Impact of mix food colors with Barly Water (Talbina) on some neurotransmitters in different brain regions, biochemical and histological structure of liver male albino rats

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Abstract: Many azo dye derivatives are used as food colorants has been neurotoxic effects. The objective of current study is to investigate the effect of chronic administration of mix Food color with Barly Water on neurotransmitters contents in different sides of brain. Particularly (cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus). Moreover, liver functions of male albino rats will be examined. Results show that the daily oral intake of mix food colors lead to decrease in the content of some neurotransmitters in brain areas at the different time, this might have caused oxidative stress leading to disturbances in neural function and decreased synthesis of neurotransmitters in the presynaptic cell. The daily oral intake of mix food colors with barly water caused a significant increase in total content of neurotransmitters in brain areas after treatment. In addition, the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase in rats oral administration with mix food colors were significant increase in treated rate. Chronic oral administration of mix food colors with barly water caused high in the level of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in treated rate. The present result in could be concluded that chronic oral administration of barly water with mix food color showed that an improvement in the tissue of liver. Finally, It was concluded that barly water improve the damage resulting from chronic intake of a mixture of food color due to its contains antioxidant substances that have the ability to capture free radicals.

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Key words: food colors, barly water, some neurotransmitters, Brain regions. liver function, male albino rats.

1. Introduction

Dyes are a class of food additives, Colored material is any substance added to food or consumed alone as a food is not considered an essential component of food components may have nutritional value or do not have any nutritional benefit and added to her food industry, such as packaging and manufacturing, setting the overall shape of food product benefit which gives the product a form attractive(1-2).

Tartrazine is used for coloring foods and beverages in yellow as well as brilliant blue FCF or Green S to create a number of green shades. tartrazine seems to cause many allergic reactions and food intolerance especially among people with asthma. tartrazine sensitivity symptoms occur either consecutively consumption of food or drinks containing tartrazine or skin exposure to substances containing tartrazine. After ingestion of tartrazine a number of health issue raised such as anxiety, headaches, blurry vision, and sleep problems (3).

One of the most azo dye used is Sunset Yellow FCF. It is used as a colorant food additive in many food products such as apricot jam, custard powders, citrus marmalade, orange sodas, sweets, energy drinks, squashes, margarine, marzipan, chips, packet soups, ice creams. It is usually used in chocolates and caramel to obtain the brown color in conjunction with amaranth dye. The EFSA acceptable daily intake for sunset yellow is 1.0 mg/kg bodyweight per day. It can inducean allergic reaction, many health problems such as diarrhea, migraines, gastric upset, swelling of the skin, nettle rash and vomiting(4).

Brilliant Blue is a colorant food additive widely used all over the world. the brilliant blue is made of aromatic hydrocarbons from petroleum(5). It is a food additive in many foods such as canned peas, dairyproducts, drinks, packet soups, sweets, icings and ice cream. The EFSA acceptable daily intake for brilliant blue is 6.0 mg/kg bodyweight per day(6). Chronic ingestion brilliant blue lead to skin irritation with redness, pain, sensitivity in the respiratory tract, which leads to coughing, reduction of breathing and digestive system disorder such as nausea, vomiting and diarrhea in humans(4).

Islam had defined barley water (talbina) which is a special diet made from fresh barley grains. The description of talbina in Sunah may show its benefits in the prevention of many diseases such asthma, allergies hypertension and obesity[7] and is used as a laxative for the intestines, calm the colon, cancer of the anti-intestinal tract, and barley contain antioxidants

and vitamins to fight free radicals that cause the destruction of the cell membrane and the incidence of certain types of cancer [8].

Barley (Hordeum vulgare), which is an annual cereal grain. Gramineae family it is important grain crops used in the Arabian Peninsula since the era of Prophet Mohammed Salam, where it appeared in many of the Hadith, Barley is used as food for humans and enters in the food industry, in the non-alcoholic beverage industry, bread and coffee malt [7-8]. barley is of functional foods, due to its content of soluble dietary fiber beta-glucan dissolved, in addition to the high content of vitamin vitamins B1, B2, B6, C, Barley is characterized as containing many nutrients fat and protein, carbohydrates amylase, dextrin, phospholipids, maltose, glucose, sulfur, niacin and beta-glucan calcium and magnesium zinc, beta carotene. In Saudi Arabia enters the barley bread and coffee industry [9-10].

Medical, Barley grain protein rich containing glutamate (glutamic acid), which works on the reconstruction of the epithelial cells lining the digestive tract, also contains a high concentration of dietary fiber, soluble and insoluble, which protect the gastrointestinal tract, and reduce inflammatory gastrointestinal tract symptoms associated with it, such as bleeding and diarrhea, help lower blood sugar and cholesterol. [11]. Moreover, it serve to reduce plasma lipids and LDL oxidation [8]. Indeed, leaves barly are used in folk medicine for treatment in Saudi Arabia. The objective of this paper was to study the modulatory neuroprotective role of barly water as an antioxidant on some neurotransmitters in different brain regions was treatment with mix food colors and biochrmical and histological structure of liver in male rats.

2. Materials and Method

2.1. Animals

male albino rats used in this study (90-100g). They were supplied with food and water ad libitum under standard conditions of light, humidity and temperature (22-25°C).

2.2. Food Colors

Tartrazine (NarmaCol, India) oral dose 25 mg/kg [3].

Sunset yellow was purchased from a local market (96%, Kamena Industries, Canada) oral dose 2.5 mg/kg [12].

Brilliant blue (NarmaCol, India) oral dose 0.4 g/kg [13].

2.3. Barley

Barley (Hordeum vulgare) was obtained from fields located in al-Quassim area, Saudi Arabia.

2.4. Method

Preparation of the barley water (Al-Talbina): soup made by of two table spoons of crushed barley grains, then add to them a glass of water, and cook over low heat for 5 minutes. [14]

2.4.1. The effect of mix Food Colors and Barley water on different sides of brain of male albino rats

The animals were randomly divided into 5 groups.

Group1: (n=6) This is as a control group, it was treated with saline vehicle and killed at the beginning of the experiment.

The other groups are normal rats, orally administered with:

Group2: (n=24) mix food colors through oral tube for 4 week and six rats were decapitated after1, 2, 3 and 4 week post treated.

Group3: (n=24) barley water through oral tube for 4 week and six rats were decapitated after1, 2, 3 and 4 week post treated.

Group 4: (n=6) mix food colors through oral tube (4 week) for histological examination.

Group5: (n=6) barley water through oral tube (4 week) for histological examination.

All rat was decapitated suddenly at the specified times. the brain was excised and on dry ice glass plate, in short time with highly care. Brain was dissected into cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus. [15]. Tissues of brain were undergoing following process before frozen put aluminum foil in dry ice for analysis NE, DA and 5-HT were extracted and estimated according to the method of Ciarlone [17]. GABA was estimated according to the method of Sutton and Simmodes [18]. The fluorescence was measured in Jenway 6200 fluorometer.

2.4.2. The effect of mix Food colors and Barley water on liver function of male albino rats

Blood samples were collected and left to coagulate by add. ethylene diamine tetracetic acid. samples are then transferred to a centrifuge at 3000 r.p.m. For 30 minutes. Serum was separated and distributed into three parts for each animal to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase in the liver [19].

2.4.3. The effect of mix food colors and barley water on histological structures of liver of male albino rats

After dissecting animals taken portion of the liver tissue from the animals treated and the control groups. And immersed in 10% buffered formalin solution. and are tissue processing for histological sections were stained with haematoxylin and eosin [20].

2.5. Statistical analysis

The experimental results were communicated as means ± standard deviation (SD). SAS version 9 software was utilized to make the examination of fluctuation of (ANOVA), $P < 0.05$ qualities were viewed as noteworthy.

3. Result

Results in Table 1 showed a noticible decrese was noticed in norepinephrine content in all of tested areas after1, 2, 3 and 4 week for mix food colors usage. A significant decrease about-80.33% was noticed in the striatum at 4 weeks. Also the usage of mix food colors for all period decrease the content of dopamine, and in the fourth week the highest decrease was noticed. It was around (-35.76%), see Table 2. Simliarly, in serotonin content was noticed due to usage of of mix food colors. The maximum decrease was around -44.01% in the cerebellum after 4 weeks, see Table 3. Moreover, results in Table 4 show gamma-butyric acid content decreased also for all brain regions during entire period. The maximal decrease was found in the cerebellum after 4 weeks (approximtally 23.81%).

In Table 5 results for the usage of mix food colors with water barly demonstrate that norepinephrine content has a significant decrease in all tested areas, for example about -19.80% in the cerebellum in at 1st week. However, an increase in norepinephrine content after 3 and 4 week was noticed, about 35.80% in the cerebellum in at 4 weeks in cerebellum. Similarly, results in Table 6 show that dopamine content reduced at 1st week in all tested areas followed by increased starting from fourth week.

Also, Table 7 shows that the daily oral administration of mix food colors with water barly caused a significant decrease in serotonin content starting from the 1st week in cerebellum, cerebral cortex and hypothalamus and increase in all areas of the brain from third to fourth week The maximal decrease in serotonin content was found in the cerebral cortex at 1st week (-12.37%) and the maximal increase in serotonin content found in brain stem at 4 weeks was (20.79). while the daily oral administration of mix food colors with water barly caused a significant decrease in gamma-butyric acid content starting from the 1st week in cerebellum and striatum and increase in all areas of the brain from third to fourth week The maximal decrease in gamma-butyric acid content was found in the cerebellum at 1st week (-10.46%) and the maximal increase in gamma-butyric acid content found in striatum at 4 weeks was (23.34). (Table 8).

For rat that were treated with mix food colors for 2 and 4 weeks, the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase increased as showen in Table 9. The incensement compared to control results were 31.06%, 54.04% and65.05%, respectively. Similarly for rat that were treated with mix food colors with water barly for 2 weeks., AST and ALT were increased by 10.18% and 10.43% respectively. For rat that treated after 4 weeks, no changes were noticed, see Table 10.

Histological changes

Table (1): Effect of chronic oral administration of mix food colors on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E	Hippocampus mean ± S.E.
1 week	C	95.382 ± 0.845	511.473 ± 1.603	56.203 ± 0.225	596.997 ± 3.242	390.050 ± 0.831	292.540 ± 1.536
	T	61.004 ± 7.169	106.667 ± 0.558	41.667 ± 0.760	487.333 ± 1.667	103.000 ± 1.239	100.000 ± 0.632
	%	-36.04 *	-79.15 *	-25.86 *	-18.37 *	-73.59 *	-65.82 *
2 weeks	C	95.358 ± 0.857	511.118 ± 1.648	54.443 ± 1.898	595.330 ± 9.485	390.490 ± 0.484	292.527 ± 1.531
	T	47.500 ± 0.764	105.333 ± 0.422	42.667 ± 0.667	456.833 ± 1.014	152.333 ± 0.882	102.833 ± 0.946
	%	-50.19 *	-79.39 *	-21.63 *	-24.53 *	-60.99 *	-64.85 *
3 weeks	C	96.688 ± 0.274	495.853 ± 1.445	55.493 ± 0.105	604.906 ± 2.337	394.485 ± 0.942	283.178 ± 0.617
	T	43.500 ± 0.764	102.000 ± 0.577	41.261 ± 1.313	402.667 ± 1.085	105.000 ± 1.350	87.333 ± 0.715
	%	-55.52 *	-79.42 *	-25.65 *	-33.43 *	-73.38 *	-69.16 *
4 weeks	C	98.485 ± 0.271	495.780 ± 1.443	55.525 ± 0.127	604.623 ± 2.261	394.618 ± 0.944	282.998 ± 0.841
	T	43.500 ± 0.764	97.500 ± 0.764	40.793 ± 0.873	402.667 ± 1.085	105.000 ± 1.350	87.333 ± 0.715
	%	-55.83 *	-80.33 *	-26.53 *	-33.40 *	-73.39 *	-69.14 *

· Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired t' test.
% : Percentage of change from control. * : Significant at $p<0.05$.

Figure 1,2 (sections 1 and 2) shows the histological structural photo sectors in the normal control liver of male albino rat show Casement region of the liver that contains the channel bile (BD) and portal vein (PV). Also notes the regularity of bars hepatic cells (HC) on the central vein (CV) and the liver cell contains the central nuclei which contain one or two nucleolus and note pockets vessels which contain cells Kupffer(KC).

Oral administration of mix food colors cause congestion of central and portal veins with cellular infiltration. Blood sinusoids and necrotic of Kupffercells(KC) as well as proliferation of bile ducts(BD) were disrupted. Hepatic cells has focal necrosis with deformed nuclei which appeared in pyknosis, see Figure 1 (sections 3 and 4).

In contrast in Figure 2(sections 3 and 4), water barly usage retain hepatic tissue natural appearance almost regularity liver cells tapes significantly with decreasing the amount of blood stagnant in the central vein (CV) and the presence of the invasion of inflammatory (LY simple) as Kpfer cells appeared (KC) form the usual and caused improvement in the portal area (PA), especially in the channel bile (BD) and hepatic portal vein (PV).

Table (2): Effect of chronic oral administration of mix food colors on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Stratum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755 ± 0.818	473.948 ± 0.856	60.488 ± 0.044	734.223 ± 2.111	451.288 ± 0.633	243.147 ± 0.863
	T	127.833 ± 0.946	424.000 ± 1.183	53.000 ± 0.730	663.833 ± 0.946	401.667 ± 0.422	199.500 ± 0.428
	%	-12.89 *	-10.54 *	-12.38 *	-10.95 *	-11.00 *	-17.96 *
2 weeks	C	145.648 ± 0.914	482.312 ± 3.336	61.240 ± 0.214	739.237 ± 4.314	451.541 ± 1.947	244.597 ± 1.448
	T	127.667 ± 0.882	424.167 ± 1.078	54.167 ± 0.703	654.000 ± 0.816	402.000 ± 0.683	199.167 ± 0.477
	%	-12.35 *	-12.06 *	-11.55 *	-11.53 *	-10.97 *	-18.57 *
3 weeks	C	146.977 ± 0.942	474.115 ± 0.911	60.715 ± 0.259	734.057 ± 2.258	451.806 ± 0.591	242.968 ± 0.843
	T	131.289 ± 0.388	424.333 ± 1.054	53.761 ± 0.505	654.157 ± 0.946	402.167 ± 0.703	157.333 ± 0.558
	%	-10.67 *	-10.50 *	-11.45 *	-10.88 *	-10.95 *	-35.25 *
4 weeks	C	147.768 ± 2.011	482.298 ± 3.043	62.466 ± 0.922	738.215 ± 4.439	451.837 ± 1.987	244.906 ± 1.544
	T	117.156 ± 1.807	424.333 ± 1.054	50.200 ± 0.317	654.157 ± 0.946	402.167 ± 0.703	157.333 ± 0.558
	%	-20.72 *	-12.02 *	-19.63 *	-11.39 *	-10.95 *	-35.76 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (3): Effect of chronic oral administration oral of mix food colors on serotonin (5-HT) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.662 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	192.878 ± 0.694	168.301 ± 1.634	57.163 ± 0.541	432.884 ± 0.514	117.798 ± 0.163	196.779 ± 3.549
	%	0.22	-1.95	-0.15	0.01	-0.30	-8.38 *
2 weeks	C	193.045 ± 0.719	171.496 ± 0.522	57.374 ± 0.463	433.106 ± 0.485	118.368 ± 0.364	215.100 ± 1.229
	T	167.500 ± 0.992	153.124 ± 1.640	41.000 ± 0.730	355.000 ± 0.577	102.671 ± 1.205	189.833 ± 0.401
	%	-13.23 *	-10.71 *	-28.54 *	-18.03 *	-13.26 *	-11.75 *
3 weeks	C	192.326 ± 0.203	173.444 ± 1.706	57.287 ± 0.176	430.635 ± 0.928	117.401 ± 0.079	216.757 ± 0.943
	T	157.796 ± 12.008	151.454 ± 1.884	50.502 ± 3.181	347.667 ± 0.955	103.328 ± 2.138	190.000 ± 0.365
	%	-17.95 *	-12.68 *	-11.84	-19.27 *	-11.99 *	-12.34 *
4 weeks	C	192.276 ± 0.075	173.669 ± 1.772	57.771 ± 0.023	430.951 ± 0.267	118.248 ± 0.380	215.868 ± 1.275
	T	107.659 ± 0.530	145.772 ± 0.757	39.581 ± 0.355	347.667 ± 0.955	92.684 ± 0.487	190.844 ± 0.862
	%	-44.01 *	-16.06 *	-31.49 *	-19.33 *	-21.62 *	-11.59 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (4): Effect of chronic oral administration of mix food colors on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	171.000 ± 0.516	151.500 ± 0.428	57.919 ± 0.149	431.393 ± 2.515	116.809 ± 0.958	215.267 ± 0.938
	%	-11.15 *	-11.74 *	1.17	-0.33	-1.14	0.22
2 weeks	C	192.544 ± 0.759	171.662 ± 0.447	57.374 ± 0.463	432.939 ± 0.370	117.868 ± 0.237	214.933 ± 1.269
	T	154.833 ± 0.601	153.833 ± 0.477	50.000 ± 0.577	382.333 ± 0.667	101.000 ± 0.577	191.000 ± 0.683
	%	-19.55 *	-10.39 *	-12.80 *	-11.69 *	-14.31 *	-11.14 *
3 weeks	C	193.611 ± 0.781	175.423 ± 1.783	57.849 ± 0.675	437.968 ± 1.007	118.436 ± 0.231	216.865 ± 0.870
	T	154.667 ± 0.667	153.500 ± 0.563	49.667 ± 0.422	382.000 ± 0.856	100.667 ± 0.667	189.167 ± 0.833
	%	-20.11 *	-12.50 *	-14.14 *	-12.78 *	-15.00 *	-12.77 *
4 weeks	C	193.379 ± 0.440	171.744 ± 1.615	57.713 ± 0.935	437.849 ± 0.198	118.118 ± 1.396	215.234 ± 1.053
	T	147.333 ± 0.667	146.333 ± 1.256	48.167 ± 0.307	374.500 ± 0.992	94.833 ± 0.792	186.833 ± 0.543
	%	-23.81 *	-14.80 *	-16.54 *	-14.47 *	-19.71 *	-13.20 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (5): Effect of chronic oral administration of mix food colors with water barly on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	96.382 ± 0.845	511.473 ± 1.803	56.203 ± 0.225	596.997 ± 3.242	390.050 ± 0.831	292.540 ± 1.536
	T	76.500 ± 0.428	452.667 ± 0.882	49.833 ± 0.307	533.667 ± 0.558	334.000 ± 0.856	252.000 ± 0.577
	%	-19.80 *	-11.50 *	-11.33 *	-10.61 *	-14.37 *	-13.86 *
2 weeks	C	96.358 ± 0.857	511.118 ± 1.648	56.110 ± 0.289	605.330 ± 9.485	390.490 ± 0.484	292.527 ± 1.531
	T	91.333 ± 0.494	502.000 ± 0.365	55.167 ± 0.477	599.167 ± 0.601	381.500 ± 0.428	290.000 ± 0.365
	%	-4.22 *	-1.78 *	-1.68	-1.02	-2.30 *	-0.86
3 weeks	C	111.000 ± 0.577	495.653 ± 1.445	55.493 ± 0.105	604.906 ± 2.337	394.485 ± 0.942	283.178 ± 0.817
	T	126.667 ± 0.667	549.667 ± 0.333	62.667 ± 0.919	667.167 ± 0.601	435.500 ± 0.847	313.500 ± 0.922
	%	14.11 *	10.90 *	12.93 *	10.29 *	10.40 *	10.71 *
4 weeks	C	96.358 ± 0.857	495.780 ± 1.443	55.525 ± 0.127	604.823 ± 2.281	394.618 ± 0.944	282.998 ± 0.841
	T	129.500 ± 1.727	555.833 ± 1.302	63.500 ± 0.428	678.333 ± 0.715	457.000 ± 0.577	329.833 ± 0.601
	%	35.80 *	12.11 *	14.36 *	12.19 *	15.81 *	16.55 *

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (6): Effect of chronic oral administration of mix food colors with water barly on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	154.833 ± 0.601	153.833 ± 0.477	50.000 ± 0.577	382.333 ± 0.667	101.000 ± 0.577	191.000 ± 0.683
	%	-19.55 *	-10.38 *	-12.66 *	-11.67 *	-14.52 *	-11.07 *
2 weeks	C	192.544 ± 0.759	171.662 ± 0.447	57.374 ± 0.463	432.939 ± 0.370	117.868 ± 0.237	214.933 ± 1.269
	T	195.167 ± 2.664	180.833 ± 0.601	57.919 ± 0.149	431.393 ± 2.516	116.809 ± 0.958	215.267 ± 0.938
	%	1.36	5.34 *	0.95	-0.36	-0.90	0.16
3 weeks	C	193.611 ± 0.781	175.423 ± 1.783	57.849 ± 0.675	437.968 ± 1.007	118.436 ± 0.231	216.865 ± 0.870
	T	197.667 ± 0.615	176.000 ± 5.033	57.919 ± 0.149	431.393 ± 2.516	116.809 ± 0.958	215.267 ± 0.938
	%	2.09 *	0.33	0.12	-1.50 *	-1.37	-0.74
4 weeks	C	193.379 ± 0.440	171.744 ± 1.615	57.713 ± 0.935	437.849 ± 0.198	118.118 ± 1.396	215.234 ± 1.053
	T	222.500 ± 0.764	192.000 ± 0.577	70.333 ± 0.422	491.333 ± 0.494	132.333 ± 0.667	245.333 ± 0.422
	%	15.06 *	11.79 *	21.87 *	12.22 *	12.03 *	13.98 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at p<0.05.

Table (7): Effect of chronic oral administration oral of mix food colors with water barly on serotonin (5-HT) content in the different brain areas of male albino rat.

Time of decapitation		Cere mean	Str mean	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	172.000 ± 1.000	168.578 ± 1.592	50.167 ± 0.401	388.000 ± 1.366	118.280 ± 0.451	202.500 ± 1.147
	%	-10.63 *	-1.79	-12.37 *	-10.82 *	0.11	-5.72 *
2 weeks	C	193.045 ± 0.719	171.496 ± 0.522	57.374 ± 0.463	433.106 ± 0.485	118.368 ± 0.364	215.100 ± 1.229
	T	192.167 ± 0.946	170.500 ± 0.671	56.500 ± 0.428	430.333 ± 0.494	117.500 ± 0.764	211.333 ± 0.558
	%	-0.45	-0.58	-1.52	-0.64 *	-0.73	-1.75 *
3 weeks	C	192.328 ± 0.203	173.444 ± 1.705	57.287 ± 0.176	430.635 ± 0.928	117.401 ± 0.019	216.757 ± 0.943
	T	212.000 ± 0.577	198.000 ± 0.601	65.500 ± 0.619	477.500 ± 0.563	131.833 ± 0.703	246.000 ± 0.577
	%	10.23 *	14.18 *	14.34 *	10.88 *	12.29 *	13.49 *
4 weeks	C	192.276 ± 0.075	173.669 ± 1.772	57.771 ± 0.023	430.951 ± 0.267	118.248 ± 0.380	215.868 ± 1.275
	T	219.833 ± 0.601	208.167 ± 0.601	69.000 ± 0.428	486.333 ± 0.494	142.833 ± 1.138	257.000 ± 0.577
	%	14.33 *	19.86 *	19.44 *	12.85 *	20.79 *	19.05 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at $p<0.05$.

Table (8): Effect of chronic oral administration of mix food colors with water barly on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457 ± 0.799	171.652 ± 0.450	57.247 ± 0.385	432.828 ± 0.319	118.155 ± 0.197	214.787 ± 1.321
	T	172.333 ± 1.145	153.833 ± 0.477	57.622 ± 0.356	433.138 ± 1.971	116.635 ± 0.890	214.403 ± 0.787
	%	-10.46 *	-10.39 *	0.66	0.07	-1.29	-0.18
2 weeks	C	192.544 ± 0.759	171.662 ± 0.447	57.374 ± 0.463	432.939 ± 0.370	117.868 ± 0.237	214.933 ± 1.269
	T	191.500 ± 0.764	172.000 ± 0.816	59.333 ± 0.843	434.833 ± 1.249	120.667 ± 0.715	213.115 ± 0.673
	%	-0.54	0.20	3.41	0.44	2.37 *	-0.85
3 weeks	C	193.611 ± 0.781	175.423 ± 1.783	57.849 ± 0.675	437.968 ± 1.007	118.436 ± 0.231	216.865 ± 0.870
	T	221.333 ± 0.955	197.667 ± 0.615	66.000 ± 0.365	489.333 ± 0.422	135.333 ± 0.494	244.333 ± 1.022
	%	14.32 *	12.69 *	14.09 *	11.73 *	14.27 *	12.67 *
4 weeks	C	193.379 ± 0.440	171.744 ± 1.615	57.713 ± 0.935	437.849 ± 0.198	118.118 ± 1.398	215.234 ± 1.053
	T	234.500 ± 0.428	211.833 ± 0.601	68.333 ± 0.667	503.833 ± 1.138	138.833 ± 0.477	248.000 ± 0.730
	%	21.28 *	23.34 *	18.40 *	15.07 *	17.54 *	15.22 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at $p<0.05$.

Table (9): Effect of chronic oral administration of mix food colors on Liver function of male albino rat.

Time of decapitation		AST(U/L) mean ± S.E.	ALT(U/L) mean ± S.E.	Alkaline phosphatase(U/l) mean ± S.E.
2 week	C	99.833 ± 0.543	78.333 ± 0.667	60.488 ± 0.044
	T	111.500 ± 0.428	97.167 ± 1.621	81.000 ± 0.365
	%	11.69 *	24.04 *	33.91 *
4 weeks	C	99.833 ± 0.543	78.333 ± 0.667	60.488 ± 0.044
	T	130.833 ± 0.307	120.667 ± 0.422	99.833 ± 0.477
	%	31.05 *	54.04 *	65.05 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t* test.
% : Percentage of change from control. * : Significant at $p<0.05$.

Table (10): Effect of chronic oral administration of mix food colors with water barly Liver function of male

Time of decapitation		AST(U/L) mean ± S.E.	ALT(U/L) mean ± S.E.	Alkaline phosphatase(U/l) mean ± S.E.
2 week	C	99.833 ± 0.543	78.333 ± 0.667	60.488 ± 0.044
	T	110.000 ± 0.422	86.500 ± 0.428	65.500 ± 0.428
	%	10.18 *	10.43 *	8.29 *
4 weeks	C	99.833 ± 0.543	78.333 ± 0.667	60.488 ± 0.044
	T	100.667 ± 0.422	75.500 ± 0.428	60.333 ± 0.667
	%	0.83	-3.62 *	-0.26

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired t' test.
% : Percentage of change from control. * : Significant at p<0.05.

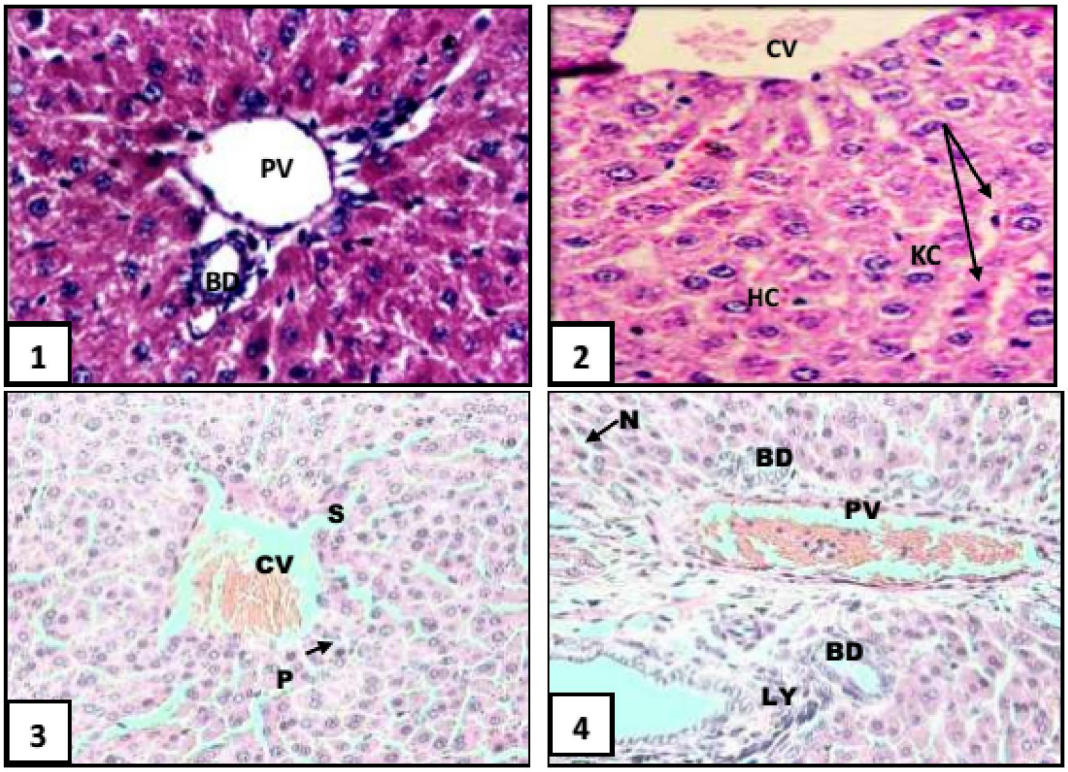

Figure 1 (sections 1 and 2) shows the histological structural photo sectors in the normal control liver of male albino rat show Casement region of the liver that contains the channel bile (BD) and portal vein (PV). Also notes the regularity of bars hepatic cells (HC) on the central vein (CV) and the liver cell contains the central nuclei which contain one or two nucleolus and note pockets vessels which contain cells Kupffer(KC).
Figure 1 (sections 3) shows the histological structural photo sectors in the liver of male albino rat treated with mix food colors led to congestion of central and portal veins with cellular infiltration. distruption of blood sinusoids. (Hx. & E, X 400).
Figure 1 (sections 4) shows the histological structural photo sectors in the liver of male albino rat treated with mix food colors led to necrotic of Kupffercells(KC), as well as proliferation of bile ducts (BD), focal necrosis of hepatic cells with deformed nuclei which appeared in pyknosis (Hx. & E, X 400).

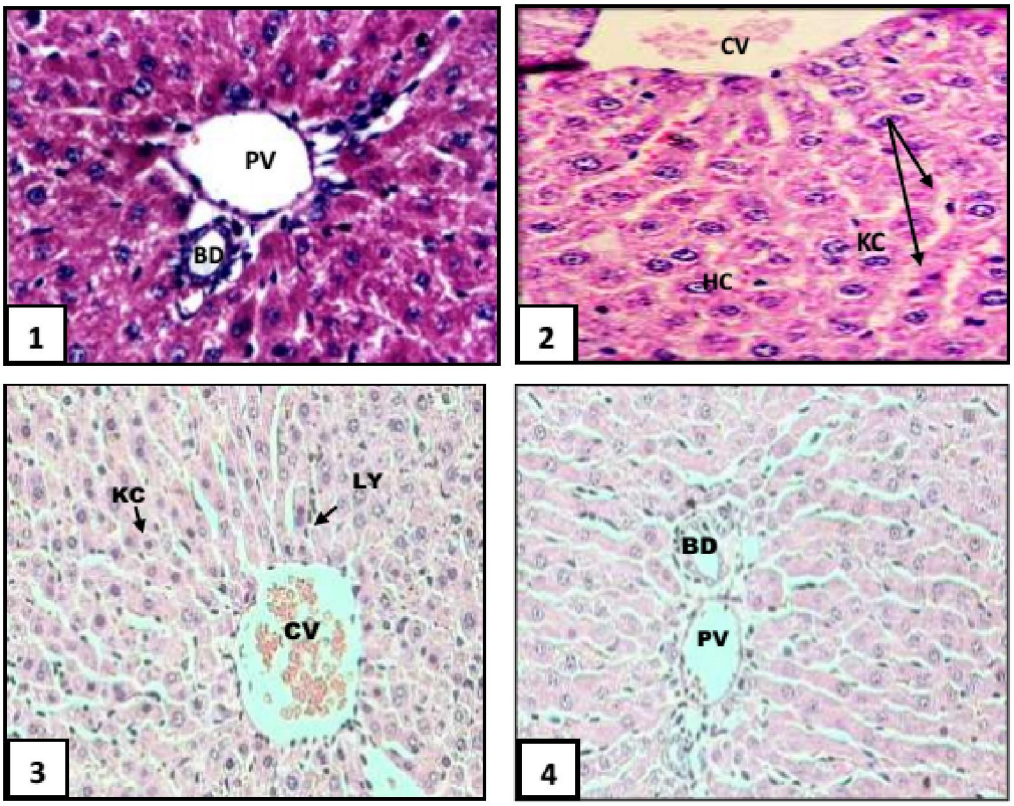

Figure 2 (sections 1 and 2): shows the histological structural photo sectors in the normal control liver of male albino rat show Casement region of the liver that contains the channel bile (BD) and portal vein (PV). Also notes the regularity of bars hepatic cells (HC) on the central vein (CV) and the liver cell contains the central nuclei which contain one or two nucleolus and note pockets vessels which contain cells Kupffer(KC).

Figure 2 (sections 3): oral administration of mix food colors with water barly retain hepatic tissue natural appearance almost regularity liver cells tapes significantly with decreasing the amount of blood stagnant in the central vein (CV) and the presence of the invasion of inflammatory (LY simple) as Kpfer cells appeared (KC) form the usual (Hx. & E, X 400).

Figure 2 (sections 4): oral administration of mix food colors with water barly improvement in the portal area (PA), especially in the channel bile (BD) and hepatic portal vein (PV) (Hx. & E, X 400).

4- Discussion

The neurotoxicity is more associated with the people who consumed food colors additive in foods and soft drinks. There is evidence in the literature such food colures can adverse reactions in the biological systems [21]. Organic Consumers Association was reported the toxic effect of combined additives such as aspartame, monosodium glutamate. It was found it may stop the nerve cells growing and interfered with proper signaling. Our findings revealed that the consume of mix food colors caused a lowering in the content of neurotransmitter in all areas of brain at different time. Food colors are cytotoxic compounds because it is able to create O2 free radicals component when it metabolized by intestinal bacteria [22], that cause oxidative stress and Suppression of ATP genesis leading to reduce synthesis or re-uptake of neurotransmitter in the presynaptic cell [14] There is a strong direct correlation between oral intake of food blend colors and the accumulation of free radicals, which cause certain diseases to humans, thereby reducing the activity of antioxidant enzymes such as the enzymes catalase, UHF, glutathione peroxidase and Algelotatheon- S- transferase in the body which leads to cell membranes and loss of function damage [23].

Our results are confirmed the recent reports that demonstrated the Lack of antioxidant enzymes activity in the liver and brain [24] resulting from chronic oral administration of mix food colors to increased oxidative stress [25].

Chronic oral administration of mix food colors with water barly caused a significant increase in the total content of norepinephrine, dopamine, serotonin and gamma-aminobutyric acid in all the tested brain areas at different time intervals. The present study showed that the mix food colors with water barly chronic oral administered may prevent induced

neurotoxicity in rats. Eating dietary antioxidants can play an important role in preventing or delaying the oxidation of vulnerable cellular substrates, and are therefore relevant for the prevention of the disease. Barley contains high amounts of phenolic compounds antioxidants [26]. And that have a role in the removal of free radicals, also contains the seeds of barley on tyrosine and tryptophan [27] both of the essential amino acids in the human, and serves as the precursor to the catecholamines and serotonin synthesis [28].

Moreover, it was reported that barley contains high amounts of myoinositol hexaphosphate, which in turn is hydrolysed to myoinositol[29]. myoinositol enters the phosphatidylinositol cycle and the formation of diacylglycerol activated protein kinase C, Inositol triphosphate (IP3) leads to increase the flow of calcium ions into cells, Such second messengers can modulate second messengers modify, noradrenergic, serotoninergic and dopaminergic systems[30]. Due phenolic compounds in water, barley activity because it contains phenolic hydroxyl group and especially phydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde, which have the ability to gain electrons. The electrons combine with free radicals, leading to reduced lipid peroxidation caused by free radicals [31.32].

The antioxidant effects of barly water were evidenced from its effects on oxidative stress biomarker. barly water contains several antioxidants [33]. and its high content of melatonin a well-known antioxidant[34]. low antioxidant in tissue increase in rat liver is the indication of hepatotoxicity In the present study, food color caused hepatocellular necrosis and vacuolation and these results go in agreement with an earlier worker who found that the synthetic food dye brilliant blue cause occurrence of histological changes in the liver of rats treated [13]. These changes includes necrosis of hepatocytes, infiltration and vacuolation. The food colors caused lymphocytic infiltration around central veins.[35] our results are also in accordance with the findings which described changes in the liver when guinea pigs received tartrazine in drinking water in a concentration of 1, 2 and 3% for 3 weeks [36]. tartrazine affected adversely and altered biochemical markers such as antioxidant enzymes in vital tissues oral administration of food colors daily to male rats suppressed the activities of antioxidant enzymes in liver. Metabolic products of mix food colors cause an increased concentration of free radicals such as hydrogen peroxide H2O2, which works to inhibited the activity of superoxide-dismutase in liver rat [22].

The results indicate that there are significant elevations in the level of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in serum of male albino rat treated with the mix food colors. damaged organs showed increase in enzyme activity. Chronic intoxication was accompanied by continuous increase in serum levels in both ALT and AST activities. the changes in serum ALT and AST activities are due to cellular degradation by mix food colors on the liver[13].

Conclusions:

The results of this research show that chronic administration of the mix food color with barley water causes increase significant in the content of some neurotransmitters in all brain regions studied with a clear improvement in liver tissue and the high level of liver enzymes, which encourage the use of barley water daily meal to reduce the effects oxidative stress on the brain, liver cells the output of the total daily consumption of food containing food dyes.

Reference:

1. SIL, V. A. and REED, E. N. O.(2010): Estudos sobre corantes artificiais em alimentos: quais os riscos mais comuns pelo consumo excessivo. Cadernos de Educação. Tecnologia e Sociedade. 2(1):10-4.
2. Cheeseman, M. A. (2012): Artificial food color additives and child behavior Environmental Health Perspectives. 20 (1): 15-16.
3. Saxena, B. and Sharma, S. (2015): Food Color Induced Hepatotoxicity in Swiss Albino Rats, Rattus norvegicus. Toxicology international.22(1):152-157.
4. Kus, E. and Eroglu, H. E. (2015): Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes Pak. J. Pharm. Sci. 28(1):227-230.
5. Kus, E. and Eroglu, H. E.(2015): Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes. Pak. J. Pharm. Sci. 28(1):227-230.
6. EFSA European Food Safety Authority (2010). Scientific Opinion on the re-evaluation of brilliant blue FCF (E133) as a food additive. EFSA panel on food additives and nutrient sources added to food (ANS). EFSA J, Parma, p.
7. Jensen, M. K. P.; Koh-Banerjee, L.; Sampson, M.; Gronbaek E. B. and Rimm, M.(2006): Whole grains, bran and germ in relation to homocysteine and markers of glycemic control, lipids and inflammation. Am. J. Clin. Nutr. 83: 275-83.
8. Ya-Mei, Y. U.; Weng-Cheng, C.; Chu-Sun, L. and Chingmin, E.(2005): Effect of young barley leaf extract and adlay on plasma lipids and LDL oxidation in hyperlipidemic smokers. Nutr. Cancer. 52: 102-108.
9. Anderson, J. W. and Hanna, T. J. (1999): Whole grains and protection against coronary heart disease: what are the active components and mechanisms. Am. J. Clin. Nutr. 70: 307-308.
10. Jacobs, D. R.; Marquart, L.; Slavin, J. and Kushi, L. H. (1998): Whole-grain intake and cancer: an expanded review and meta-analysis. Nutr. Cancer. 30: 85-96.
11. McIntosh, G.; Whyte, J.; McArthur R. and Nestel, P. (1991): Barley and wheat foods: influence on plasma

cholesterol concentrations in hypercholesterolemic men. Am. J. Clin. Nutr. 53: 1205-1209.
12. Fouad, D.; Alobaid, H. and Al-Jafary, A. A. (2013): Effect of melatonin on the oxidative stress induced by the food additive (C. I. Food Yellow 3) on some bloodparameters and antioxidant enzymes in male rat kidney. Life Science J.10(4):343-350.
13. Mahmoud, H. N.(2006): Toxic effects of the synthetic food dye brilliant blue on liver, kidney and testes. J Egypt Soc. Toxicol.34:77–84.
14. Bawazir, A. E.(2012): Effect of chocolate brown HT with olive oil on some neurotransmitters in different brain regions, physiological and histological structure of liver and kidney of male albino rats. J of Evolutionary Bio. Res.4(1):13-23.
15. Glowinski, J. and Iversen, L. L. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [3H] dopamine and [3H] dopa in various regions of the brain. J. Neurochem., 13: 655-669.
16. Chang, C. C., 1964. A sensitive method forspectrofluorometric assay of catecholamines. Int. J. Neuropharmacol., 4: 643-649.
17. Ciarlone, A. E. (1978). Further modification of a fluorometric method for analyzing brain amines. Microchem. J. 23: 9 - 12.
18. Sutton, I. and Simmonds, M. A. (1973): Effects of acute and chronic ethanol on the gama-aminobutyric acid system in rat brain. Biochem. Pharmacol., 22: 1685 - 1692.
19. Christic, J. H. and Michelson, E. H. (1975): Transaminase levels in the digestive gland-gonad of Schistosoma mansoni infected Biomplalaria alexandrina. Comp. Biochem. Physiol. 50: 233 - 236.
20. Harris, H. F. (1900): On the rapid conversion of haematoxylin into haematin staining reactions. J. Appl. Microsc. Lab. Methods. 3: 777.
21. Branen, A. L.; Davidson, M. and Salminen, S. (2001): Food additives. Marcel Dekker London, 2nd Edition, pp. 1-8.
22. Amin, K. A.; Hameid, H. A. and AbdElsttar, A. H.(2010): Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology. 48: 2994-2999.
23. Moskovitz J.; Yim, M. B. and Chock, P. B. (2002): "Free Radicals and Disease," Archives of Biochemistry and Biophysics. 397 (2): 354-359.
24. Gao, Y.; Li, C.; Shen, J.; Yin, H.; An, X. and Jin, H.(2011): Effect of food azo dye tartrazine on learning and memory functions in mice and rats and the possible mechanisms involved. Journal of Food Science. 76(6): 125-129.
25. El-Tohamy, M. M.(2012): The mechanisms by which oxidative stress and free radical damage produces male infertility. Life Science J. 9(1): 674-88.
26. [26] – Holtekjolen, A. K.; Baevere, A. B.; Rodbotten, M.; Berg, H. and Knutsen, S. H.(2008):"Antioxidant Properties and Sensory Profiles of Breads Containing Barley Flour," Food Che mistry. 110(2): 414-421.
27. Davy, A.; Sorensen, M.; Svendsen, I.; Cameron-Mills, V. and Simpson, D. (2000): Prediction of protein cleavage sites by the barley cysteine endoproteases EP-A and EP-B based on the kinetics of synthetic peptide hydrolysis. Plant Physiol. 122:137-145.
28. Jin, G. and Lin, X. (2004): The electrochemical behavior and amperometric determination of tyrosine and tryptophan at a glassy carbon electrode modified with butyrylcholine. Electrochem. Commun. 6:454-460.
29. Rimsten, L.; Haraldsson, A.; Andersson, R.; Alminger, M.; Sandberg, A. and Åman, P. (2002): Effects of malting on β-glucanase and phytase activity in barley grain. J. Sci. Food Agric. 82:904-912.
30. Einat, H.; Karbovski, H.; Korik, J.; Tsalah, D. and Belmaker, R. H. (1999): Inositol reduces depressive-like behaviors in two different animal models of depression. Psychopharmacology (Berl) 144:158-162.
31. Stohs, S. J. and Bagchi, D.(1995):"Oxidative Mechanism in the Toxicity of Metal Ions," Free Radical Biology and Medicine.18(2): 321-336.
32. Thomas, S. S.; Michaelh, H. G. and Jennife, M. A.(2005): "Antio- xidant Properties of Malt Model Systems," J Agriculture and Food Chemistry. 53(12): 4938-4945.
33. Qingming, Y.; Xianhui, P.; Weibao, K.; Hong, Y.; Zidan, S.; Li, Z.; Yanan, Z.; Yuling, Y.; Lan, D. and Guoan, L. (2010): Antioxidant activities of malt extract from barley (Hordeum vulgare L.) toward various oxidative stress in vitro and in vivo. Food Chem. 118:84-89.
34. Badria, F. A. (2002): Melatonin, serotonin and tryptamine in some Egyptian food and medicinal plants. J. Med. Food 5:153-157.
35. Soltan, S. A. and Shehata, M. N.(2012): The effects of using color foods of children on immunity properties and liver, kidney on rats. Food Nutr Sci.3:897–904.
36. Rus, C.; Gherman, V.; Miclaus, A.; Mihalca, G. and Nadas. C. (2009): Comparative toxicity of food dyes on liver and kidney in Guinea pigs histopathological study. Ann. R.S.C.B.15:161–165.

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