated capillaries. Fenestrated capillaries are characterized by the presence of diaphragms that cover the pores, while open pore capillaries lack a diaphragm and just have an open pore. Endothelial cells exhibit significantly enhanced permeability due to their open pores. Furthermore, the presence of extensive inter-cellular clefts and a reduced number of tight junctions leads to an increase in permeability. The permeability level is sufficient to facilitate the easy passage of small and medium-sized proteins, such as albumin, into and out of the bloodstream. Dilation of sinusoids is a heterogeneous enhancement of the hepatic. After sectioning, fatty body contain spore was observed in the liver tissues of experimental group-4 when compare to the liver cell of control group was shown in the figure-7 (a & e), respectively. This type of spore is seen in the cell of sinusoidal dilatation liver that damage or affects the outflow of blood. The findings of (Klastskin and Oconn ,1993) substantiated this outcome, since they ascribed the expansion of hepatic arteries and sinusoids to the direct toxic impact of the toxins, resulting in damage to the hepatocytes. (El-Malky et. al.,2014) observed the expansion and enlargement of the central vein in rats that were given an excessive amount of a commercial yellow dye. So, from this result, it can be suggested that this color create the sinusoidal dilatation in the liver.



Histopathological analysis of KidneyLipofuscin pigment

Lipofuscin refers to small granules of yellow-brown pigment that consist of lipid-containing remnants from the process of lysosomal digestion. It is regarded as one of the pigments associated with aging or "wear-and-tear", commonly present in the liver, kidney, heart muscle, retina, adrenals, nerve cells, and ganglion cells. The kidney cell of group-4 rats was examined using a microscope, as depicted in figure-2 (k & k1), in comparison to group-1 rats. Compare to the control group it was clearly seen that there was a number of spot of chromatin in the kidney cell of the experimental rats (Figure: 2-k_1). The result indicates thatconsuming color may accumulate in the kidney cell and made such type of chromatin spot which one absent in the control rat (Figure:2-k).

Hyalinization

Hyalinization is the process in which the walls of arteries thicken due to the accumulation of deposits that appear as a uniform pink hyaline substance when stained using conventional methods. It is a form of arteriolosclerosis, which specifically denotes the calcification of the arteriolar wall. It results in the escape of plasma chemicals through the vascular endothelium and the abnormal growth of muscle cells, typically as a result of hypertension. This condition is characterized by the constriction of arterioles, leading to a widespread reduction in blood supply to the kidneys. As a result, there is a loss of nephrons and a gradual decline in kidney function. The kidney cell of group-1 and group-4 rats was examined under a microscope. In the experimental group-4, the color component was seen to be present in the kidney tissues (Figure: 2-k_2), however no color spread region was observed in the kidney tissue of the control group-1 rats (Figure: 2-k). In cases of kidney hyalinization, the color is evenly distributed and the cells appear in a uniform and homogenized state. This work somewhat aligns with the findings reported by (Aboel-Zahab H, et. al.,1997), who noticed the accumulation of brown pigment in the portal tracts and Kupffer cells of the liver, as well as in the interstitial tissue and renal tubular cells of the kidney. Based on this result, it may be inferred that prolonged consumption of this particular color may lead to the development of hyalinization.

Interstitial inflammation:

Following a six-month period of extensive feeding treatment, the kidney cell from group-1 and group-4 rats was dissected and examined under a microscope. The cell from group-4 exhibited a higher presence of tiny granules compared to the control group (group-1). Our results were similarly to that of (Hesham A Eissa, *et. al*.,2010) who mentioned that drinking red grape drink color for 3 week showed vacuolar degeneration, diffusion of inflammatory cells. The color component was spread on the kidney tissues of the experimental group-4 (Figure-2-k_3), whereas, the color spread region was not observed or seen in the tissue of the control rat group-1(Figure- k). Based on this result, it may be inferred that prolonged use of this sort of hue may lead to the development of hyalinization. It can be inferred that the color of the food consumed by rats may have inflammatory effects on the kidneys.

Mesangial expansion

Mesangial cells are specialized renal cells that compose the mesangium of the glomerulus. The vascular pole of the renal corpuscle is formed by the mesangial matrix and they. Mesangial cells can be classified as either extra-glomerular mesangial cells or intra glomerular mesangial cells, depending on their position in relation to the glomerulus (Schlo, D., & Banas, B., 2009). They provide structural support to the glomerular capillaries, regulate glomerular filtration, form the mesangial matrix, and ensure that the filter remains free of debris (Schell, et. al., 2014). Kidney cell of the rat group-4 & group-1 was sectioned and analyzed; glomerular cell was observed that the mesangium cells were expand of the group-4 rat compared to group-1 in the Figure-2-k_4 & k, respectively that impact on the glomerular filtration.

CONCLUSION

Based on these results, the weight and biochemical parameters of rats that consumed color were not altered during a six-month period. However, these parameters showed small changes after consuming diets

containing color for six months. On the other hand, histopathological reports of liver and kidney tissues indicated that consuming color was accumulated in the liver and kidney cells (Figure-1b and Figure-8k_1). Generally, there is no gap biochemical parameters since tissues or organ are not infected or damaged. In this regard, there is slightly correlation to biochemical parameters between histopathological tests. The histopathological results clearly indicated that when artificial chocolate color was fed to the rat the color consumed and accumulated in the accumulating organ like liver and kidney. The findings also demonstrated that the color was developed or caused by a different type of liver and kidney diseases like Peripheral Hepatic Congestion, Fatty liver, Sinusoidal dilatation of liver, Interstitial inflammation, Hyalinization of kidney cell, Mesangial expansion, *etc*. The color is consumed and accumulated in the accumulating organ. When low dose but long time the artificial color containing foods will be taken, the color will be accumulated, and the above liver and kidney related diseases will develop and may be converted into liver cirrhosis or cancer. Based on the findings of this investigation, it can be inferred that artificial color containing foods should be avoided to lead a healthy life as well as to save money and develop the country.

Conflict of interest: All the authors claim that there are no conflicts of interest.

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