PHYSIOLOGICAL EFFECTS OF SOME ARTIFICIAL AND NATURAL FO COLORING ON YOUNG MALE ALBINO RATS

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ABSTRACT

Food colorants are widely added to food in order to attract the consumer. Recent researches have incriminated these additives for causing some problems to human health. This study was conducted to determinate the physiological effects of some natural (curcumin, carrotin and curcumin) and synthetic (tetrazine, sunset yellow and erythosine) food colorants on some hematological and biochemical parameters of male albino rats (rattus norvegicus, spraguedawlay strain). Results revealed that administration of synthetic food colorants decreased the percentage of high density lipoprotein cholesterol (HDL-C), glutathione secretion (GSH), superoxide dismutase (SoD), and plasma immune-system and significantly increased plasma lipid lipoprotein, total cholesterol (LDL-C), lipid peroxidase, blood glucose, plasma urea and creatinine and increased activities of alkaline phosphatase, acid phosphatase, and lactate dehydrogenase. Hence, it is recommended to avoid adding synthetic colorants as additive to foodstuff, while natural colorants seemed to be the best choice as food colorants.

Keywords: Food coloring, Carrotin, Tetrazine, Albino rat, Curcumin, Albumin.

Contribution/ Originality

This study is emphasizing the adverse effects of the synthetic food colorants on human health as previously reported in the literature.

1. INTRODUCTION

Food additives are natural or manufactured substances, which are added to food to restore colors lost during processing (Soltan and Shehata, 2012). The current preference for naturally derived food colorants is because of their trustfulness when added to human diet. Several studies have incriminated synthetic colorants to cause some adverse effects to human health. Recently,

natural colorants are becoming the preferable choice in term of food coloring because of their high safety. Colorants of natural origin have been used to provide color in foods, drugs and cosmetics for the thousands of years. Ash from fires, mineral compounds and plants were probably among the first materials used for cosmetic purposes (Gaunt *et al.*, 1972). Synthetic colorants were developed to provide a more economical and extensive array of colorants (Salah, 1994). By the early 1995, natural and synthetic colour additives were used extensively to color foods, drugs and cosmetics (Hallagan *et al.*, 1995). Color is an important characteristic and selection criterion for food choice. Recent studies have highlighted this importance and have shown how selection may change among certain populations, and over time (Clydesdale, 1993). Color is important for cosmetic purposes among many populations, and also for safety purposes such as the identification of pharmaceuticals. Color additives exempt from certification are used for a wide variety of purposes in foods, drugs and cosmetics (Van Bever *et al.*, 1989). Although all color additives are alike in terms of the FDA's regulatory definition, they are regulated in two classes; the certified (synthetic) color additives, and the color additives exempt from certified (natural). Colorants play a significant role in enhancing the aesthetic appeal of food. Foods that are aesthetically pleasing are more likely to be consumed and to contribute to a varied diet (Newsome, 1986; Hallagan *et al.*, 1995). Nowadays, natural dyes are commonly used in the cosmetic industry due to no side effects, UV protection and anti-aging properties (Chengaiah *et al.*, 2010). A review article (Burgos-Moron *et al.*, 2010) reported that curcumin has an efficient and safe for the prevention and treatment of several diseases including cancer. However, previous studies reported that curcumin from Curcuma longa has antioxidant, anti-inflammatory, anti cancer, hepatoprotective, Alzheimer's disease, it has anti-inflammatory effects in arthritis, possibly inhibits prostaglandin synthesis pathway of Cox-2 without causing ulcers in the GI tract (Molnar and Garai, 2005). Finally it has anti-platelet, anti viral, anti fungal, anti bacterial effects (inhibits Helicobacter Pylori) and powerful antiseptic agent (Siva, 2007). On the other hand, there are accumulating evidences that curcumin may not be so effective and safe. Moreover, curcumin was recently found to be an active iron chelator *in vivo* and induced a state of overt iron deficiency anemia in mice fed with diets poor in iron (Jiao *et al.*, 2009). It has been concluded that food color (tartrazine and carmoisine) affect and alter bioelements levels in vital organs e.g. liver, kidney and brain (Cemek *et al.*, 2014).

Although the importance of food colorants, a wave of awareness and concern about the many adverse effects of synthetic food colorants on human health are ever growing (Van Bever *et al.*, 1989). Recently, there is a sharp increase in the use of synthetic food colorants especially in food mostly consumed by children (Salah, 1994). More attention must be focused on the physiological and pathological effects of colour additives (Ganong, 1991). Previous studies (Reyes *et al.*, 1996; Zraly *et al.*, 2006; Al-Shinnawy, 2009) investigated the metabolic and toxicological disorders induced by the administration of specific food colorant additives to rats and other mammals. However, nutritional hazards have been detected in the liver and kidney due to the uncontrolled use of synthetic (El-Malky *et al.*, 2014). Therefore, the present investigation was planned to

compare and demonstrate the effects of three natural and three synthetic color additives on the physiological functions of small albino rats.

2. MATERIAL AND METHODS

2.1. Colorant Samples

The natural pigment (curcumin, carotene and anthocyanin) used in the present work, were provided from P. Robertel and Co. g France. The synthetic dyes (tetrazine, sunset yellow and erythrosine) were obtained from imperial chemical industries, England. tartrazine / carotin , sunset yellow / curcumin and erythosine / anthocyanin mixtures were prepared by adding each one to the other in ratio of 1:1 (w/w). The colorant samples were used to give yellow and red food stuff colorants.

2.2. Experimental Animals

Health adult male albino rats (60 animals) rattus norvegicus, spraguedawlay strain weighing 100-150 g were obtained from the animal house of Nutrition Institute Cairo and housed in the laboratory animal center of faculty of agricultural, Cairo university Giza Egypt.

2.3. Method of Application

The animal were kept under normal health laboratory conditions for two weeks in their cages prior to the experiment of acclimatization rats were housed individually in a room with a well aerated under hygienic conditions . they were allowed free access to tap water and fed on diet consisting of a mixture of casein 20 % , cotton seed oil 10% and cellulose 5 % salts mixture 4 % vitamins mixture 1% and starch 60% (Lane-Part and Pearson, 1971)

2.4. Colorant Treatments

Rats were divided into 10 groups (6 rats each). The first group served as normal health control, the second group was ingested orally with dose of curcumin which 10 mg /kg body weight. The third group was ingested orally with the dose of carrotin which was 10mg/kg body weight. The fourth group was ingested orally with the dose of anthocyanin which was 10mg/kg body weight. The fifth group was ingested orally with the dose of tetrazine which. 0.1g/kg body weight. Sixth group ingested orally with the dose of sunset yellow which was 10 mg/kg body weight. Seventh group was ingested orally with the dose of erythrosine which was 10 mg/kg body weight. The eighth group was ingested orally with the dose of mixture of tetrazine/carrotin which was 20 mg/kg body weight. The ninth group ingested orally with mixture of sunset yellow/curcuminwhich was 20 mg/kg body weight. Tenth group was ingested orally with the dose of mixture of erythosine/anthocyanin which was 20 mg/kg body weight. The colorant samples and their mixtures were mixed with ml corn oil. one dose was induced every two days during the experimental period (12 weeks) either for individual or mixture colorant and water were supplied ad libitum for the each rat was weighted every weeks and it's daily food intake was

determined . Feed efficiency was calculated as the following equation. (Body weight gain/ food intake). At the end of the experimental period (12 weeks), animals were killed by decapitation. Blood was collected some of which was centrifuged at 3000 rpm to obtain the plasma which was kept frozen at -20̊c until used for analysis. The other blood was used to determine the bloodpicture.

Liver were rapidly removed weighted , Put in ice-cold 1.15% kcl and stored at -20̊c for homogenization to determine the activities of catalase and superoxide dismutase as well as liver content of GSH (glutathione reduced) and also lipid peroxide. Estimation of body weight gain and internal organ to body weight ratio. Some vital organs such as liver, heart and kidneys were removed after sacrificing animals and their weight were recorded but liver was kept for some analysis. Methods of analysis (Hematological and biochemical studies)

2.5. Determination of Acid Phosphatase Activity

Plasma acid phosphatase and alkaline activity was determined by enzymatic – kinetic method using kit as described by.

2.6. Determination of Lactate Dehydrogenase (LDH)

Plasma lactate dehydrogenase (cardiac enzyme) was determined by kinetic method using kit as kinetic determination of LDH activity.

2.7. Determination of Plasma Glucose

Plasma glucose was determined according to the method of Fossati and Prencipe (1982).

2.8. Determination of Kidneys Function
2.8.1. Determination of Plasma Uric Acid

Plasma uric acid was determined according to the method of Barham and Trinder (1972).

2.9. Determination of Blood Urea

Blood uric acid was determined according to the method of Fawcett and Scott (1960).

2.10. Determination of Creatinine

Creatinine was determined according to the method of Fossati and Prencipe (1982).

2.11. Determination of Total Lipid Pattern

Total lipids was determined according to the method of Barham and Trinder (1972).

2.12. Determination of Triglycerides

Triglycerides were determined according to the Fossati and Prencipe (1982).

2.13. Determination of Total Cholesterol

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Total cholesterol was determined according to the method of Richmond (1973).

2.14. Determination of High Density Lipoprotein-Cholesterol (HDL)

HDL- cholesterol was estimated by enzymatic colorimetric method according to the method of Wieland and Seidel (1983).

2.15. Determination of Low Density Lipoprotein –Cholesterol (LDL)

LDL-cholesterol was estimated by enzymatic colorimetric method according to the method of Wieland and Seidel (1983).

2.16. Determination of 1gg, 1gA, 1gM (RID)

The kit is intended for measuring plasma 1gG ,1gA and 1gM according to Barham and Trinder (1972) methods.

2.17. Determination o Superoxide Dismutase Activity (SOD) in Liver

Superoxide dismutase (sod) activity was determined in erythrocyte lysate according to Nishikimi et al. (1972).

2.18. Determination of Catalase in Liver

Plasma catalase activity was determined according to Barham and Trinder (1972) using method kit.

2.19. Determination of Reduced Glutathione in Liver

Reduced glutathione (GSH) was colorimetrically determined according to the method described by Barham and Trinder (1972).

3. STATISTICAL ANALYSIS

The obtained data were statistically analyzed using the method of Snedecor and Cochran (1967). And LSD (least squared difference) test was used to compare the significant differences between means of treatments. Values are expressed as mean ± SD and the significant difference at $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Effect of Natural and Synthetic Colorants on Plasma Lipid Lipoprotein

It is clear that natural food colorants treatment resulted in no effect on total lipids of all treated groups comparing to the normal health control (Table 1). On the other hand, change of total lipids was more evident in those groups treated with synthetic colorants especially after sunset yellow administration (total lipids percentage was increased to 131%). Yet, it was less pronouncing for groups treated with mixed colorants (tetrazine/carrotin, yellow/curcumin and

erythosine/anthocyanin) with total lipids percentages of 110%, 113% and 112%, respectively. With respect to total cholesterol, comparing to normal health control, the best food intake results were noticed in groups that administered with natural colorants followed by groups that treated with mixed colorants and the lowest food intake were remarkably evident in groups that ingested orally with synthetic colorants. However, the highest increased in total cholesterol was noticed in case of sunset yellow treatment which amounted to 129% comparing to the normal health control. The same result has been found when assessing total triglycerides. No changes in plasma total phospholipids of all groups have been occurred by food colorants treatment (Table 1). High density lipoprotein cholesterol (HDL-C) has decreased in case of groups that treated with synthetic food colorants especially in groups treated with tetrazine (decrease percentage was 78%). On contrast, low density lipoprotein cholesterol (LDL-C) has increased in groups treated with synthetic food colorants especially in groups that treated with sunset yellow (increase percentage was 142%). The same result has been observed in case of determining the very low density lipoprotein cholesterol (VLDL-C) (Table 1).

The increase in serum cholesterol and triglyceride levels obtained in this study are in accordance with results reported by previous studies (Abou El-Zahab *et al.*, 1997; Himri *et al.*, 2011) who observed significant increases in serum total lipids, cholesterol and triglycerides in rats whose diets were supplemented with some food colorants in varying concentrations. The current results of this study are in a contrary with Sharma *et al.* (2006) who reported that two doses of tomato red (blend of Carmoisine and ponceau 4R) showed a significant decrease in serum total cholesterol and triglycerides when Swiss albino mice consumed these colorants for 21 days as short term or 42 days as long term. Also, these results are in opposite with those reported by Ashour and Abdelaziz (2009) who noticed a significant reduction in serum total cholesterol and triglycerides level when food color azo dye (fast green) was consumed orally to male albino rats for 35 days.

4.2. Effect of Natural and Synthetic Colorants on Liver Lipid per Oxidation

Regarding to the effect of oral administration of colorants on glutathione (GSH) secretion, the data obtained revealed a marked decrease in GSH of synthetic colorants treated groups comparing to normal health control (Table 2), GSH percentages were 72%, 69% and 68% for tetrazine, sunset yellow and erythosine, respectively. Comparing to normal health control, slight decrease of GSH was observed in the three groups of rats that treated with natural colorants, GSH percentages were 97%, 98% and 98% for curcumin, carrotin and curcumin, respectively. With regard to mixed colorants (tetrazine/carrotin, yellow/curcumin and erythosine/anthocyanin), liver/body weight percentages were 83%, 84% and 83%, respectively.With respect to lipid peroxide, percentages were found to be 222%, 204% and 210% for tetrazine, sunset yellow and erythosine, respectively. Comparing to normal health control, slight increase of lipid peroxide was observed in the three groups of rats that treated with natural colorants, lipid peroxide percentages were 104%, 102% and 104% for curcumin, carrotin and

curcumin, respectively. With regard to mixed colorants (tetrazine/carrotin, yellow/curcumin and erythosine/anthocyanin), lipid peroxide percentages were 145%, 139% and 145%, respectively (Table 2).No changes have been noticed in superoxide dismutase (SoD) (antioxidant enzyme) percentages in groups of rats that treated with natural food colorants comparing to normal health control (Table 2). For groups of rats that treated with synthetic food colorants, SoD percentages were found to be 56%, 57% and 53%, for tetrazine, sunset yellow and erythosine, respectively. With regard to mixed colorants (tetrazine/carrotin, yellow/curcumin and erythosine/anthocyanin), SoD percentages were 75%, 67% and 68%, respectively. Catalase (antioxidant enzyme) has remarkably decreased in groups treated with synthetic food colorants, percentages were found to be 71%, 64% and 66%, for tetrazine, sunset yellow and erythosine, respectively. With regard to mixed colorants (tetrazine/carrotin, yellow/curcumin and erythosine/anthocyanin), catalase percentages were 88%, 86% and 85%, respectively. No changes were observed in groups supplemented with natural food colorants comparing to control (Table 2). Moriarty-Craige and Jones (2004) reported that GSH plays a crucial role in protecting the intestines against oxidative damage that originates from possible toxic compounds in food. Besides, GSH is a necessary thiol compound that promotes normal development and function of the intestines. Previous *in vitro* and *in vivo* studies show that aromatic amines were possibly responsible for the endotoxic and carcinogenic effects of azo dyes and that they significantly decreased GSH levels (Valentovic et al., 2002). Amin et al. (2010) showed that, when young male rats were given low (15 mg/kg BW) and high (500 mg/kg BW) doses of tartrazine, the high dose decreases GSH levels in liver homogenates significantly, as compared to control. Depletion of GSH puts cells at oxidative risks. Cemek et al. (2014) concluded that tartrazine and carmoisine concentrations were not sufficient to change the GSH levels in tissues.

4.3. Effect of Natural and Synthetic Colorants on Blood Glucose, Activities of Alkaline Phosphate, Acid Phosphate and Lactate Dehydrogenase

Slight differences have been recorded between normal health control rats and groups of rats that treated with natural food colorants with respect to plasma glucose. However, the highest increased in blood glucose was noticed in rat groups that treated with synthetic colorants especially in case of sunset yellow treatment which amounted to 126% comparing to the normal health control. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia (Al-Shinnawy, 2009).

Alkaline phosphatase, acid phosphatase and lactate dehydrogenase activities has been increased in all treated groups, but activities of these enzymes were increased remarkably in case of rat groups that treated with synthetic food colorants (Table 3). The remarkable increase has been observed in acid phosphatase activity in groups treated with curcumin, carrotin and curcumin, which increased to 256%, 289% and 278%, respectively (Table 3). This study agreed with the findings reported by Mahmoud (2006) who found a significant increase in alkaline

phosphatase activity for brilliant blue dye and attributed that to the defect in liver function. Alkaline phosphatase has several physiological functions in bone cells, it splits inorganic phosphates from organic phosphate which is a potent inhibitor of mineralization (Mahmoud, 2006).

4.4. Effect of Natural and Synthetic Colorants on Kidney Function in Plasma

Comparing to normal health control, plasma urea was remarkably increased in case of the three synthetic colorants treated groups (130%, 139% and 135% for tetrazine, sunset yellow and erythrosine, respectively) (Table 4). While no changes were observed in rats administered with natural colorants comparing to the normal health control. In groups treated with the three mixed colorants (tetrazine/carrotin, yellow/curcumin and erythosine/anthocyanin), plasma urea was increased to 111%, 113% and 115%, respectively. High increase was observed in uric acid in the three groups of rats that treated with the synthetic colorants, percentages were: 186%, 206% and 197% for tetrazine, sunset yellow and erythrosine, respectively (Table 4).

High increase of creatinine was also observed in groups treated with synthetic colorants comparing the other groups (Table 4). This increase was more pronouncing in case of tetrazine treatment (percentage was 188%) followed by erythrosine (percentage was 184%) and then sunset yellow (percentage was 179%). These results are in agreement with the findings reported by Mackenzie *et al.* (1992) and El-Malky *et al.* (2014) who found a significant elevation in urea and creatinine levels in plasma and attributed that to impairment of renal functions. Through this work, the previous data confirmed the destructive effect of the synthetic tetrazine, sunset yellow and erythosine colorants on liver and kidney functions. Moreover, this study also agreed with the findings reported by Helal *et al.* (2000) who found that food colorants (both natural: tumeric, carmine chlorophyll and synthetic: fast green, annatto and sunset-yellow) increased serum creatinin except tumeric and sunset yellow. On the other hand, he reported that both of them revealed no effect on serum uric acid but highly increased serum urea.

4.5. Effect of Natural and Synthetic Colorants on 1 M Immune-System in Plasma

As regard to the effect of oral administration of colorants on plasma immune-system, the data obtained (Table 5) revealed that the plasma immunoglobulin IgG has decreased to 88%, 84% and 78% in groups that supplemented with synthetic colorants tetrazine, sunset yellow and erythosine, respectively, whereas no significant changes were observed in groups treated with mixed food colorants. Absolutely no IgG changes were observed in groups that treated with natural food colorants. On the other hand, a marked decrease in the plasma immunoglobulin IgM of groups that treated with synthetic colorants comparing to the normal health control. For each synthetic colorant, the decreasing percentage rate for IgM was 63%, 61% and 56% for tetrazine, sunset yellow and erythosine, respectively. The increasing percentage rate for IgM was ranged between 86% and 91% after treatment with mixed colorants. On the other hand, no significant changes were observed in IgM after treatment with natural colorants comparing to the normal

health control (Table 5). No significant changes on IgA were observed in groups treated with natural colorants and a synthetic colorant tetrazine, the lowest percentage level of the IgA was 64% and 67% after the administration with both sunset yellow and erythrosine, respectively. The higher IgG percentage (118%), comparing to the control, was observed in groups treated with a mixed colorant yellow/curcumin, whereas the other mixed colorants (tetrazine/carrotin and erythosine/anthocyanin) showed lower percentages (84% and 87%, respectively). Soltan and Shehata (2012) reported that the high concentration of synthetic food colorants lead to increase number of WBC as the result to the response of the immune system to the inflammation.

5. CONCLUSION AND RECOMMENDATIONS

It could be concluded that the present study indicated that synthetic colorants are adversely affecting hepatic and renal parameters comparing to natural colorants. Synthetic colorants were found to increase total lipids, total cholesterol, LDL-C, lipid peroxide, activities of alkaline phosphatase; acid phosphatase; lactate phposphatase, and plasms urea. While decreased each of HDL-C, GSH, SoD, catalase content, IgA, IgM and IgA. Therefore, it is necessary be aware about the hazardous effects of consuming such synthetic food colorants. And more attention should be paid towards using natural colorants.

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Table-1. Effect of natural and synthetic colorants on plasma lipid lipoprotein of male albino rats.

treatment	total lipids		total chlestrol		total triglyceride		total phospholpid		HDL-C		LDL-C		VLDL-C	
	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%	mg/dl	%
control	300±17	100 c%	167±9.1	100 c%	110±6.6	100 b%	100±5.4	100 a%	45±3.0	100 a%	36±1.7	100 c%	22±1.8	100 b%
n (1)	299±15	100 c%	170±8.4	102 c%	112±7.1	102 b%	99±5.6	99 a%	46±2.5	102 a%	35±1.9	97 c%	22.4±1.1	102 b%
n (2)	301±16	100 c%	169±8.6	101 c%	115±7.1	105 b%	106±6.0	106 a%	46±3.1	102 a%	34±2.0	94 c%	23±1.5	105 b%
n (3)	300 v 13	100 c%	166±9.0	99 c%	109±5.4	99 b%	102±5.9	102 a%	44±2.1	98 a%	34±1.4	94 c%	21.8±1.1	99 b%
s (1)	360±15	120 a%	211±8.9	126 a%	130±6.8	118 a%	110±6.2	110 a%	35±2.7	78 b%	47±2.1	131 a%	26±1.3	118 a%
s (2)	194±18	131 a%	215±10.2	129 a%	129±7.1	117 a%	104±6.0	104 a%	37±2.9	82 b%	51±2.2	142 a%	25.8±1.5	117 a%
s (3)	371±19	124 a%	209±11.0	125 a%	133±6.1	121 a%	105±5.3	105 a%	36±3.0	80 b%	46±2.1	128 a%	26.6±1.2	121 a%
m (1)	331±21	100 b%	182±10.1	109 c-b%	119±5.3	108 a-b%	105±5.5	105 a%	41±2.1	91 a-b%	40±2.1	111 b%	23.8±1.1	108 a-b%
m (2)	340±20	113 b%	190±12.0	114 b%	120±6.1	109 a-b%	103±6.1	103 a%	42±2.2	93 a-b%	41±1.8	114 b%	24±1.2	109 a-b
m (3)	336±18	112 b%	197±8.9	118 b%	121±5.9	110 a-b%	104 v 6.7	104 a%	41±2.3	91 a-b%	42±1.9	117 b%	24.2±1.2	110 a-b%

Relative to normal health control and each value represented the mean of 6 rats (mean ± SD)
Means in the same column followed by the same latter are not significantly different at ($P < 0.05$)
n (1) = curcumin, n (2) = carrotin, n(3) = anthocyanin.
s (1) = tetrazine, s (2) = sunset yellow, s (3) = erythrosine.
m (1) = tetrazine/carrotin, m (2) = yellow/curcumin, m (3) = erythosine/anthocyanin.

Table-2. Effect of natural and synthetic colorants on liver lipid per oxidation of male albino rats.

treatment	GSH		Lipid peroxide		SoD		catalase	
	mg/g liver	%	n mol /g liver	%	u/g liver	%	u/g liver	%
control	7.11±0.41	100 a%	4.82±0.20	100 d %	1199±67	100 a%	92±4.6	100 a%
n(1)	6.91±0.36	97 a%	5.00±0.31	104 d %	1201±56	100 a%	89±5.1	97 a%
n(2)	7.02±0.39	98 a%	4.91±0.24	102 d%	1205±66	101 a%	95±4.2	103 a%
n(3)	7±0.31	98 a%	5.01±0.21	104 a%	1210±59	101 a%	91±5.1	99 a %
s(1)	5.11±0.24	72 c%	10.71±0.53	222 a%	671±41	56 c%	65±3.2	71 c%
S(2)	4.92±0.21	69 c%	9.84±0.60	204 a%	682±36	57 c%	59±3.0	64 c%
s(3)	4.81±0.30	68 c%	10.13±0.61	210 a-b%	640±34	53 c%	61±2.9	66 c%
m(1)	5.91±0.25	83 c%	7.00±0.36	145 c%	900±47	75 b%	81±4.1	88 b%
m(2)	6.00±0.26	84 c%	6.72±0.41	139 c%	7.99±40	67 b%	79±4.2	86 b%
m(3)	5.87±0.30	83 c%	7.01±0.31	145 c%	812±42	68 b%	78±4.0	85 b%

Relative to normal health control and each value represented the mean of 6 rats (mean ± SD).
Means in the same column followed by the same latter are not significantly different at ($P < 0.05$).
n (1) = curcumin, n (2) = carrotin, n(3) = anthocyanin.
s (1) = tetrazine, s (2) = sunset yellow, s (3) = erythrosine.
m (1) = tetrazine/carrotin, m (2) = yellow/curcumin, m (3) = erythosine/anthocyanin.

Table-3. Effect of natural and synthetic colorants on blood glucose, activities of alkaline phosphate, acid phosphate and lactate dehydrogenase of male albino rats.

treatments	plasma glucose		alkaline phosphatase		acid phosphase		lactate dehydrogenase LDH	
	mg/dl	%	iu/l	%	iu/l	%	u / l	%
control	91 ± 5.2	100 c %	120 ± 7.1	100 c %	18.0 ± 0.77	100 a %	200 ± 11.0	100 c %
n (1)	96 ± 6.1	105 c %	131 ± 6.2	109 c %	17.62 ± 0.81	98 a %	220 ± 12.0	110 c %
n (2)	92 ± 6.2	101 c %	125 ± 6.2	104 c %	17.71 ± 0.90	98 a %	211 ± 11.0	106 c %
n (3)	94 ± 5.9	103 c %	128 ± 5.9	107 c %	18.01 ± 0.88	100 a %	199 ± 13.0	100 c %
s (1)	110 ± 6.7	121 a %	191 ± 8.8	159 a %	46.11 ± 2.17	256 b %	281 ± 15.0	141 a %
s (2)	115 ± 7.1	126 a %	200 ± 9.2	167 a %	43.01 ± 1.99	289 b %	300 ± 16.0	150 a %
s (3)	111 ± 6.9	122 a %	187 ± 9.3	156 a %	50.0 ± 2.41	278 a-b %	292 ± 17.0	146 a %
m (1)	102 ± 5.2	112 b %	156 ± 8.1	130 b %	27.00 ± 1.11	150 c %	240 ± 16.0	120 b %
m (2)	103 ± 6.1	113 b %	161 ± 9.0	134 b %	24.11 ± 1.02	134 d %	251 ± 13.0	126 b %
m (3)	105 ± 6.4	115 b %	163 ± 8.0	136 b %	25.61 ± 1.17	142 d-c %	255 ± 14.0	128 b %

Relative to normal health control and each value represented the mean of 6 rats (mean ± SD)
Means in the same column followed by the same latter are not significantly different at (P < 0.05)
n (1) = curcumin, n (2) = carrotin, n(3) = anthocyanin.
s (1) = tetrazine, s (2) = sunset yellow, s (3) = erythrosine.
m (1) = tetrazine/carrotin, m (2) = yellow/curcumin, m (3) = erythosine/anthocyanin.

Table-4. Effect of natural and synthetic colorants on kidney function in plasma of male albino rats

treatment	ured		Uric acid		creatinine	
	Mg/dl plasma %	%	Mg/dl plasma %	%	Mg/dl plasma	%
control	54.11±2.77	100 a %	2.91±1.91	100 c %	0.80±0.05	100
n (1)	55.00±3.00	102 a %	3.00±2.00	103 c %	0.82±0.04	103
n (2)	55.11±3.01	102 a %	2.97±1.71	102 c %	0.79±0.04	99
n (3)	54.81±2.96	101 a %	2.90±1.51	100 c %	0.78±0.03	98
s (1)	70.11±3.33	130 a %	5.41±3.00	186 a %	1.50±0.08	188
s (2)	75.21±3.65	139 a %	6.00±3.01	206 a %	1.43±0.08	179
s (3)	73.22±4.00	135 a %	5.72±2.97	197 a %	1.47±0.07	184
m (1)	60.11±3.01	111 b %	3.79±2.00	130 b %	0.98±0.05	123
m (2)	61.01±2.89	113 b %	3.81±1.79	131 b %	1.01±0.06	126
m (3)	62.00±2.87	115 b %	4.00±1.92	137 b %	0.92±0.05	115

Relative to normal health control and each value represented the mean of 6 rats (mean ± SD)
Means in the same column followed by the same latter are not significantly different at (P < 0.05)
n (1) = curcumin, n (2) = carrotin, n(3) = anthocyanin.
s (1) = tetrazine, s (2) = sunset yellow, s (3) = erythrosine.
m (1) = tetrazine/carrotin, m (2) = yellow/curcumin, m (3) = erythosine/anthocyanin.

Table-5. Effect of natural and synthetic colorants on 1 m immune-system in plasma of male albino rats.

treatment	lgG		lgM		lgA	
	mg/dl plasma	%	mg/dl plasma	%	mg/dl plasma	%
control	2500 ± 201	100 a	231 ± 17	100 a	345 ± 18	100 b
n (1)	2477 ± 178	99 a	229 ± 19	99 a	350 ± 19	101 b
n (2)	2511 ± 131	100 a	235 ± 16	102 a	349 ± 20	101 b
n (3)	2497 ± 142	100 a	226 ± 11	98 a	351 ± 21	102 b
s (1)	2201 ± 99	88 a-b	145 ± 12	63 c	240 ± 17	96 c
s (2)	2101 ± 151	84 b	140 ± 10	61 c	221 ± 17	64 d
s (3)	1955 ± 101	78 b	130 ± 9	56 c	232 ± 16	67 d
m (1)	2351 ± 182	94 a	200 ± 14	87 b	291 ± 18	84 c
m (2)	2371 ± 173	95 a	211 ± 13	91 b-a	289 ± 17	118 a
m (3)	2281 ± 191	91 a	198 ± 14	86 b-a	300 ± 16	87 c

Relative to normal health control and each value represented the mean of 6 rats (mean ± SD)
Means in the same column followed by the same latter are not significantly different at (P < 0.05)
n (1) = curcumin, n (2) = carrotin, n (3) = anthocyanin.
s (1) = tetrazine, s (2) = sunset yellow, s (3) = erythrosine.
m (1) = tetrazine/carrotin, m (2) = yellow/curcumin, m (3) = erythosine/anthocyanin.