

Effects of Food Colour Allura Red (No. 129) on some neurotransmitter, Antioxidant Functions and Bioelement Contents of Kidney and Brain Tissues in male albino Rats

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Abstract: Currently, Allura Red (E 129) is a colourant which typically added in food products, drugs and cosmetics. This study exam the influence of chronic ingestion of allura red at 200mg/kg for 8 weeks in rat via determination of some neurotransmitter, antioxidant levels in brain, kidney tissues. Our results show that neurotransmitter contents decreased in treated group, while urea and creatinin increased in the serum. The brain and kidney tissue MDA and GSH levels were a significant decrease after 8 weeks. histological examinations showed kidney damages, allura red induces adverse effects on brain, and kidney.

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Keywords: Effect; Food; Colour; neurotransmitter; Antioxidant Function; Bioelement Content; Kidney; Brain; tissue; Rat

1. Introduction

Food additives play important role to meet the requirements of growing population during production and presentation of plentiful, tasty and nutritious food [1]. Azo dyes, which is kind of artificial food, widely used in the textile, leather, paper, food, pharmaceutical and cosmetic industries [2]. Azo colors are derived from Azo groups ($-N=N-$) bound to aromatic rings in their molecular structures [3]. The most common a red colorant derived from Azo is allura red [4].

After consumed azo dyes researchers have been studied the safety of this dyes. Studies reported that dyes may cause illnesses such as anemia, pathological lesions in the brain, liver, kidney and allergic reactions as nettle rash and asthma [5-6]. Therefore, the effect of Allura red has been re-evaluated by the Panel on Food Additives and Nutrient Sources. Adverse impact in vitro genotoxicity and the effects on nuclear DNA have been reported (Allura Red E 129). Moreover, previous studies reported the changes in antioxidant enzyme activity. These changes occur due to the interaction between free radicals produced by azo dyes and intact molecules. [7-8-9-10].

Studies have been recommended that further information of statistical analysis of the long-term effect for allura red is need (Allura Red E 129). The aim of this study was to exam the effects of allura red on rat serum and brain, kidney tissues via measuring some neurotransmitter contents in different brain regions, antioxidant biomarkers and kidney functions.

2. Materials And Method

2.1. Chemicals

Allura Red was purchased from Roha Dychem Pvt. Ltd., Maharashtra [India].

2.2. Animals

Male albino rats, *Rattus rattus* (90-100g) were used in this study. Rats were feed and lived under standard conditions (22-25°C) in the laboratory of faculty of Science - Al-Faisalyyah campus King Abdul-Aziz University.

2.3. Experimental Design

The animals were randomly divided into four groups.

Group1: (n=6) was used as control, and killed at the beginning of the experiment.

Group2: (n=48) was orally administered with allura red via gavage tube for 8 week and six rats were decapitated after 1, 2, 3,4,5,6,7 and 8 week post treated to examination of neurotransmitter in brain.

Group3: (n=6) was orally administered allura red via gavage tube for 8 week and six rats were decapitated after 4 and 8 week post treated for examination of function kidney.

Group4: (n=6) was orally administered allura red via gavage tube for 8 week and six rats were decapitated after treated for antioxidant and histological examination.

2.4. Method

2.4.1. The effect of allura red on examined brain regions

Rats were suddenly decapitated in a certain times. The brain was carefully excised then dissected into a number of areas: cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus [11]. Filter papers were used to wipe tissue, and then plastic films and aluminum foil were used to wrapped and quickly frozen. We analysis using fluorometer (Jenway 6200) serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) [12-13], gamma aminobutyric acid (GABA) [14], and histamen [15].

2.4.2. The effect of allura red on kidney function

The blood samples were collected and stored to coagulate at room temperature. Samples were centrifuged at 3000 r.p.m. for 30 min. serum were kept it to subsequent analysis for the measurement of urea and creatinine [16].

2.4.3. The effect of allura red on histological structures of kidney

After 2 months from decapitated, kidney of each rat were excised. For histopathology, 10% neutral buffered formalin was added to a portion of each kidney then were further processed using standard method. Each portion were divided to 5 μ m thicknesses and stained by with haematoxylin-eosin. Slides were examined using light microscope [17].

2.4. The effect of allura red on biochemical analysis of brain and kidney

At the end of the experiment blood samples were collected for analysis the indicators of oxidative stress, malonaldehyde (MDA) and glutathione peroxidase (GSH). Methode by [18] was performed to measure the level of MDA. a spectrophotometric method [19] has been used to measure the level of GSH.

2.5. Statistical analysis

Results are expressed as means \pm standard deviation (SD) and $P < 0.05$ values were regarded as significant.

3. Result

3.1. Neurotransmitter Levels in Tissues brain

Table (1) shows that allura red leads to a significant reduce in serotonin (5-HT) content in all examined areas from 2nd week till the last week of the experimental duration, the maximum decrease (-78.51%) in 5-HT content was found after 7 weeks in the cerebral cortex.

Table (2) shows that allura red leads to a significant decrease in 5-hydroxyindoleacetic acid (5-HIAA) content starting from the 1st week in striatum, cerebral cortex and hippocampus and from the third week in all brain area till the end of the experiment duration. Maximum decrease (-91.73%) in 5-HIAA content was found after 8 weeks in the hippocampus.

Table (3) shows allura red leads to a noticeable decrease in GABA level from the 1st week in striatum and hypothalamus, from the 2nd week in all tested areas except hippocampus, and from 3th week till the end of the experimental duration in other tested areas. Maximum decrease (-58.07%) in GABA content was found after 8 weeks in hypothalamus.

Table (4) shows that the chronic administration of allura red leads to a significant decrease in histamine level from the 1st week in brain stem and hippocampus, and in the other tested areas from the 2nd week till the end of experimental duration. Maximum decrease (57.07%) in histamine content found after 8 week in striatum.

Table (1): Effect of chronic oral administration of the allura red (200 mg/ kg bw) on serotonin (5-HT) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean \pm S.E.	Striatum mean \pm S.E.	Cerebral cortex mean \pm S.E.	Hypothalamus mean \pm S.E.	Brain stem mean \pm S.E.	Hippocampus mean \pm S.E.
1 week	C	192.711 \pm 0.708	172.162 \pm 0.564	57.041 \pm 0.330	432.606 \pm 0.326	117.868 \pm 0.237	214.933 \pm 1.269
	T	181.055 \pm 5.314	162.009 \pm 5.414	55.528 \pm 1.045	429.983 \pm 2.289	113.430 \pm 2.473	185.051 \pm 0.760
	%	-6.05	-5.90	-2.65	-0.61	-3.76	-13.90 *
2 weeks	C	192.457 \pm 0.799	171.652 \pm 0.450	57.247 \pm 0.385	432.828 \pm 0.319	118.155 \pm 0.197	214.787 \pm 1.321
	T	144.952 \pm 0.370	127.153 \pm 1.338	43.708 \pm 0.673	375.506 \pm 1.028	105.701 \pm 1.623	183.304 \pm 1.605
	%	-24.68 *	-25.92 *	-23.65 *	-13.24 *	-10.54 *	-14.66 *
3 weeks	C	192.983 \pm 1.154	172.160 \pm 2.053	56.796 \pm 1.677	433.823 \pm 6.329	118.436 \pm 0.231	215.584 \pm 1.789
	T	136.760 \pm 1.042	111.826 \pm 0.375	41.325 \pm 0.410	350.869 \pm 0.265	88.407 \pm 0.530	182.013 \pm 1.992
	%	-29.13 *	-35.05 *	-27.24 *	-19.12 *	-25.35 *	-15.57 *
4 weeks	C	193.393 \pm 0.781	177.560 \pm 0.689	55.580 \pm 0.649	446.555 \pm 1.027	118.436 \pm 0.231	216.865 \pm 0.870
	T	130.323 \pm 0.663	96.110 \pm 1.214	43.841 \pm 0.312	246.499 \pm 1.181	56.116 \pm 0.690	179.561 \pm 2.248
	%	-32.61 *	-45.87 *	-21.12 *	-44.80 *	-52.62 *	-17.20 *
5 weeks	C	193.727 \pm 0.750	177.770 \pm 0.838	55.686 \pm 0.650	446.692 \pm 1.078	118.452 \pm 0.224	216.982 \pm 0.835
	T	113.757 \pm 0.548	55.661 \pm 1.120	22.870 \pm 0.342	206.901 \pm 1.422	34.091 \pm 0.713	174.437 \pm 0.717
	%	-41.28 *	-68.69 *	-58.93 *	-53.68 *	-71.22 *	-19.61 *
6 weeks	C	194.705 \pm 1.028	176.674 \pm 0.809	55.348 \pm 0.813	445.328 \pm 1.454	118.987 \pm 1.129	217.618 \pm 1.262
	T	93.604 \pm 0.679	45.714 \pm 1.112	16.087 \pm 0.221	198.499 \pm 1.531	46.174 \pm 0.787	93.692 \pm 0.898
	%	-51.93 *	-74.13 *	-70.94 *	-55.43 *	-61.19 *	-56.95 *
7 weeks	C	194.551 \pm 0.970	176.549 \pm 0.744	55.292 \pm 0.847	445.568 \pm 1.396	118.821 \pm 1.018	217.498 \pm 1.334
	T	77.095 \pm 0.626	45.797 \pm 1.410	11.881 \pm 0.217	171.540 \pm 0.374	31.928 \pm 0.321	84.597 \pm 0.181
	%	-60.37 *	-74.06 *	-78.51 *	-61.50 *	-73.13 *	-61.10 *
8 weeks	C	193.811 \pm 0.781	177.560 \pm 0.689	55.580 \pm 0.649	446.555 \pm 1.027	118.436 \pm 0.231	216.865 \pm 0.870
	T	71.952 \pm 0.154	42.002 \pm 0.618	12.180 \pm 0.318	368.192 \pm 4.968	29.623 \pm 0.156	75.653 \pm 0.974
	%	-62.84 *	-76.34 *	-78.09 *	-17.55 *	-74.99 *	-65.12 *

- 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 (2): Effect of chronic oral administration of the allura red (200 mg/ kg bw) on 5-hydroxyindoleacetic acid (5-HIAA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean \pm S.E.	Striatum mean \pm S.E.	Cerebral cortex mean \pm S.E.	Hypothalamus mean \pm S.E.	Brain stem mean \pm S.E.	Hippocampus mean \pm S.E.
1 week	C	101.241 \pm 0.785	112.879 \pm 0.240	30.362 \pm 0.079	183.611 \pm 1.372	81.871 \pm 0.129	151.557 \pm 0.653
	T	95.594 \pm 2.895	95.462 \pm 1.634	25.496 \pm 0.291	179.598 \pm 3.674	77.351 \pm 2.095	134.172 \pm 0.530
	%	-5.58	-15.43 *	-16.03 *	-2.19	-5.52	-11.47 *
2 weeks	C	102.408 \pm 0.576	112.649 \pm 0.572	30.412 \pm 0.138	185.902 \pm 0.864	82.994 \pm 0.553	151.836 \pm 0.621
	T	71.046 \pm 0.758	81.060 \pm 0.850	22.495 \pm 0.190	154.658 \pm 0.818	68.600 \pm 0.192	134.949 \pm 3.764
	%	-30.62 *	-28.04 *	-26.03 *	-16.81 *	-17.34 *	-11.12 *
3 weeks	C	101.814 \pm 0.053	112.823 \pm 0.492	31.174 \pm 0.067	183.361 \pm 0.748	82.443 \pm 0.647	153.695 \pm 0.783
	T	65.217 \pm 0.722	67.890 \pm 0.764	21.577 \pm 0.160	107.446 \pm 0.790	54.831 \pm 0.714	137.953 \pm 2.627
	%	-35.94 *	-39.83 *	-30.78 *	-41.40 *	-33.49 *	-10.24 *
4 weeks	C	102.041 \pm 0.293	112.823 \pm 0.492	30.352 \pm 0.092	185.775 \pm 0.778	82.944 \pm 0.573	151.676 \pm 0.522
	T	60.877 \pm 0.691	61.205 \pm 0.886	23.770 \pm 0.261	43.511 \pm 0.530	28.275 \pm 0.353	129.442 \pm 0.189
	%	-40.34 *	-45.75 *	-21.68 *	-76.58 *	-65.91 *	-14.66 *
5 weeks	C	101.137 \pm 2.138	114.371 \pm 1.037	31.604 \pm 0.285	184.104 \pm 1.209	82.213 \pm 0.719	152.384 \pm 0.738
	T	45.031 \pm 0.694	28.182 \pm 0.844	20.058 \pm 0.148	28.674 \pm 0.197	20.519 \pm 0.205	107.325 \pm 0.416
	%	-55.48 *	-75.36 *	-36.53 *	-84.43 *	-75.04 *	-29.57 *
6 weeks	C	102.908 \pm 1.049	112.149 \pm 0.952	30.579 \pm 0.293	186.069 \pm 0.991	83.161 \pm 0.517	151.836 \pm 0.621
	T	35.887 \pm 0.577	10.874 \pm 0.099	15.279 \pm 0.310	35.303 \pm 0.588	35.937 \pm 0.429	31.855 \pm 0.305
	%	-65.13 *	-90.30 *	-50.03 *	-81.03 *	-56.79 *	-79.02 *
7 weeks	C	100.171 \pm 1.800	113.601 \pm 0.492	31.333 \pm 0.058	183.361 \pm 0.748	81.990 \pm 0.564	151.748 \pm 0.169
	T	21.269 \pm 0.243	11.566 \pm 0.228	11.716 \pm 0.095	19.470 \pm 0.093	22.002 \pm 0.286	22.234 \pm 0.226
	%	-78.77 *	-89.82 *	-62.61 *	-89.38 *	-73.17 *	-85.35 *
8 weeks	C	101.932 \pm 0.451	112.823 \pm 0.492	31.355 \pm 0.164	183.361 \pm 0.748	81.583 \pm 0.552	152.764 \pm 0.488
	T	16.902 \pm 0.414	10.609 \pm 0.095	11.722 \pm 0.236	19.324 \pm 1.972	20.883 \pm 0.405	12.634 \pm 0.622
	%	-83.42 *	-90.60 *	-62.62 *	-89.46 *	-74.40 *	-91.73 *

- 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 of the allura red (200 mg/ kg bw) on gama-aminobutyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean \pm S.E.	Striatum mean \pm S.E.	Cerebral cortex mean \pm S.E.	Hypothalamus mean \pm S.E.	Brain stem mean \pm S.E.	Hippocampus mean \pm S.E.
1 week	C	192.457 \pm 0.799	171.652 \pm 0.450	57.247 \pm 0.385	432.828 \pm 0.319	118.155 \pm 0.197	214.787 \pm 1.321
	T	186.892 \pm 3.904	148.623 \pm 0.540	58.079 \pm 0.369	221.678 \pm 0.344	115.224 \pm 4.719	215.638 \pm 1.244
	%	-2.89	-13.42 *	1.45	-48.78 *	-2.48	0.40
2 weeks	C	192.544 \pm 0.759	171.662 \pm 0.447	57.208 \pm 0.366	432.939 \pm 0.370	117.868 \pm 0.237	214.600 \pm 1.407
	T	171.608 \pm 4.783	145.681 \pm 1.738	47.677 \pm 0.208	220.937 \pm 0.420	102.831 \pm 3.244	211.472 \pm 0.792
	%	-10.87 *	-15.14 *	-16.66 *	-48.97 *	-12.76 *	-1.46
3 weeks	C	193.611 \pm 0.781	175.423 \pm 1.783	57.849 \pm 0.675	437.968 \pm 1.007	118.436 \pm 0.231	216.865 \pm 0.870
	T	172.176 \pm 1.089	139.180 \pm 0.929	45.671 \pm 0.244	218.254 \pm 3.968	102.274 \pm 3.265	194.849 \pm 5.679
	%	-11.07 *	-20.66 *	-21.05 *	-50.17 *	-13.65 *	-10.15 *
4 weeks	C	193.379 \pm 0.440	171.744 \pm 1.615	57.713 \pm 0.935	437.849 \pm 0.198	118.118 \pm 1.398	215.234 \pm 1.053
	T	167.939 \pm 0.984	134.565 \pm 1.081	44.396 \pm 0.232	213.033 \pm 0.793	101.717 \pm 3.267	192.936 \pm 3.171
	%	-13.16 *	-21.65 *	-23.07 *	-51.35 *	-13.89 *	-10.36 *
5 weeks	C	191.573 \pm 3.038	173.106 \pm 2.084	57.865 \pm 0.232	440.913 \pm 2.801	117.905 \pm 0.418	214.923 \pm 1.045
	T	162.239 \pm 0.964	132.210 \pm 0.789	43.538 \pm 0.320	202.083 \pm 0.659	101.160 \pm 3.577	190.859 \pm 0.305
	%	-15.31 *	-23.62 *	-24.76 *	-54.17 *	-14.20 *	-11.20 *
6 weeks	C	193.350 \pm 0.430	171.462 \pm 1.337	57.767 \pm 0.948	437.948 \pm 0.178	118.268 \pm 1.384	214.953 \pm 1.066
	T	155.318 \pm 1.899	117.677 \pm 0.843	42.474 \pm 0.368	198.670 \pm 2.123	100.046 \pm 3.700	191.554 \pm 0.238
	%	-19.67 *	-31.37 *	-26.47 *	-54.64 *	-15.41 *	-10.89 *
7 weeks	C	194.551 \pm 0.970	174.250 \pm 0.873	57.549 \pm 0.881	437.000 \pm 1.369	118.821 \pm 1.018	217.498 \pm 1.334
	T	144.392 \pm 0.912	112.550 \pm 0.474	40.557 \pm 0.219	194.207 \pm 1.352	99.474 \pm 0.391	184.544 \pm 0.669
	%	-25.78 *	-35.41 *	-29.53 *	-55.56 *	-16.28 *	-15.15 *
8 weeks	C	194.705 \pm 1.028	174.279 \pm 0.878	57.580 \pm 0.862	436.683 \pm 1.454	118.987 \pm 1.129	217.285 \pm 1.478
	T	135.631 \pm 0.416	108.405 \pm 0.418	38.217 \pm 0.231	183.121 \pm 0.442	94.424 \pm 0.496	172.692 \pm 0.921
	%	-30.34 *	-37.80 *	-33.63 *	-58.07 *	-20.64 *	-20.52 *

- 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 the allura red (200 mg/ kg bw) on histamine 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	227.528 ± 1.088	197.673 ± 1.008	82.269 ± 0.452	202.614 ± 6.536	236.849 ± 0.696	175.452 ± 0.400
	T	217.788 ± 4.934	190.730 ± 6.362	81.190 ± 0.434	194.497 ± 5.597	212.679 ± 0.243	153.345 ± 1.825
	%	-4.28	-3.51	-1.31	-4.01	-10.21 *	-12.60 *
2 weeks	C	226.458 ± 0.768	198.263 ± 0.868	83.174 ± 1.348	195.152 ± 0.621	231.694 ± 2.743	174.237 ± 0.852
	T	203.565 ± 2.418	164.444 ± 1.405	74.047 ± 0.807	162.529 ± 1.319	186.758 ± 0.244	144.884 ± 0.661
	%	-10.11 *	-17.06 *	-10.97 *	-16.72 *	-19.39 *	-16.85 *
3 weeks	C	225.626 ± 0.521	198.263 ± 0.868	84.434 ± 0.898	196.804 ± 0.188	234.348 ± 1.135	174.737 ± 0.756
	T	198.548 ± 0.292	153.639 ± 0.741	72.189 ± 0.203	142.022 ± 0.528	158.299 ± 0.922	141.905 ± 0.602
	%	-12.00 *	-22.51 *	-14.50 *	-27.84 *	-32.45 *	-18.79 *
4 weeks	C	226.627 ± 1.337	196.860 ± 0.764	83.370 ± 0.973	196.330 ± 0.452	235.350 ± 0.527	175.558 ± 0.704
	T	199.399 ± 0.380	134.101 ± 1.626	71.034 ± 0.371	137.205 ± 1.302	152.418 ± 0.769	140.010 ± 0.932
	%	-12.01 *	-31.88 *	-14.80 *	-30.12 *	-35.24 *	-20.25 *
5 weeks	C	225.321 ± 0.679	198.504 ± 0.742	84.542 ± 0.853	197.121 ± 0.416	234.365 ± 1.140	175.447 ± 0.713
	T	192.460 ± 0.251	117.546 ± 0.568	70.443 ± 0.102	131.900 ± 0.907	148.990 ± 0.641	135.068 ± 0.730
	%	-14.58 *	-40.78 *	-16.68 *	-33.09 *	-36.43 *	-23.02 *
6 weeks	C	226.091 ± 1.075	197.673 ± 1.008	82.375 ± 0.912	195.584 ± 1.450	234.593 ± 0.824	175.431 ± 0.753
	T	184.921 ± 0.502	103.932 ± 0.865	69.474 ± 0.138	116.088 ± 1.845	147.589 ± 1.032	126.852 ± 0.653
	%	-18.21 *	-47.42 *	-15.66 *	-40.65 *	-37.09 *	-27.69 *
7 weeks	C	227.041 ± 0.999	198.326 ± 1.013	82.851 ± 1.008	196.366 ± 1.300	235.075 ± 1.097	176.279 ± 0.878
	T	176.419 ± 0.970	93.262 ± 0.636	59.831 ± 0.289	104.466 ± 0.755	137.448 ± 0.468	114.543 ± 0.733
	%	-22.30 *	-52.98 *	-27.78 *	-46.80 *	-41.53 *	-35.02 *
8 weeks	C	225.154 ± 0.802	199.004 ± 0.706	84.542 ± 0.853	197.121 ± 0.416	235.199 ± 1.593	175.614 ± 0.800
	T	167.927 ± 0.468	85.437 ± 0.687	57.965 ± 0.340	86.081 ± 1.014	132.247 ± 0.358	106.773 ± 0.766
	%	-25.42 *	-57.07 *	-31.44 *	-56.33 *	-43.77 *	-39.20 *

- 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 $p < 0.05$.

3.2. Urea and Creatinin Levels in the serum

Table 5 shows that there are noticeable changes in the content of urea and creatinin in the serum when

they feed by allura red for 4 and 8 weeks. The rising was 101.70% and 115.12% respectively compared to standard.

Table (5): Effect of chronic oral administration of the allura red (200 mg/ kg bw) on kidney function of male albino

Time of decapitation		Urea(mg/dl) mean ± S.E.	creatinine(mg/dl) mean ± S.E.
4 week	C	37.100 ± 0.379	0.810 ± 0.400
	T	72.500 ± 0.764	1.733 ± 0.049
	%	95.42 *	113.99 *
8 weeks	C	37.267 ± 0.481	0.827 ± 0.009
	T	75.167 ± 0.833	1.778 ± 0.005
	%	101.70 *	115.12 *

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test.

% : Percentage of change from control.

3.3. MDA and GSH content in brain and kidney tissue

MDA and GSH levels in brain and kidney in control and experimental groups are shown in the

Table 6. After 8 weeks, MDA and GSH content have significantly reduced. In brain MDA and GSH decreased to 01.29% and -19.12% respectively and in kidney to -11.56% and -13.50%.

Table (6): Effect of chronic oral administration of the allura red (200 mg/ kg bw) on brain and kidney tissue malondialdehyde MDA and glutathione (GSH) levels of male albino rat.

Time of decapitation		MDA(nmol/mg) mean \pm S.E.	GSH(nmol/mg) mean \pm S.E.
brain	C	108.500 \pm 0.428	151.667 \pm 0.494
	T	97.330 \pm 0.365	122.667 \pm 0.760
	%	-10.29 *	-19.12 *
kidney	C	53.333 \pm 0.667	139.500 \pm 0.428
	T	47.167 \pm 0.703	120.667 \pm 0.422
	%	-11.56 *	-13.50 *

- 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 $p < 0.05$.

3.4. Histological changes

Results in figure 1 and 2 (sections 1 and 2) demonstrate the histological structural photo sectors in standard kidney of male albino rat. Results display developed glomerulus(G), Bowman's capsule, proximal and distal convoluted tubules(PT, DT). oral administration of allura red led to abnormal glomerular filtration. renal corpuscles atrophy, in addition several corpuscles and tubular necrosis, deformation and degradation of renal tubules hepatocyte enlargement and distant nuclei and hemorrhage occurs between the walls of the cells and the disappearance of hthall alfrshaaeh border to nearby pipes A clear distortions and decomposition in glomeruli and wallet of Bowman and the widening voids intra portfolio and capillary tuft decomposition Figure 3 and 4 (sections 3 and 4).

4- Discussion

It is a well known fact that humans are significantly exposed to food additives as a constituent of many foods and drinks consumed every day. There is lot of evidence that several kinds of food additives can initiate adverse reactions in the biological systems of humans. When intake Azo dyes, it can reach directly the intestine ingestion or the bile. It may reaction with acid, digestive enzymes, and microflora [4]. Azoreductases and peroxidases reacts with Azo dyes and produce semiquinone radicals and aromatic amines. These products generated superoxide radicals, hydroxyl radicals, and H₂O₂, which maybe weakens cellular defense and caused oxidative stress-related disorders [3].

Resulted in this study show that daily oral administration of allura red leads to noticeable decreasing in the content of serotonin, 5-hydroxyindoleacetic acid, gamma-aminobutyric acid and histamine in all the examined areas at different

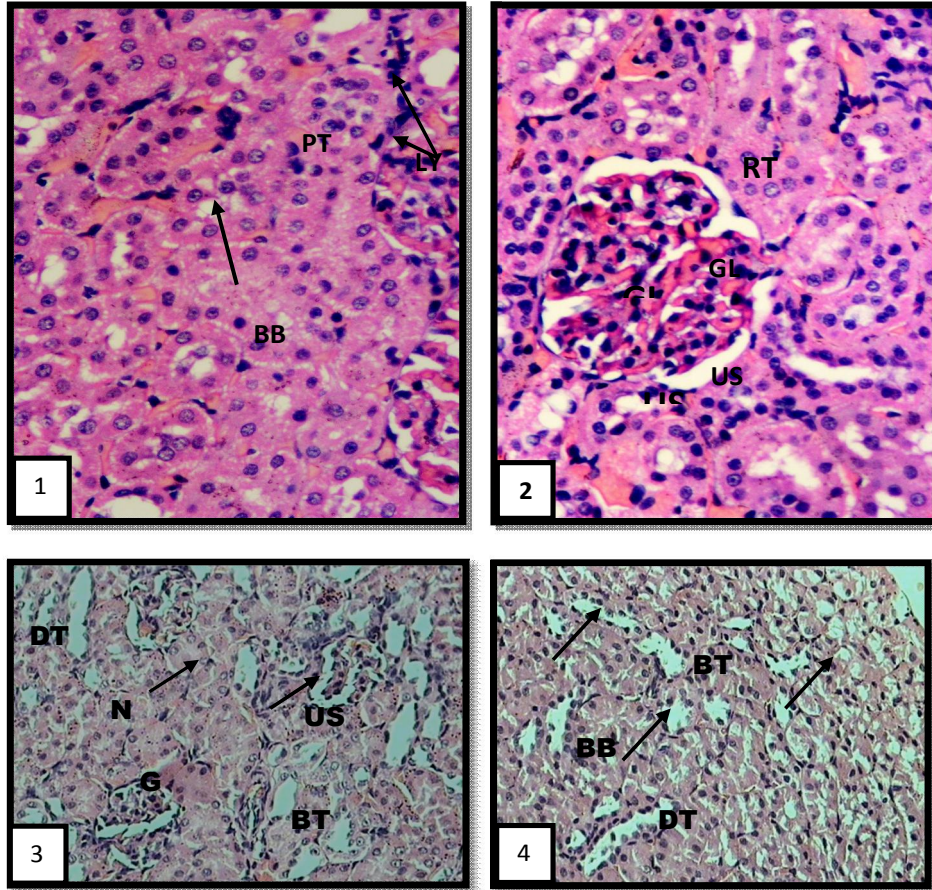
time. The decreases in neurotransmitter may explain due to free radicals that generated when azo dyes metabolized by intestinal bacteria[4]. These free radicals inhibited formation of Adenosine triphosphate ATP that cause reduces synthesis or re-uptake of neurotransmitter in the presynaptic cell [20].

MDA and GSH plays an important role intestines. They protect the intestines from oxidative damage that caused from free radicals in artificial colure food [22]. Reduction of MDA and GSH caused at oxidative risks in cells. This study found changes in MDA and GSH contents in examined tissues. Allura red caused reduce in the content of MDA and GSH in brain and kidney. This may explained by free redical resulted from azo dyes. In brain, these free radical inhibited endogenous antioxidant defense enzymes then caused brain tissue damage. Results were confirmed previous study that show the combinations of high consuming of tartrazine and brilliant blue may negatively impact on developmental and adult hippocampal region in nervous system [21]. In addition, in vitro and in vivo studies using tartrazine show significantly reduced GSH levels that caused by azo dyes. Study was concluded that azo dyes may have responsible for the endotoxic and carcinogenic effects [23]. In Kidney, incubation of hepatocytes with aromatic amines generated by azo dyes caused a reduce in the mitochondrial membrane potential and followed with cytotoxicity ensued [24-25].

Serum creatinine and urea has been increased significantly due to daily oral admistartion of allura red in rat through two months. This result confirmed previous study that noticed a significant changes in serum creatinine and urea content when consumed Fast Green and ingested tartrazine [26][4]. Moreover, renal dysfunction reported due to an increase content of urea or creatinine in the plasma [27] [25].

The histopathologic result of renal section indicated that renal tubules had deformed and degraded. The deformation cause cirrhosis in cells peel and necrosis in the pipe walls and and alternate

the shape of the tube cells. These data consist with previous study that showed tubules degraded when used tartrazine at doses for 90 days(10 mg/Kg/day) [28].



Control group:

Fig.1: Cross section in the cortex and renal tubules shows the appearance of the border in proximal tubule(arrow) distal tubule X400 (H & E)

Fig. 2: Cross section shows installation of the glomerulus and Bowman wallet natural clearly visible strands of capillaries for filtering urine from the blood arrow (H & E) X400

treatment group

Fig.3: Cross section in the area of crust shows deformation and degradation of renal tubules and the severity of the deformity leading to cirrhosis in cells Peel and necrosis in the pipe walls and clear change in shape of the tube and the integration of cells and causing hemorrhage between the walls of the cells (arrow) (H E &) X400

Fig.4: Cross section in cortex shows deformation and degradation of renal tubules hepatocyte enlargement and distant nuclei and hemorrhage occurs between the walls of the cells and the disappearance of hthall alfrshaah border to nearby pipes A clear distortions and decomposition in glomeruli and wallet of Bowman and the widening voids intra portfolio and capillary tuft decomposition(arrow) (H E &) X400

6. Conclusion and Recommendations

Food colours are added commonly in food products. The influence of consumed Allura red was examined in this study. Allura red has adverse impact on some neurotransmitter in brain and biochemical marker contents of kidney and brain tissue based on consumption period, level of dose. Allura red causes depletion antioxidant defence. Further studies may need to clarify the causes and the mechanisms of allura red reactions. In addition, consumer awareness regarding the effects of this dyes was recommended. Moreover, the type and concentration of each material added to food should be mentioned to prevent a variety of disorders beginning by weaker defence system and tissue damage.

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