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THE EFFECT OF SOME NATURAL AND SYNTHETIC COLORANTS ON BLOOD PARAMETERS OF MALE ALBINO RATS

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ABSTRACT

The present study investigated the effect of some natural colorants (curcumin, β-carotene, Hibiscus water extract) and synthetic one (Quinoline Yellow) on some biochemical and hematological parameters in male albino rats with histopathological examination of sections from liver. The natural and synthetic colorants were given daily for 4 weeks 2 at doses: 100 and 200 mg/kg body weight (bw), while Hibiscus cold and hot water extract at 350 and 400 mg/kg bw respectively. Feed efficiency ratio was increased significantly with curcumine and Quinoline Yellow (100 mg/kg/bw) and both doses of β-carotene compared with the control group. AST was not significantly changed in male rats administrated with natural and synthetic colorants, while a significant increase in ALT and ALP was noticed in rats treated with β-carotene and Quinoline Yellow at dose 200 mg/kg bw respectively. Natural and synthetic colorants at different doses did not cause any significant difference in total protein, albumin and blood urea nitrogen contents for treated rats. As exception, β-carotene 200 mg/kg caused significant decrease in total protein and albumin when compared with control rats. A significant increase in total bilirubin concentration was found in rats treated with both Hibiscus cold and hot extracts compared with the control animals. The levels of total hemoglobin (Hb) were highly when rats treated with both doses of Quinoline Yellow and both Hibiscus cold and hot extract. The results did not demonstrate any significant effects of both natural and synthetic colorants on % deferential leucocyte count comparing with control. Histopathological examination of liver

of rats exposed with high dose of natural and synthetic colorants showed mild pathological changes. It's concluded that these colorants must be used under limited restrictions and recommendations to avoid any damage or diseases that hurt human health.

INTRODUCTION

Natural and synthetic colour additives were used extensively to colour foods, drugs and cosmetics (Hallagan *et al.*, 1995). Colour is an important characteristic and selection criterion for food choice. The studies of Clydesdale (1993) have highlighted this importance and have shown how selection may change among certain populations and over time. Colorants have been used for many years in the pharmaceutical industry in order to add colour to many medicinal products, as well as to ensure the same colour for all the batches of a given product (Rowe *et al.*, 2003).

Curcumin, commonly called diferuloylmethane, is a hydrophobic polyphenol which responsible for the yellow color of turmeric, and is used widely in the food industry as a natural food coloring agent and curry powder as spice (Buescher and Yang 2000). Natural curcumin isolated from powdered rhizome of *Curcuma longa* L. (Zingiberaceae). It has been identified as the active principle of turmeric, chemically; it is a bis-α, β- unsaturated β- diketone that exhibits keto-enol tautomerism. The yellow-pigmented fraction of turmeric contains curcuminoids, which are chemically related to its principal ingredient, curcumin. The major curcuminoids present in turmeric are demethoxy curcumin (curcumin II), bisdemethoxy curcumin (curcumin III), and cyclocurcumin (Kiuchi *et al.*, 1993).

Carotenoids are a group of natural pigments present in fruits and vegetables imparting it their colours from yellow to red. The predominant carotenoids observed in the plasma are β-carotene, lycopene, lutein and α-carotene, accounting for more than 90% of the circulating carotenoids in humans (Rock, 1997). Some dietary carotenoids, such as β-carotene, serve as an important source of vitamin A, which is the major known function of carotenoids in humans. β -carotene is a hydrocarbon $C_{40}H_{56}$ that has a β-ionone structure as the terminal ring system at each side of the polyene chain. Carotenoids containing at least one unsubstituted β-ionone ring and a polyene chain are potential precursors of vitamin A. The preformed

vitamin A is only present in animal products (e.g. liver, eggs, milk products), thus, in countries where the intake of animal products is low, carotenoids have to meet (i.e. by 80% or more in Asia and Africa) the vitamin A requirements (Woutersen *et al.*, 1999).

Hibiscus sabdariffa, commonly known as Roselle or red Sorrel, is widely grown in Central and West Africa and South-East Asia. The red and fleshy cup-shaped flower calyces are consumed worldwide as a cold beverage or a hot drink. These extracts are also used in folk medicine to treat many complaints that include high blood pressure, liver disease and fever (Wang *et al.*, 2000). The pigments contained in the flowers of Hibiscus species are anthocyanins such as cyaniding -3 -glucoside and delphinidin -3-glocoside (Nakamura *et al.*, 1990).

Quinoline Yellow is a quinophthalone synthetic yellow dye prepared by sulfonating 2-(2-quinolyl) indan-1, 3-dione. It consists essentially of a mixture of sodium disulfonates, monosulfonates and trisulfonates (Code of Federal Regulations, 2000) and the quantities of these components vary widely according to the synthesis conditions (The Merck Index, 2001). Quinoline yellow is an acid dye which is used in coloring fibers, leather; paper, agrochemicals, fertilizers, detergents, wood, and ink, externally applied cosmetics, etc. The wide use of the dye in industry and its water-soluble nature maximize its chances to be found as a contaminant in industrial effluents. Though it is considered less toxic than its spirit soluble counterpart, its oral consumption is prohibited, particularly for infants and children (Coleman, 1991).

Therefore, the present study aimed to evaluate the effect of some natural colorants (curcumin, β-carotene, Hibiscus water extract) and synthetic one (Quinoline Yellow) on blood of male albino rats and the relation of these colorants with some common diseases. Some hematological and biochemical parameters were determined in the blood together with histological examination of sections from liver of rats of different groups used in the present work.

MATERIALS AND METHODS

1. Chemicals

The natural colorants curcumin and β-carotene were purchased from Sigma chemical company, St Louis, MO, USA, where the calyx of

Hibiscus sabdariffa was purchased from local market. The synthetic colorant Quinoline Yellow was obtained from Merck Limited, India.

2. Animals

54 male albino rats (Sprague-Dawely), *Rattus norvegicus albinu* s, weighting 140-160 gm were housed in the biological laboratory of Chemistry department, Faculty of Agriculture, Minia University. Rats were kept under the laboratory conditions for two weeks as an acclimatization period. They were housed in special healthy standard cages and maintained on *ad libitum* for water and a standard rat chow diet which contains 17% protein. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

3. Experimental design

Rats were randomly divided into nine groups as the following; Group (1): control group which was given distilled water. Group (2): rats of this group were given daily 100 curcumin mg/kg body weight. Group (3): rats of this group were given daily 200 curcumin mg/kg body weight. Group (4): in this group rats were administrated daily with 100 β-carotene mg/kg body weight. Group (5): in this group rats were administrated daily with 200 β-carotene mg/kg body weight. Group (6): albino rats were given daily 100 Quinoline Yellow mg/kg body weight. Group (7): albino rats were given daily 200 Quinoline Yellow mg/kg body weight. Group (8): the male rats of this group received daily 350 *Hibiscus sabdariffa* cold water extract mg/kg body weight. Group (9): the male rats of this group received daily 400 *Hibiscus sabdariffa* hot water extract mg/kg body weight.

The body weight and food consumption were recorded through out the period of experiment. At the end of experiment (4 weeks) blood samples were taken from the retro-orbital plexus from all animals of each group after anesthetized by diethyl ether. Each sample was divided into two portions: the first portion was used for hematological, Immunological studies and determination of hemoglobin using 10% ethylenediaminetetraacetic acid (EDTA) as anticoagulant and the second portion of the sample was centrifuged at 3000 rpm for 15 min, the obtained supernatant (serum) kept at -20°C until used in biochemical analysis. Animals were dissected as quickly

as possible and liver, kidney, and testis were excised, wiped with filter paper and weighted.

4. Hematological and Immunological parameters

Blood collected in 10% EDTA was analyzed for red blood cells (RBCs), white blood cells (WBCs) as described by Dacie and Lewis (1984) and platelets count as described by Wu and Hoak (1974), while hemoglobin concentration (Hb) was measured according to VanKampen and Zijlstra (1961). Hematocrit value (HCT %) or packed cell volume (PCV) was determined by centrifuging blood in heparinized microhematocrit tube (capillary tubes of 1mm internal diameter and 7.5 cm length) for 5 minutes at 15,000 r.p.m (Dacie and Lewis, 1991). The percentage of each type of the total leucocyte population in relation to the total count of counted WBCs was determined according to Schalm *et al.* (1975).

5. Biochemical methods analysis

Total protein, albumin, and blood urea nitrogen (BUN) were determined according to the methods of Gornal *et al.* (1949), Doumas *et al.* (1971) and Fawcett (1960) respectively. Liver function as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with colorimetric method (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) was measured colorimetrically (Belfield and Golberg, 1971). Total and direct bilirubin was determined colorimetrically (Walters and Gerarde, 1970).

6. Histopathological Determinations

Sections were taken from the liver of rats in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin and examination was done through the light electric microscope (Banchroft *et al.*, 1996).

7. Statistical analysis

Data were subjected to the ANOVA analysis using the MSTAT C program version 3 and means were compared using L.S.D – rang according to (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

1. Changes in body weight and food consumption:

Data in Table (1) reveal no significant difference in the body weight gain allover the course of administration. Feed efficiency ratio was increased significantly in group 3 (curcumine 100 mg/kg/bw), in group 4 and 5 (both doses of β-carotene) and in group 6 (Quinoline Yellow 100 mg/kg bw), on the other hand the ratio significantly decreased with Hibiscus hot extract compared with the control group. Our results were in agreement with Eboh et *al.* (1997) who found that β-carotene not affected body weight gain. Ganiger *et al.* (2007) stated that there were no treatment related effects in group mean body weights and net body weight gains for animals treated with curcumin. Essa and Subramanian (2006) found that there is no significant change in body weight of animals in the experimental groups treated with Hibiscus extract when compared with controls. Hansen *et al.* (1960) found that there is no treatment related effects were observed on body weight, feed intake in the rats treated with Quinoline Yellow.

Table (1) Effects of curcumin, β-carotene, Hibiscus extract and Quinoline Yellow on body weight and feed efficiency ratio of male rats.

Groups	Initial weight (g)	Final weight (g)	Daily body weight gain (g)	Daily feed intake (g)	Feed efficiency ratio (%)
Control	$160.^{a}$=13.60	261.8^{a}=16.69	3.636^{c}	19.820	18.345^{d}
Curcumin 100 mg/kg	160^{a} =13.01	269.2^{a}=11.63	3.902^{abc}	18.480	21.115^{bc}
Curcumin 200 mg/kg	160^{a} =10.47	269.6^{a}=10.6	3.558^{cd}	18.910	18.814^{cd}
β-carotene 100 mg/kg	145^{a} =6.11	263.4^{a}=9.90	4.350^{a}	17.280	25.174^{a}
β-carotene 200 mg/kg	140^{a} =3.62	265.8^{a}=8.09	4.164^{ab}	18.970	21.950^{b}
Quinoline Yellow100 mg/kg	143^{a} =6.93	258.8^{a}=6.44	4.158^{ab}	18.660	22.283^{b}
Quinoline Yellow 200 mg/kg	141^{a} =4.29	244^{a}=4.94	3.698^{bc}	17.980	20.567^{bcd}
Hibiscus cold extract	157^{a} =9.81	263.6^{a}=14.29	3.808^{bc}	18.520	20.561^{bcd}
Hibiscus hot extract	160^{a} =10.71	247.2^{a}=12.02	3.114^{d}	19.870	15.672^{e}
LSD at$_{0.05}$	29.56	28.32	0.464		2.504

Each value represents mean of 6 replicants =Standard Error (SE).

2. Changes in relative weight of some internal organs:

Data given in Table (2) indicate that curcumin, β-carotene, Hibiscus extract and Quinoline Yellow did not cause significant changes in liver, kidney and testis relative weight to the total weight of the treated albino rats through the experimental period than control group. As exception, curcumin and Quinoline Yellow when administrated at the higher dose 200 mg/kg caused significant decrease in relative weight of liver after 4 weeks of treatment. On the other hand administration of β-carotene at dose 200 mg/kg bw caused significant increase in relative testis weight comparing with control group. The achieved results are in agreement with previous studies as Eboh *et al.* (1997) who stated that relative liver weight was not significantly affected by β–carotene (0.01% and 0.02%) in broilers. Inano *et al.* (1999) showed that the treatment with curcumin increased liver weight significantly ($P < 0.01$) in rats fed the diet containing 1% curcumin for 1 year. Ishida et *al.* (2004) stated that none of the doses (20, 100, 300 and 900 mg/kg) of curcumin effect on liver weight of mice. Following 30 days of oral administration, *H. sabdariffa* aqueous extract given to rats at 250 and 1000 mg/kg/day did not demonstrate any significant effects on body weight, liver weight, % relative liver weight body weight gain as well as relative food consumption and relative water consumption (Prommetta *et al.*, 2006). In an oral feeding study, rats (5 per sex) were fed diets containing 0, 125, 250, 500, 1000, 2500 mg/kg bw/day methylated Quinoline Yellow for 90 days. No treatment-related effects were observed in relative organ weights (Hansen *et al.*, 1960).

Table (2) Effects of curcumin, β-carotene, Hibiscus extract and Quinoline Yellow on relative weight of Liver, kidney and testis to total body weight of male albino rats.

Groups	Liver(%) weight	Kidney (%) weight	Testis(%) weight
Control	$3.868^{ab} \pm 0.053$	$0.582^{a} \pm 0.013$	$0.945^{b} \pm 0.02$
Curcumin 100 mg/kg	$3.507^{c} \pm 0.082$	$0.706^{c} \pm 0.037$	$0.946^{c} \pm 0.02$
Curcumin 200 mg/kg	$3.004^{c} \pm 0.058$	$0.804^{a} \pm 0.030$	$0.998^{ab} \pm 0.05$
β-carotene 100 mg/kg	$3.989^{a} \pm 0.318$	$0.584^{a} \pm 0.016$	$1.074^{ab} \pm 0.12$
β-carotene 200 mg/kg	$3.594^{ab} \pm 0.161$	$0.558^{a} \pm 0.020$	$1.134^{a} \pm 0.09$
Quinoline yellow100 mg/kg	$3.593^{ab} \pm 0.0617$	$0.656^{a} \pm 0.021$	$1.099^{ab} \pm 0.04$
Quinoline yellow 200 mg/kg	$3.069^{c} \pm 0.106$	$0.519^{a} \pm 0.016$	$1.052^{ab} \pm 0.01$
Hibiscus cold extract	$3.567^{ab} \pm 0.108$	$0.859^{a} \pm 0.062$	$1.078^{ab} \pm 0.02$
Hibiscus hot extract	$3.409^{bc} \pm 0.106$	$1.056^{a} \pm 0.029$	$0.974^{ab} \pm 0.01$
$LSDat_{0.05}$	0.415	0.638	0.152

Each value represents mean of 6 replicants ±SE.

4. Changes in Biochemical parameters:

Serum biochemical parameters such as AST (GOT), ALT (GPT) and ALP were not significantly changed in male rats administrated with natural and synthetic colorants. However a significant increase in ALT was noticed in rats treated with β-carotene 200 mg/kg bw. The same icrease in ALP was achieved in rats treated with Quinoline Yellow 200 mg/kg bw (Table 3). There are no changes in laboratory indexes including serum concentrations of GOT (AST) and GPT (ALT) in the β–carotene treated group. (Murata *et al.*, 1994). Essa *et al.* (2005) stated that rats treated with ethanolic extract of *H. Sabdariffa* (250 mg/kg bw) alone showed no significant differences in levels of AST, ALT and ALP when compared with control rats. Also, Prommetta *et al.* (2006) indicated that administration of *H. sabdariffa* aqueous extract at the levels of 250 and 1000 mg/kg/bw did not cause any significant effects on serum AST, ALT and ALP of the treated rats comparing with control group.

Table (3) changes in the enzyme activities of serum (AST), (ALT) and (ALP) of male rats administered with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract.

Groups	ALT (U/L)	AST (U/L)	ALP (IU/L)
Control	56.8^{defgh} ±4.60	17.436^{cdef}±0.63	83.677^{ghi}±5.05
Curcumin 100 mg/kg	62.2^{abcdef}±6.00	16.006^{efgh}±1.05	69.406^{hi} ±2.86
Curcumin 200 mg/kg	64.6^{abcde} ±4.82	18.78^{bcd}±0.63	87.040^{gh} ±5.32
β-carotene 100 mg/kg	67.2^{abcd}±2.12	17.01^{cdefgh}±0.39	75.263^{hi}±6.05
β-carotene 200 mg/kg	73^{a} ±1.29	17.82^{cdefg}±0.29	93.466^{g} ±7.04
Quinoline yellow100 mg/kg	55.6^{defghi}±4.81	16.852^{cdefgh}±0.54	80.802^{ghi}±3.28
Quinoline yellow 200 mg/kg	62.2^{abcdef}±11.16	16.958^{cdefgh}±0.19	124.079^{f} ±2.70
Hibiscus cold extract	$60.6^{abcdefghi}$±6.89	17.000^{cdefgh}±0.99	80.325^{ghi}±3.65
Hibiscus hot extract	65.8^{abcd} ±11.87	17.246^{cdefgh}±0.32	83.677^{ghi}±5.05
LSD at 0.05	6.695	1.08	7.02

Each value represents mean of 6 replicants ±SE

Changes in total serum protein and serum albumin of rats of different groups as shown in Table (4). Our data indicate that cucrumin, β-carotenc, Quinoline Yellow and Hibiscus extract at different doses to male rats did not cause any significant difference in total protein and albumin contents throughout the experimental course. As exception, β-carotene 200 mg/kg caused significant decrease in total protein and albumin when compared with control rats. Similar finding was reported by Eboh *et al.* (1997) who observed that total protein and albumin concentrations in blood of broilers was

not significantly affected by 0.01% and 0.02% of β–carotene. Also, Usoh *et al.* (2005) and Prommetta, *et al.* (2006) stated that administration of *H. sabdariffa* extract did not cause any significant effects on total serum protein and albumin of rats when compared with control group.

Data presented in Table (4) show that the serum total and direct bilirubin concentration in rats administrated with cucumine, β-carotene and Quinoline Yellow was higher than those recorded for control animals but the increase was not significant. A Statistically significant increase in total bilirubin concentration was found in rats treated with both Hibiscus cold and hot extracts compared with the control animals. The same significant increase in direct bilirubin was noticed in rats treated with both doses of synthetic colorant. These results are confirmed with the histopathological examination which revealed congestion in the central and portal veins, associated with mononuclear leucocytes inflammatory cells infiltration in between hepatocytes. On the contrary Prommetta, *et al.*, (2006) showed that both dosages of *H. sabdariffa* aqueous extract (250 and 1000 mg/kg/day) did not cause any significant effects total bilirubin and direct bilirubin of treated male rats when compared with non-treated control group.

The obtained results showed that the administration of rats with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract at different doses did not cause any significant changes in blood urea nitrogen (Table 4). These results coincided with those obtained by Eboh *et al.* (1997) who founded that level of blood urea nitrogen (BUN) of broilers was not significantly affected by administration with β-carotene at levels 0.01% and 0.02% when compared with control group. Also, Jagadeesh *et al.* (2009) stated that administration of male rats with curcumin at dose 100 mg/kg/ body weight for 14 weeks didn't cause any significant differences in BUN when compared with non treated control. Essa *et al.* (2005) and Prommetta *et al.* (2006) showed that treating male rats with ethanolic and aqueous extract of *H. sabdariffa* respectively showed no significant differences in levels of blood urea nitrogen when compared with control rat. But in the contrast, a significant ($p<0.05$) dose dependent increase in serum urea levels were seen from the results of Orisakue *et al.* (2003) for rats received different doses of 1.15; 2.3; 4.6 g/kg bw of *H. sabdariffa* dried extract .

Table (4) changes in serum total protein, albumin, total bilirubin, direct bilirubin and BUN in rats treated with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract.

Groups	Total protein (g/dl)	Albumin(g/dl)	Total bilirubin (g/dl)	Direct bilirubin (g/dl)	BUN (mg/dl)
Control	7.13^{abc}±0.236	4.15^{cdef}±0.39	0.86^{def}±0.12	0.32^{cdef}±0.12	7.63^{bcde}±0.52
Curcumin 100 mg/kg	6.79^{dcd}±0.18	3.75^{defgh}±0.62	0.99^{cd}±0.12	0.35^{cdef}±0.02	7.92^{abcde}±3.0
Curcumin 200 mg/kg	7.21^{abc}±0.45	5.18^{ab} ±0.305	1.05^{cd}±0.23	0.48^{bcd}±0.04	8.97^{a}±0.46
β-carotene 100 mg/kg	6.50^{cde}±0.04	3.54^{efgh} ±0.16	1.01^{cd} ±0.04	0.33^{cdef}±0.04	8.06^{abcd}±0.15
β-carotene 200 mg/kg	5.89^{fg}±0.13	3.13^{gh}±0.28	1.13^{cd}±0.11	0.54^{bc} ±0.02	8.57^{abc}±0.24
Quinoline yellow100 mg/kg	6.76^{abcd}±0.18	3.76^{defgh}±0.44	1.05^{cd}±0.135	0.66^{ab} ±0.04	8.15^{abcd}±0.37
Quinoline yellow 200 mg/kg	6.82^{abcd}±0.12	4.80^{bc}±0.08	1.205^{c} ±0.05	0.92^{a} ±0.01	8.81^{ab}±0.36
Hibiscus cold extract	6.74^{abcd}±0.18	4.16^{cdef}±0.03	1.78^{b} ±0.04	0.46^{bcde}±0.16	8.83^{ab}±0.42
Hibiscus hot extract	6.94^{abcd}±0.36	4.31^{bcdef}±0.33	1.90^{b} ±0.25	0.51^{bcd} ±0.19	8.80^{ab}±0.16
LSD $at_{0.05}$	0.482	0.6496	0.203	0.1542	0.590

Each value represents mean of 6 replicants ±SE

5. Changes in hematological and immunological parameters:

The changes in RBCs, WBCs, Hb, PCV and platelets are presented in Table (5) which indicated narrow ranges in group treated with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract comparison with the normal values in non-treated control animals. Statistical studies of the data revealed no significant differences between them. Where, the levels of total hemoglobin (Hb) were highly when rats treated with both doses of Quinoline Yellow and both Hibiscus cold and hot extract. In the study of Sarada *et al.* (2002) β-carotene given at 10 mg/kg/bw to reduce the oxidative stress induced by hypoxia was evaluated on male albino rats. Hemoglobin concentration, red blood cell (RBC) and white blood cell (WBC) count were increased under hypoxia. β-carotene supplementation did not alter the changes in hemoglobin (Hb) concentration, RBCs and WBCs count.

Data shown in Table (6) represent the effect of curcumin, β-carotene, Quinoline Yellow and Hibiscus extract on the % differential leucocyte count. The results did not demonstrate any significant effects of both natural and synthetic colorants on % deferential leucocyte count comparing with control group. Our results are parallel with those reported by Prommetta *et al.* (2006) stated that oral administration of *H. sabdariffa* aqueous extract to rats at 250 and 1000 mg/kg/day did not show any significant effects on hemoglobin (Hb),

hematocrit (HCT%), red blood cell (RBC) count, platelet count, white blood cell (WBC) count and % differential WBCs. Hansen *et al.* (1960) showed that In an oral feeding study of rats with diets containing 0, 0.25, 0.5, 1, 2, and 5 % methylated Quinoline Yellow for 90 days. No significant effects were observed on blood cell counts for treated rats compared with non treated control group.

Table (5) Hematology data of male rats administered with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract.

Groups	Platlets (m/mm^3)	WBCs (m/mm^3)	RBCs (M/mm^3)	Hb (g/dl)	PCV (ml/dl)
Control	309.40^{a}±56.91	8.42^{ab}±0.72	5.72^{a} ±0.47	11.66^{c} ±0.12	41.40^{a}±1.03
Curcumin 100 mg/kg	325.60^{a}±29.63	7.38^{b}±1.42	5.54^{a} ±0.26	11.82^{bc} ±0.18	41.00^{a}±1.31
Curcumin 200 mg/kg	339.20^{a}±41.88	7.44^{b}±0.98	5.92^{a} ±0.45	12.32^{abc}±0.34	47.00^{a}±2.31
β-carotene 100 mg/kg	357.00^{a}±25.93	7.68^{b} ±1.6	7.16^{a}±0.42	13.50^{a} ±0.42	45.20^{a}±2.40
β-carotene 200 mg/kg	316.00^{a}±31.64	8.04^{b}±0.34	5.74^{a} ±0.86	11.46^{c} ±0.81	41.00^{a}±1.20
Quinoline yellow100 mg/kg	320.00^{a}±10.10	10.16^{a}±1.8	7.38^{a} ±0.72	12.86^{ab} ±0.25	43.40^{a}±3.14
Quinoline yellow 200 mg/kg	370.60^{a}±87.62	9.20^{ab}±0.51	6.02^{a} ±0.51	13.12^{a} ±0.37	44.80^{a}±1.29
Hibiscus cold extract	361.60^{a}±17.48	7.68^{b} ±1.65	6.02^{a} ±0.70	12.86^{ab} ±0.53	43.60^{a}±2.21
Hibiscus hot extract	363.00^{a}±36.79	7.82^{b} ±0.94	5.94^{a} ±1.82	13.28^{a} ±0.53	44.40^{a}±0.49
LSD $at_{0.05}$	76.00	3.810	1.772	1.076	5.854

Each value represents mean of 6 replicants ±SE

Table (6) Effects of curcumin, β-carotene, Quinoline Yellow and Hibiscus extract on differential leucocytes count in male albino rat.

Groups	Neutrophils	Eosinophil	Basophils	Lymphocytes	Monocytes
Control	55.2^{bc} ±1.94	3.0^{abc} ±0.24	1.2^{a}±0.20	36.6^{abc}±2.66	2.8^{abc}±0.20
Curcumin 100 mg/kg	52.6^{c} ±1.44	3.4^{a} ±0.245	1.6^{a}±0.24	40.2^{ab}±1.75	2.6^{abc}±0.24
Curcumin 200 mg/kg	51.0^{c} ±2.31	3.4^{a} ±0.245	1.6^{a} ±0.25	41.4^{a} ±0.75	2.4^{bc}±0.24
β-carotene 100 mg/kg	52.6^{c} ±1.92	3.2^{ab}±0.375	1.4^{a} ±0.24	39.6^{ab} ±1.13	3.2^{ab} ±0.49
β-carotene 200 mg kg	60.8^{a}±0.448	2.4^{c}±0.245	1.6^{a} ±0.25	33.4^{d} ±0.68	2.6^{c} ±0.20
Quinoline yellow100 mg/kg	60.4^{a}±0.246	2.6^{bc}±0.238	1.4^{a} ±0.25	30.4^{d} ±0.68	3.2^{ab} ±0.49
Quinoline yellow 200 mg/kg	59.2^{ab}±0.491	3.0^{abc} ±0.24	1.4^{a} ±0.25	35.2^{bcd}±1.16	3.6^{a} ±0.40
Hibiscus cold extract	59.2^{ab} ±1.24	3.2^{ab} ±0.20	1.6^{a} ±0.25	33.0^{cd} ±1.00	3.0^{abc}±0.31
Hibiscus hot extract	53.6^{c} ±1.81	3.2^{ab}±0.200	1.4^{a} ±0.25	35.6^{bcd}±2.70	3.0^{abc} ±0.31
LSD$at_{0.05}$	3.889	0.651	0.698	4.765	0.951

Each value represents mean of 6 replicants ±SE

6. Histopathological results:

Effect of treatments with curcumin, β-carotene, Quinoline Yellow and Hibiscus water extract on histopathological examination of the liver shown in the Figure (I). Histopathological changes in liver Hematoxylin and Eosin (H.&E.) stained sections in treated and control

animals were evaluated in order to screen for possible inflammatory response.

Fig (I1) showing the normal histological structure of the central vein (CV) and surrounding hepatocytes (h) in the liver of rat from control group. Fig (I2) showing degeneration in the hepatocytes (d) in the liver of rat from curcumin 100 mg/kg group. Fig (I3) for the liver of rat from curcumin 200 mg/kg group showing fibrosis in the portal area (arrow) surrounding the portal vein (PV). Fig (I4) for the liver of rat from β-carotene 100 mg/kg group showing inflammatory cells infiltration in the portal area (m) with dilatation in bile duct (d). Fig (I5) showing moderate fibrosis in the portal area (arrow) with inflammatory cells infiltration in the liver of rat from β-carotene 200 mg/kg group. Fig (I6) for the liver from rat in Quinoline yellow 100 mg/kg group showing mononuclear leucocytes inflammatory cells infiltration (m) in between the hepatocytes. Fig (I7) for the liver of rat from Quinoline yellow 200 mg/kg group showing congestion and dilatation of portal vein (PV) with inflammatory cells infiltration in the portal area (m) as well as the hepatic parenchyma (arrow). Fig (I8) showing mild dilation in the central vein (CV) in the liver of rat from Hibiscus cold extract group. Fig (I9) showing dilatation in central veins (CV) and sinusoids (S) in the liver of rat from Hibiscus hot extract group.

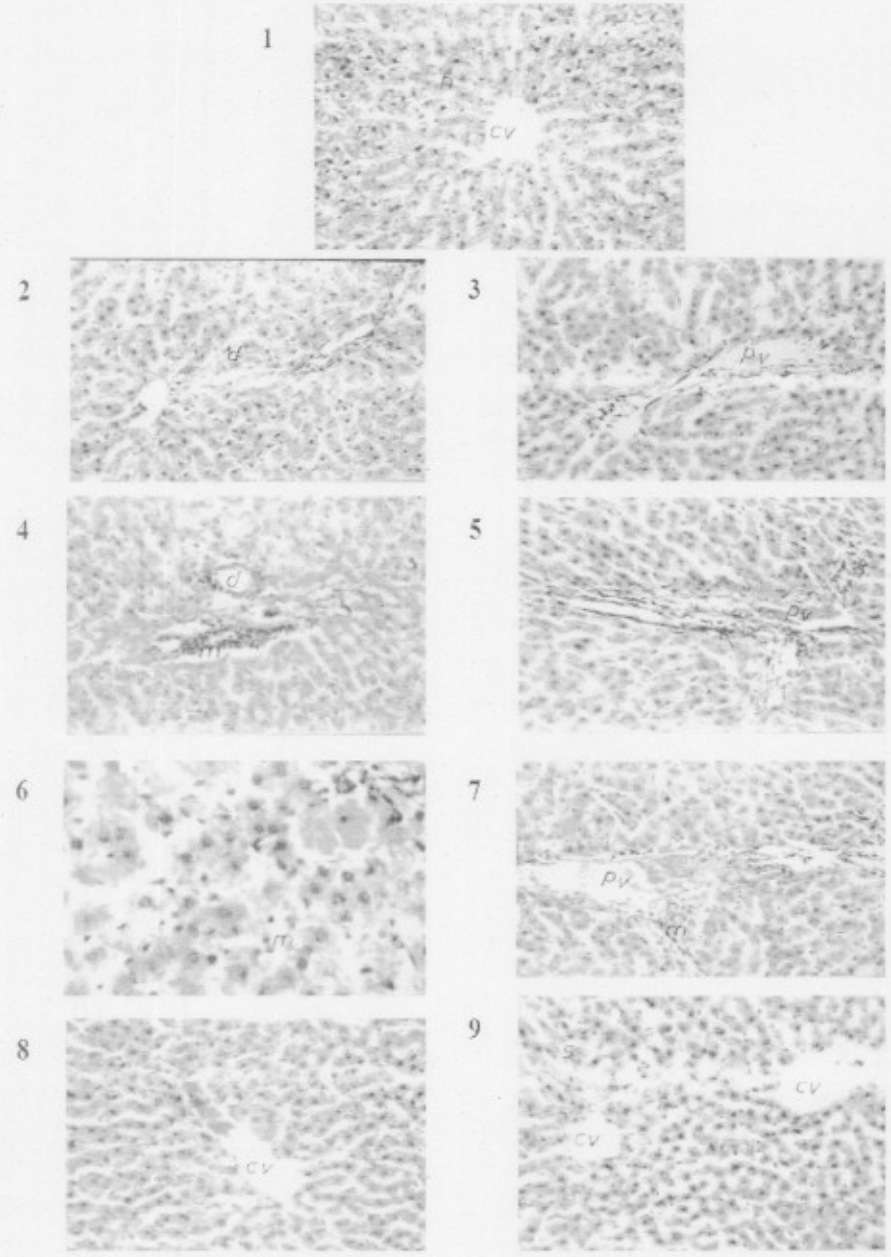

Figure (I): A photomicrograph of the liver sections of the control and administrated with curcumin, β-carotene, Quinoline Yellow and Hibiscus extract rats. (H&E, x 100).

In summary, the biochemical, hematological and histopathological investigations in this biological experiment has been explained the effect of some natural colorants (curcumin, β-carotene, Hibiscus water extract) and synthetic one (Quinoline Yellow) on blood of male albino rats. The results showed that high doses of both natural and synthetic colorants caused some significant changes in the offer mentioned parameters, so we concluded that these colorants must be used under limited restrictions and recommendations to avoid any damage or diseases that hurt human health.

REFERENCES

Banchroft, J. D.; Stevens, A. and Turner D. R. (1996). Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Belfield, A. and Goldberg, D. (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme, 12, 561-566.

Buescher, R. and Yang, L. (2000) Tumeric Natural food colorants. In: Lauro, G.J.; Francis, F.J. (Eds.) Marcel Dekker, New Yourk, 205-226.

Clydesdale, F. M. (1993) Color as a factor in food choice. Critical Reviews in Food Science and Nutrition, 33(1): 83-101.

Code of Federal Regulations, Title 21, Part 74.1710, US Government Printing Office, Washington, DC, 2000.

Coleman, M.P. (1991) Surgery. In: Cancer Risk After Medical Treatment Coleman MP (ed) pp 127–152. Oxford: Oxford University Press.

Dacie, J. V. and Lewis, S. M. (1984). Practical Haematology. 6th ed., Edinburgh: Churchill Livings tone, London.

Dacie, J. V. and Lewis, S. M. (1991). Practical Haematology. 7th ed., Edinburgh: Churchill Livingstone, London.

Doumas B. T.; Watson, W. A. and Biggs, H. G. (1971). Albumin standards and measurement of serum albumin with bromcresol green. Clin Chim Acta; 31: 87–96.

Eboh, G. O.; Kubena, L. F.; Chinnah, A. D. and Bailey, A. (1997). Effects of β-Carotene and Canthaxanthin on Aflatoxicosis in Broilers. Poultry Science, 76:1337–1341.

Essa, M. M. and Subramanian, P. (2006). *Hibiscus sabdariffa* affects ammonium chloride induced hyperammonemic rats. CAM, 1 -5.

Essa, M.; Subramanian, P.; Suthakar, G.; Manivasagam, T.; Dakshayani, K. B.; Sivaperumal, R.; Subash, S. and Vinothini, G. (2005). Influence of *Hibiscus sabdariffa* (Gongura) on the levels of circulatory lipid peroxidation products and liver marker enzymes in experimental hyperammonemia. J. Appl. Biomed, 4: 53–58 ISSN 1214-0287.

Fawcett, J. K. and Scott, J. E. (1960). A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13, 156-159.

Ganiger, S.; Malleshappa, H. N.; Krishnappa, H.; Rajashekhar, G.; Rao, V. R. and Sullivan, F. (2007). A two generation reproductive toxicity study with curcumin, turmeric yellow, in Wistar rats, Food and Chemical Toxicology, 45: 64–69.

Gomez, K.A. and Gomez, A. A. (1984). Statistical Procedures in Agricultural Research New York, Chichester, etc.: Wiley 2nd edition, pp 680.

Gornall, A. G.; Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751-766.

Hallagan, J.B.; Allen, D.C. and Borzelleca, J.F. (1995). The safety and regulatory status of food, drug and cosmetics colour additives exempt from certification. Food and Chemical Toxicology, 33:515-528.

Hansen, W. H.; Wilson, D. C. and Fitzhugh, O. C. (1960). Sub-acute oral toxicity of ten D and C coal-tar colours. Fed. Proc. 19, 390 (as referred to by JECFA, 1975).

Inano, H.; Onoda, M.; Inafuku, N.; Kubota, M.; Kamada, Y.; Osawa, T.; Kobayashi, H. and Wakabayashi, K. (1999). Chemoprevention by curcumin during the promotion stage of tumorigenesis of mammary gland in rats irradiated with g-rays. Carcinogenesis, 20 (6):1011–1018.

Ishida, T.; Taketoh, J.; Nakatsune, E.; Kan-O, S.; Naito, E.; Takeda, S.; Mutoh, J.; Ishii, Y. and Yamada, H. (2004). Curcumin anticipates the suppressed body weight gain with 2,3,7,8- Tetra

chloro dibenzo p- Dioxin in Mice. Journal of health science, 50 (5):474-482.

Jagadeesh, M.C.; Sreepriya M.; Bali, G. and Manjulakumari, D. (2009). Biochemical studies on the effect of curcumin and embelin during N-nitrosodiethylamine/Phenobarbital induced-hepatocarcinogenesis in Wistar rats. African Journal of Biotechnology, 8 (18):4618-4622.

Kiuchi, F.; Goto, Y.; Sugimoto, N.; Akao, N.; Kondo, K. and Tsuda, Y. (1993). Nematocidal activity of tumeric: synergistic action of curcuminoids. Chem. Pharm Bull (Tokyo), 41(9): 1640-1943.

Murata, T.; Tamai, H.; Morinobu, T.; Manago, M.; Takenaka, H.; Hayashi, K. and Mino, M. (1994). Effect of long-term administration of β-carotene on lymphocyte subsets in humans.: American Society for Clinical Nutrition, 60:597-602.

Nakamura, Y.; Hidaka, M.; Masaki, H.; Seto, H. and Uozumi, T. (1990). Major anthocyanin of the flowers of hibiscus (*Hibiscus sabdariffa* L.). Agricultural Biological Chemistry, 54:3345-3346.

Orisakwe, O. E.; Hussaini, D. C.; Orish, V. N.; Obi, E. and Udemezue, O. O. (2003). Nephrotoxic Effects of *Hibiscus Sabdariffa* Calyxin Rats. European Bulletin of Drug Research, 11(4) .99-103.

Prommetta, P.; Phivthong-ngam, L.; Chaichantipyuth, C.; Niwattisaiwong, N. and Lawanprasert, S. (2006). Aqueous extract of the calyces of *Hibiscus subdariffa* L. effects on hepatic cytochrome P450 and subacute toxicity in rats. Thai J. Pharm. Sci., 30: 8-18.

Reitman, S. and Frankel, S. (1957). A colourimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28, 56-63.

Rock, C.L.; Lovalvo, J.L.; Emenhiser, C.; Ruffin, M.T.; Flatt, S.W. and Schwartz, S.J. (1998) Bioavailability of beta-carotene is lower in raw than in processed carrots and spinach in women. Journal of Nutrition, 128:913–916.

Rowe, R.C.; Sheskey, P. J. and Weller, P. J. (2003) Handbook of Pharmaceutical Excipients, fourth ed. Pharmaceutical Press and American Pharmaceutical Association. Pharmaceutical Press, London, 83 - 85.

Sarada, S. K. S.; Dipti, P.; Anju, B.; Pauline, T.; Kain, A. K.; Sairam, M.; Sharma, S. K.; Ilavazhagan, G.; Kumar, D. and Selvamurthy, W. (2002). Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. Journal of Ethno pharmacology, 79 (2):149-153.

Schalm, O. W.; Jain, N. C. and Carroll, E. J. (1975). Veterinary Haematology. 3rd ed., Lea and Febiger, Philadelphia, 66-180.

The Merck Index, 13th ed., Merck, Whitehouse Station, NJ, 2001, p. 1446.

Usoh, I.F.; Akpan, E. J.; Etim, E. O. and Farombi, E. O. (2005). Antioxidant Actions of Dried Flower Extracts of *Hibiscus sabdariffa* L On Sodium Arsenite - Induced Oxidative Stress in Rats, Pakistan Journal of Nutrition,4 (3): 135-141.

VanKampen, E. J. and Zijlstra, W. G. (1961). Standardization of hemoglobinometry. II. The hemiglobincyanide method. Clin Chim Acta., 6: 538 544.

Walters, M.I. and Gerarde, H. W. (1970). An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchem J., 15:231–243.

Wang, C.J.; Wang, J.M.; Lin, W.L.; Chu, C.Y.; Chou, F.P. and Tseng, T.H. (2000). Protective effect of Hibiscus anthocyanins against tert- butyl hydroperoxide – induced hepatic toxicity in rats, Food and Chemical Toxicology, 38: 411-416.

Woutersen, R.; Wolterbeek, A.P.M. and Appel, M.J. (1999). Safety evaluation of synthetic beta carotene. Critical Reviews in Toxicology, 29:515-542.

Wu, K. K. and Hoak, J. C. (1974). A new method for quantitative detection of platelet aggregates in patients with arterial insufficiency. Lancet, 11: 924-926.

تأثير بعض الملونات الطبيعية و الصناعية علي مكونات الدم في ذكور جرذان الألبينو

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اظهرت الدراسة الحالية تأثير بعض الملونات الطبيعية (كيوركيومين - بيتا كاروتين و المستخلص المائي للكركدية) و كذلك المادة الملونة الصناعية اصفر الكينولين علي بعض الصفات البيوكيميائية و الهيماتولوجية في ذكور جرذان الألبينو مع اجراء دراسات هستولوجية لبعض قطاعات الكبد. اعطيت الملونات الطبيعية و الصناعية للجرذان يومياً لمدة 4 اسابيع في جرعتين: 100 و 200 مللجم/كجم من وزن الجسم بينما اعطي مستخلص الكركدية في جرعتين 350 و 400 مللجم للمستخلص البارد و الساخن علي التوالي. و كان من أهم النتائج: زيادة معدل الكفاءة الغذائية للجرذان في مجموعات الكيوركيومين واصفر الكينولين بمعدل100 مللجم/كجم من وزن الجسم و كذلك مجموعتي البيتا كاروتين مقارنة بالمجموعة الضابطة. لم تحدث تغيرات معنوية في نشاط انزيم AST عند معاملة الجرذان بكل من الملونات الطبيعية و الصناعية بينما حدث زيادة معنوية في نشاط انزيمي ALT, ALP في الجرذان المعاملة باصفر الكينولين و البيتا كاروتين بمعدل200 مللجم/كجم من وزن الجسم علي التوالي. لم تظهر معاملة ذكور الجرذان البيضاء بالملونات الطبيعية او الصناعية اي تاثير علي تركيز البروتين الكلي - الالبيومين و اليوريا و كان الاستثناء من ذلك هو الانخفاض المعنوي في تركيز البروتين الكلي و الالبيومين للجرذان التي عوملت بالبيتا كاروتين بمعدل200 مللجم/كجم من وزن الجسم. لوحظ كذلك زيادة معنوية في تركيز البيلروبين الكلي للجرذان المعاملة بمستخلصي الكركدية البارد و الساخن مقارنة بالمجموعة الضابطة. تركيز الهيموجلوبين في الدم كان مرتفعا في كل من مجموعتي اصفر الكينولين و كذلك مجموعتي مستخلص الكركدية البارد و الساخن بينما لم توضح معاملة ذكور الجرذان البيضاء بالملونات الطبيعية او الصناعية اي تاثير علي العد النوعي لكرات الدم البيضاء. اظهرت الدراسات الهستولوجية للكبد وجود بعض التغيرات الطفيفة عند معاملة الجرذان بالجرعة المرتفعة للملونات الطبيعية و الصناعية. و يمكن تلخيص ذلك علي انه يجب استخدام هذه الملونات تحت ظروف و توصيات محددة لتجنب حدوث خطر او مرض يهدد صحة الانسان.