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Full Length Research Paper

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

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The present study aimed to investigate the effect of chronic administration of chocolate brown HT or chocolate brown HT with oil on norepinephrine (NE), dopamine (DA) and gamm-aminobutyric acid (GABA) contents in different brain areas (cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus) and liver and kidney functions of male albino rats. In addition, histopathological examinations of the liver and kidney of the male albino rats were carried out. The results show that chronic oral administration of chocolate brown HT (200 mg/kg body weight (b.wt.)) caused a significant decrease in the content of norepinephrine, dopamine and gamm-aminobutyric acid in all the tested regions. This may be attributed to the inhibition of ATP formation leading to decreased synthesis or re-uptake of NE, DA and GABA in the presynaptic cell. Chronic oral administration of chocolate brown HT (200 mg/kg b.wt.) with olive oil caused no significant change in the total content of norepinephrine, dopamine and gamm-aminobutyric acid in all the tested brain areas at different time intervals. The results also revealed that the urea content and level of aspartate aminotransferase (AST) and alkaline phosphatase significantly increased while alanine aminotransferase (ALT) and creatinine significantly decreased in chocolate brown HT treated rat. On the other hand, administration of chocolate brown HT in combination with olive oil resulted in the amelioration of the functional analysis of the brain, liver and renal tissues which were confirmed by histological observation of the hepatic and renal tissues. Finally, it was concluded that olive oil ameliorated the unusual findings of chronic administration of chocolate brown HT due to its antioxidant capacity of capturing free radicals.

Key words: Chocolate brown HT, olive oil, NE, DA, 5-HT, GABA, brain regions, male albino rats.

INTRODUCTION

Chocolate brown HT (CI (1975) No. 20285) is the disodium salt of 4,4-[(2,4-dihydroxy-5-(hydroxymethyl-1,3-phenylenebisazo)di(naphthalenel- sulphonic acid). This is used in ice-cream, soft drinks, piddles and sauces, fish and meat spreads, dessert mixes, sugar confectionery, flour confectionery and preservatives (Food Additives and Contaminants Committee, 1979). The coloring material is approved by Mangham et al. (1987) who reported no toxicological findings except delayed sternbrae and abnormal caecum weight of rat pups. Administration of Chocolate brown HT (purity minimal 85%) to Proton strain rats at levels of 0.5, 1.0 and 2.0% for a period of 12 weeks was found to reveal no adverse effect except liver and renal function of increased urinary glutamic-oxaloacetic transaminases activity. In the gross and histological studies, brown pigmentation was evident in the Kupffer cells of the liver, the proximal convoluted tubules of the kidney and the lymph nodes especially of the small intestine. The intensity of this effect was proportional to the dietary level administered and the pigment was only very occasionally present, and in trace amounts, in animals of the 0.5%

group. However, no adverse effects were detected (Hall and Lee, 1966).

Feeding rats with Chocolate brown HT at dietary levels of 0.0, 0.02, 0.06, 0.20, 0.60, 1.0 and 2% for 90 days was found to reveal no adverse effects except slight growth retardation observed in males at the 1 and 2% levels and in females the highest level became significant. Haematological examination revealed slight but significant decreases in haemoglobin, red cell count and haematocrit in male rats on the highest dietary level. In the biochemical studies, reductions in the blood urea levels occurred in both sexes and were significant in all groups except at the 0.06 and 0.6% levels (Chambers et al., 1966).

Oxidative stress is responsible for many damaging processes in the body which can lead to several types of diseases, such as atherosclerosis, cancer and neurodegenerative diseases. Ageing is also a result of oxidative stress, in the form of mitochondrial damage caused by oxidative injury (Pérez-Jiménez et al., 2005). The definition of oxidative stress can be referred to as “an imbalance between the antioxidant and oxidant systems in the body favoring the oxidant system” (Fitó et al., 2007).

Since several beneficial health effects from olive oil cannot be found in seed oils, which also contain high amounts of oleic acid but limited amounts of micronutrient (majority lost during the refinement of seed oils) gives an increasing interest of the effects of micronutrients in olive oil. Virgin olive oil (VOO) contains more micronutrients (Annunziata et al., 2007). With increased longevity, a number of related pathologies are becoming a current problem. Although a diet high in VOO has been associated with a reduced risk of age-related cognitive decline. The mono unsaturated fatty acid (MUFA) is present in a higher degree in VOO than in other oils decrease the levels of free radicals thereby reducing the damage caused by oxidative stress (Pérez-Jiménez et al., 2005). If one could decrease oxidative stress in the body, the rate of ageing would slow down and the incidents of the related diseases would decrease (Fitó et al., 2007).

Since olive oil is a wild oil commonly available in Saudi Arabia and especially in the Mediterranean and its leaves are used in folk medicine for treatment, the aim of this study was to evaluate a chronic effect of Chocolate brown HT with olive oil on norepinephrine (NE), dopamine (DA), serotonin (5-HT) and gamm-aminobutyric acid (GABA) contents in different brain areas, biochemical and histological structure of liver and kidney of male albino rats.

MATERIALS AND METHODS

Animals

The experimental animals used in this study were male albino of the Wistar strain rats, *Rattus norvegicus* (90 to 100 g). They were supplied with food and water *ad libitum* under standard conditions of light, humidity and temperature (22 to 25°C).

Chocolate brown HT

Brown HT or Chocolate Brown HT (CI(1975) NO.20285) is the disodium salt of 4,4-[(2,4-dihydroxy-5-(hydroxymethyl-1,3-phenylenebisazo)di(naphthalenel-sulphonic acid). Banned in Denmark, Belgium, France, Germany, Switzerland, Sweden, Austria, USA, Norway.

Olive oil

It is an oil obtained from the olive (*Olea europaea*; family Oleaceae), a traditional tree crop of the Mediterranean Basin. It is commonly used in cooking, cosmetics, pharmaceuticals, and soaps and as a fuel for traditional oil lamps.

Methods

The effect of Chocolate brown HT and olive oil on different brain regions of male albino rats

The animals were randomly divided into 3 groups. The first group (n=6) treated with saline vehicle was killed at the beginning of the experiment and this served as the control. The second group (n=24) was normal rats orally administered with Chocolate brown HT (200 mg/kg b.wt.) through gastric tube for 4 weeks. The third group (n=24) was normal rats orally administered with olive oil (0.75 ml/kg) and Chocolate brown HT through gastric tube for 4 weeks (Luciane et al., 2006). The rat was killed by sudden decapitation at the designed period. The brain was rapidly and carefully excised and then dissected on dry ice glass plate according to the method of Glowinski and Iversen (1966) into the following regions: cerebellum, striatum, cerebral cortex, hypothalamus, brain steam and hippocampus. The brain tissues were wiped dry with a filter paper, weighed, wrapped in plastic films and then in aluminum foil and quickly frozen in dry ice pending analysis. NE, DA and 5-HT were estimated according to the method of Ciarlone (1978). GABA was estimated according to the method of Sutton and Simmodes (1973). The fluorescence was measured in Jenway 6200 fluorometer.

The effect of Chocolate brown HT and olive oil on liver and kidney function of male albino rats

Blood sampling: The portion of blood samples were collected and allowed to coagulate at room temperature; EDTA (ethylene diamine tetracetic acid) was added to the other portion of blood and centrifuged at 3000 r.p.m. for 30 min. The clear, non-haemolysed supernatant sera and plasma were quickly removed and divided into four portions for each animal, and stored at -20°C for subsequent analysis for the measurement of AST, ALT and Alkaline phosphatase in the liver, urea and creatinine in kindey (Christic and Michelson, 1975).

The effect of Chocolate brown HT and olive oil on histological structures of liver and kindey of male albino rats

After the sacrifice of animals, part of the liver and kidney tissues from each animal from treated and control was removed and immersed in 10% buffered formalin solution. Each part of the liver and kidney tissues were kept in separate numbered small glass bottles and then embedded in paraffin, and sectioned. Four sections (5 microns in thickness) were taken from each liver and kidney tissues, each section being at a distance of at least 500u from the proceeding, one sections were stained with haematoxylin and eosin (Harris, 1900).

Statistical analysis

Values reported were means ± SE (n = 6). The results were statistically analyzed using the Student's t-test (Hill, 1971) for unpaired data, with P value of less than 0.05 considered significant.

RESULTS

The present results in Table 1 showed that the daily oral administration of exhibited Chocolate brown HT (200 mg/kg b.wt.) caused a significant decrease in norepinephrine content in all of tested areas after 2, 3 and 4 week. The maximal decrease ($p<0.001$) in norepinephrine content was found in the cerebellum, striatum, brain stem and hippocampus at 4 weeks (55.83, 80.33, -73.39 and- 69.14, respectively). As shown in Table 2, the daily oral administration of Chocolate brown HT (200 mg/kg b.wt.) was significantly decrease in dopamine content starting from the second, third and fourth week in cerebellum, striatum, cerebral cortex, hypothalamus, brain stem and hippocampus. The maximal decrease ($p<0.001$) in dopamine content was found in the striatum, hypothalamus and brain stem at 4 weeks (-59.37, -59.81 and -51.88, respectively). Also, Table 3 shows that the daily oral administration of Chocolate brown HT (200 mg/kg b.wt.) caused a significant decrease in gamma-butyric acid content starting from the second to fourth week in striatum, cerebral cortex, hypothalamus, brain stem and hippocampus. The maximal decrease in gamma-butyric acid content was found in the striatum and hypothalamus after 4 weeks (-59.44 and -56.49, respectively) while the daily oral administration of Chocolate brown HT (200 mg/kg b.wt.) caused a significant ($p<0.05$) decrease in serotonin content starting from the third and four week in all brain area till the end of the experiment. The maximal decrease ($p<0.001$) in serotonin content was found in the Striatum and hypothalamus after 4 weeks (44.15 and 30.75%) (Table 4).

The present results from Table 5 showed that the daily oral administration of olive oil and Chocolate brown HT (50 mg/kg b.wt.) caused a significant decrease in norepinephrine content in all tested areas after 2, 3 and 4 week. The maximal decrease ($p<0.05$) in norepinephrine content was found in the cerebellum, striatum, brain stem and hippocampus at 4 weeks (-29.59, -11.37, -54.68 and-8.46, respectively).

As shown in Table 6, the daily oral administration of olive oil and Chocolate brown HT (50 mg/kg b.wt.) caused a significant increase in dopamine content starting from the third and fourth week in hypothalamus and brain stem. The maximal increase ($p<0.001$) in dopamine content found in hypothalamus at 4 weeks was 152.96. Also, Table 7 shows that the daily oral administration of olive oil and Chocolate brown HT (200 mg/kg b.wt.) caused a significant increase in gamma-butyric acid content starting from the second to fourth week in striatum and low decrease in other areas of the brain. The maximal increase in gamma-butyric acid content was found in the cerebellum after 4 weeks (72.69), while the daily oral administration of olive oil and Chocolate brown HT (200 mg/kg b.wt.) caused a significant ($p<0.001$) increase in serotonin content starting from the third and fourth week in the cerebellum and less decrease in other brain. The maximal increase ($p<0.001$) in serotonin content was found in the cerebellum after 4 weeks (38.09) (Table 8).

The results (Table 9) show that there are significant ($P<0.001$) elevations in the level of aspartate aminotransferase (AST) and alkaline phosphatase in the serum of male albino rat treated with Chocolate brown HT (200 mg/kg b.wt.) for 4 weeks. This increase was 678.11 and80.47%, respectively when compared with the control. Also, there was significant ($P<0.05$) decrease in the level of alanine aminotransferase (ALT) in the serum of male albino rat. Urea content in serum of male albino rat treated with olive oil (Table 5) significantly ($P<0.01$) increase than in the untreated control rat with percentage 192% ($P<0.01$) while creatinine significantly ($P<0.05$) increase in treated rate.

The results in Table 10 showed that there were significant ($P<0.001$) elevations in the level of aspartate aminotransferase (AST) and alkaline phosphatase in the serum of male albino rat treated with olive oil and chocolate brown HT (200 mg/kg b.wt.) for 4 weeks. This increase was 444.46 and 113.19%, respectively when compared with the control. Also, significant ($P<0.05$) increase in the level of alanine aminotransferase (ALT) in the serum of male albino rat was noticed. Urea content in the serum of male albino rat treated with olive oil and chocolate brown HT was significantly ($P<0.01$) increase than in the untreated control rat with percentage 84% ($P<0.01$) while creatinine significantly ($P<0.05$) decrease in treated rate.

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 (S) which contain cells Kupffer (KC).

Oral administration of Chocolate brown HT led to minor alteration of hepatic blood vessel and slight collections of leukocytes in between blood sinusoids (Figure 1 (sections 3 and 4)). Administration of Chocolate brown HT in combination with olive oil ameliorated the histological findings compared with the control (Figure 1: sections 5 and 6).

The present results showed that Figure 2 (section 1) histological structural picture of the sector in the renal tissues control sample shows the renal glomerulus (GL) and the void urine (US) and intertubular urinary (RT) and Figure 2 (section 2) shows picture of the sector in the

Table 1. Effect of chronic oral administration of Chocolate brown HT colour (200 mg/kg b.wt.) 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.
	C	95.38±0.58	511.47±1.8	56.20±0.23	595.99±3.2	390.05±0.83	292.54±1.54
1 week	T	75.90±0.58	513.01±1.04	56.54±0.76	604.94±1.58	386.17±1.88	292.65±0.54
	%	0.54	0.30	0.60	1.33	1.0	0.04
	C	95.36±86	511.12±1.65	54.45±1.90	605.33±9.49	390.49±0.48	292.53±1.53
2 week	T	60.54±6.7	98.5±1.7	41.67±0.75	487.33±1.67	103±0.48	100±0.63
	%	36.52	80.7	23.47	19.49	73.6*	65.82*
	C	98.69±0.27	495.65±1.4	55.49±0.11	604.91±2.34	394.49±0.94	283.18±0.82
3 week	T	47.5±0.76	105.33±0.42	42.67±0.67	456.83±1.01	152.33±0.88	102.83±0.94
	%	51.87	78.75	23.11	24.48	61.38*	63.69
	C	98.49±0.27	495.78±1.4	55.52±0.13	606.83±2.26	394.62±0.94	282.99±0.84
4 week	T	43.5±0.75	97.5±0.76	40.80±0.87	402.7±1.1	105±1.39	87.33±0.72
	%	55.83	80.33	26.53	33.4*	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.

Table 2. Effect of chronic oral administration of Chocolate brown HT (50 mg/kg b.wt.) 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.
	C	146.76±0.82	473.98±0.86	60.49±0.04	734.22±2.11	451.28±0.63	241.15±0.86
1 week	T	145.63±0.54	476.25.±1.3	60.49±0.74	726.24±3.55	453.98±1.8	241.43±0.66
	%	-0.77	0.49	0.15	-1.09	0.60	-0.71
	C	145.64±0.91	482.3±3.34	61.24±0.21	739.24±4.31	451.54±1.95	244.59±1.45
2 week	T	123.64±0.76	401.5±0.56	53.33±0.67	552.83±0.95	400.83±0.40	199.33±0.33
	%	15.21	16.76	12.91	25.22	11.23	18.51
	C	146.97±0.94	474.12±0.91	60.72±0.26	734.06±2.26	451.61±0.59	242.96±0.84
3 week	T	122.17±0.87	222.83±0.94	53.76±0.51	343.17±0.80	215.83±0.48	156.17±0.40
	%	16.88	53	11.45	51.9	52.21	35.73
	C	146.10±1.16	482.78±3.19	62.328±0.95	738.22±4.44	451.63±1.98	244.91±1.54
4 week	T	117.10±1.8	196.17±0.91	50.2±0.32	296.67±0.67	217.33±0.84	157.33±0.56
	%	19.81	59.37	19.45	59.81	51.88	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 of Chocolate brown HT (50 mg/kg b.wt.) on gamma-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.
	C	192.46±0.8	171.652 ± 0.450	57.24±0.385	432.828±0.319	118.155±0.197	214.933±1.269
1 week	T	192.35±0.54	171.688±1.024	57.919±0.149	431.393±2.515	116.809±0.958	110.333±0.422
	%	0.06	0.02	1.17	0.33	1.14	48.67 *
	C	192.55±0.8	171.66 2± 0.447	57.374±0.463	432.939±0.370	117.868±0.237	216.865±0.870
2 week	T	137.83±0.31	99.667± 0.333	41.833±1.740	253.500±0.764	83.333±0.760	85.500±0.563
	%	28.41	41.94 *	27.09 *	41.45 *	29.30 *	60.57 *
	C	193.6±0.78	175.423±1.783	57.849±0.675	437.968±1.007	118.436±0.231	215.234±1.053
3 week	T	142.83±0.95	92.333± 0.843	40.833±0.401	202.333±0.843	72.500±0.764	65.667±0.333
	%	26.23	47.37 *	29.41 *	53.80 *	38.79 *	69.49 *
	C	193.38±0.34	171.744±1.615	57.713±0.935	437.849±0.198	118.118±1.398	214.933±1.269
4 week	T	140.5±0.34	69.667±12.341	34.000±0.816	190.500±0.342	65.667±0.211	110.333±0.422
	%	27.34	59.44 *	41.09 *	56.49 *	44.41 *	48.67 *

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 oral of Chocolate brown HT (50 mg/kg b.wt.) 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.
	C	171.652±0.450	192.457±0.799	432.828±0.319	57.247±0.385	214.787±1.321	118.155±0.197
1 week	T	168.301±1.634	182.756±4.869	431.746±1.004	56.723±0.267	213.043±0.298	117.798±0.163
	%	1.95	5.04	0.25	0.92	0.81	0.30
	C	171.496±0.522	193.045±0.719	433.106±0.485	57.374±0.463	215.100±1.229	118.368±0.364
2 week	T	154.334±2.666	182.925±4.810	432.680±0.413	54.049±1.950	204.858±4.789	106.093±0.069
	%	10.01 *	5.24	0.10	5.80	4.76	10.37 *
	C	173.444±1.705	192.326v0.203	430.635±0.928	57.287±0.176	216.757±0.943	117.401±0.079
3 week	T	152.288±1.447	170.018±7.740	429.894±3.439	53.405±2.007	208.125±4.029	104.945±0.712
	%	12.20 *	11.60 *	0.17	6.77	3.98	10.61 *
	C	173.669±1.772	192.276±0.075	430.951±0.267	57.771±0.023	215.868±1.275	118.248±0.380
4 week	T	145.679±0.781	107.381±0.313	385.847±5.326	40.005±0.195	192.869±1.830	92.950±0.584
	%	16.12 *	44.15 *	10.47 *	30.75 *	10.65 *	21.39 *

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 olive oil and Chocolate brown 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.
	C	54.443±1.898	511.118±1.648	95.358±0.857	292.540±1.536	390.490±0.484	596.997±3.242
1 week	T	38.333±1.054	453.000±0.966	74.500±.922	73.833±0.872	88.167±0.601	422.167±0.601
	%	29.59 *	11.37 *	21.87 *	74.76 *	77.42 *	29.28 *
	C	55.493±0.105	495.653±1.445	98.688±0.274	292.527±1.531	390.490±0.484	605.330±9.485
2 week	T	51.833±0.872	454.167±1.302	98.182±0.341	83.833±1.302	101.000±0.516	451.833±0.872
	%	6.60*	8.37*	0.51	71.34*	74.14 *	25.36 *
	C	55.525±0.127	495.780±1.443	98.485±0.271	283.178±0.817	394.485±0.942	604.906±2.337
3 week	T	55.195±0.163	456.333±1.174	98.182±0.341	129.000±0.365	132.333±0.760	502.667±0.843
	%	0.59	7.96 *	0.31	54.45 *	66.45 *	-16.90 *
	C	54.443±1.898	511.118±1.648	95.358±0.857	282.998±0.841	394.618±0.944	604.623±2.261
4 week	T	38.333±1.054	453.000±0.966	74.500±0.922	203.500±0.847	178.833±0.477	553.500±0.764
	%	29.59 *	11.37 *	21.87 *	28.09 *	54.68 *	8.46 *

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 olive oil and Chocolate brown 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.
	C	473.948± 0.856	146.755±0.818	734.223 ± 2.111	60.488 ± 0.044	243.147 ± 0.863	451.288 ± 0.633
1 week	T	395.167± 0.477	112.167±0.601	402.833 ± 0.477	33.500 ± 0.764	152.500 ± 0.764	291.667 ± 0.494
	%	16.62 *	23.57 *	45.13 *	44.62 *	37.28 *	35.37 *
	C	482.312± 3.336	145.648±0.914	739.237 ± 4.314	61.240 ± 0.214	244.597 ± 1.448	451.541 ± 1.947
2 week	T	405.667± 0.667	97.000±0.365	551.333 ± 0.558	73.000 ± 0.730	204.000 ± 0.856	321.500 ± 0.428
	%	15.89 *	33.40 *	25.42 *	-19.20 *	16.60 *	28.80 *
	C	474.115± 0.911	146.977±0.942	734.057 ± 2.258	60.715 ± 0.259	242.968 ± 0.843	451.606 ± 0.591
3 week	T	449.167± 0.703	137.333±0.558	555.833 ± 0.601	122.667 ± 0.760	345.167 ± 0.307	446.500 ± 0.563
	%	5.26 *	6.56 *	24.28 *	-102.04 *	-42.06 *	1.13 *
	C	482.780± 3.194	146.100±1.156	738.215 ± 4.439	62.328 ± 0.946	244.905 ± 1.544	451.637 ± 1.987
4 week	T	450.500± 1.057	140.667±0.333	557.667 ± 0.882	157.667 ± 0.211	302.333 ± 0.760	446.667 ± 0.494
	%	6.69	3.72 *	24.46 *	-152.96 ***	-23.45 *	1.10

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 of olive oil and Chocolate brown on gama-butyric acid (GABA) content in the different 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.
	C	57.247± 0.385	171.652± 0.450	192.457± 0.799	214.787± 1.321	118.155± 0.197	432.828± 0.319
1 week	T	51.000± 0.516	108.833± 0.477	125.167± 1.400	153.500± 0.764	76.000± 0.365	252.167± 0.703
	%	10.91 *	36.60 *	34.96 *	28.53 *	35.68 *	41.74
	C	57.374± 0.463	171.662± 0.447	192.544± 0.759	214.933± 1.269	117.868± 0.237	432.939± 0.370
2 week	T	68.667± 0.422	145.833± 0.401	132.667±.615	154.333± 0.333	85.667± 0.494	303.167± 0.792
	%	-19.68 *	15.05 *	31.10 *	28.19 *	27.32 *	29.97 *
	C	57.849± 0.675	175.423± 1.783	193.611± 0.781	216.865± 0.870	118.436± 0.231	437.968± 1.007
3 week	T	84.667± 0.422	171.167± 0.703	146.333± 0.494	181.500± 5.530	114.000± 0.856	401.500± 0.563
	%	-46.36 *	2.43	24.42 *	16.31 *	3.75	8.33 *
	C	57.713± 0.935	171.744± 1.615	193.379± 0.440	215.234± 1.053	118.118± 1.398	437.849± 0.198
4 week	T	99.667± 0.333	171.167± 0.703	153.333± 0.760	194.500± 1.607	139.167± 0.601	352.500± 0.764
	%	-72.69 *	0.34	20.71 *	9.63 *	-17.82 *	19.49 *

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 oral of olive oil and Chocolate brown 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.
	C	57.247±0.385	171.652±0.450	192.457±0.799	214.787v1.321	118.155±0.197	432.828±0.319
1 week	T	56.485±0.302	169.082±1.528	189.587±1.744	211.215±1.931	116.453±0.890	429.482±2.424
	%	1.33	1.50	1.49	1.66	1.44	0.77
	C	56.594±1.008	170.315±1.639	191.029±0.352	213.653±0.436	118.123±0.223	433.014±1.290
2 week	T	77.833±0.946	151.333±0.494	212.833±0.946	203.000±0.856	126.167±0.307	197.833±0.401
	%	-37.53 *	11.14 *	-11.41 *	4.99 *	-6.81 *	54.31 *
	C	57.604±0.246	173.099±1.479	191.972±0.481	214.949±0.886	117.983±0.607	433.014±0.404
3 week	T	67.167±0.307	149.000±0.683	162.947±1.831	173.667±0.803	126.167±0.307	170.333±0.494
	%	-16.60 *	13.92 *	15.12 *	-19.21 *	-6.94 *	60.66 *
	C	58.055±0.382	173.099±1.479	192.856±0.299	217.158±0.610	117.865±0.257	431.432±1.369
4 week	T	80.167±0.307	184.000±0.816	250.667±0.333	183.667±0.803	134.000±0.516	170.333±0.494
	%	-38.09 *	-6.30 *	-29.98 *	15.42 *	-13.69 *	60.52 *

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

Table 9. Effect of chronic oral administration of Chocolate brown HT (50 mg/kg b.wt.) on Liver and kidney 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.	Urea (mg/dl) mean ± S.E.	Creatinine (mg/dl) mean ± S.E.
2 week	C	60.488±0.044	78.333±0.667	99.833±0.543	0.417±0.031	97.333±0.494
	T	470.167±0.307	69.167±0.307	179.000±0.365	1.000±0.043	123.167±0.477
	%	-677.28***	11.70 *	-79.30 **	-140.00 **	-26.54 *
4 week	C	60.488±0.044	78.333±0.667	99.833±0.543	0.417±0.031	97.333±0.494
	T	470.667±0.333	70.000±0.365	180.167±0.654	1.217±0.048	124.000±0.365
	%	-678.11 ***	10.64 *	-80.47 **	-192.00 **	-27.40 *

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 of olive oil and Chocolate brown HT (50mg/kg b.wt.) on Liver and kidney 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.	Urea (mg/dl) mean ± S.E.	Creatinine (mg/dl) mean ± S.E.
2 week	C	60.488±0.044	78.333±0.667	99.833±0.543	0.417±0.031	97.333±0.494
	T	328.333±0.333	205.333±0.422	210.333±0.422	0.767±0.021	62.167±0.307
	%	-442.80 *	-162.13 *	-110.68 *	-84.00 *	36.13 *
4 week	C	60.488±0.044	78.333±0.667	99.833±0.543	0.417±0.031	97.333±0.494
	T	329.333±0.667	206.333±0.494	212.833±0.703	0.767±0.033	63.167±0.307
	%	-444.46 *	-163.40 *	-113.19 *	-84.00 *	35.10 *

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.

renal tissues control sample intertubular wrapped nearby (PT) and the edge Alfaragonip (BB) (shares) and intertubular wrapped remote (DT) also notes lymphocytes (Ly) and fibrous tissue interface.

In renal tissues, Chocolate brown HT-treatment possessed stricking alterations in renal tissues characterized by lobulated glomeruli, mild interstitial leukocyte infiltration, light hyaline deposits within few renal tubules and mild erosion of epithelial cells lining the tubules (Figure 2; sections 3 and 4).

However, administration of Chocolate brown HT in combination with olive oil ameliorated the renal tissues compared with the control (Figure 2; sections 5 and 6).

DISCUSSION

The present findings revealed that chronic oral administration of Chocolate brown HT (200 mg/kg b.wt.) caused a significant decrease in the content of norepinephrine, dopamine and gamma-aminobutyric acid in all the tested areas at different time. Chronic oral administration of Chocolate brown HT caused the inhibition of ATP formation leading to decreased synthesis or re-uptake of NE, DA and GABA in the presynaptic cell. The brain is highly susceptible to oxidative stress due to its enrichment with non-heme iron that is catalytically involved in the production of free radicals (Yousuf et al., 2005). The toxicology of this colouring has been reviewed in detail by Mangham et al. (1987). Chronic oral administration of Chocolate brown HT (200 mg/kg b.wt.) with Olive oil caused no significant change in the total content of norepinephrine, dopamine and gamma-aminobutyric acid in all the tested brain areas at different time intervals. The present study showed that the Chocolate brown HT chronic oral administered olive oil may prevent -induced testes toxicity in mice.

The beneficial health effects of olive oil are due to its high content of antioxidative substances, as well as its high content of monounsaturated fatty acids, that is., oleic acid (Pérez-Jiménez et al., 2007). Oleic acid andand linoleic acid (Olive oil) induced increases in free intracellular calcium concentrations Ca2+ by recruiting calcium from endoplasmic reticulum pool via instill 1,4,5-triphosphate production followed by calcium influx via opening of store-operated calcium channels. Oleic acid and linoleic acid also induced phosphorylation of Src-protein-tyrosine kinases, particularly of Fyn59 and Yes62. Linoleic acid -evoked phosphorylation of Fyn59 and Yes62 was implicated in the activation of SOC channels.

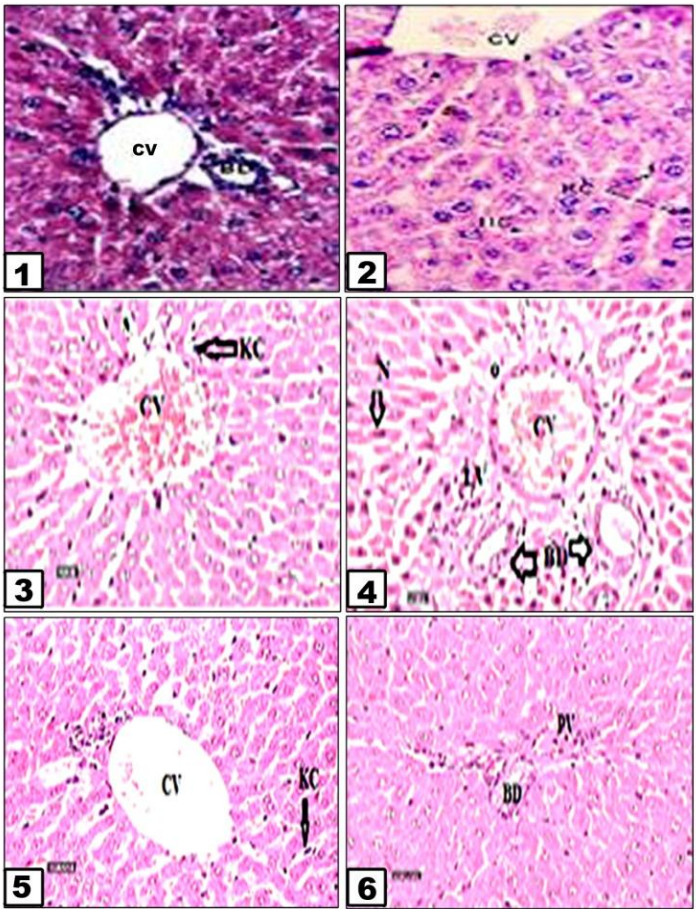

Figure 1. Photomicrographs of histological sections of liver of Wistar rat.
Sections 1 and 2 is the control showing normal hepatocytes and hepatic strands; sections 3 and 4, chocolate brown HT-treatment showing congested blood vessels and grouping foci of leukocytes; sections 5 and 6 Chocolate brown HT and olive oil-treatment showing amelioration of hepatic picture.

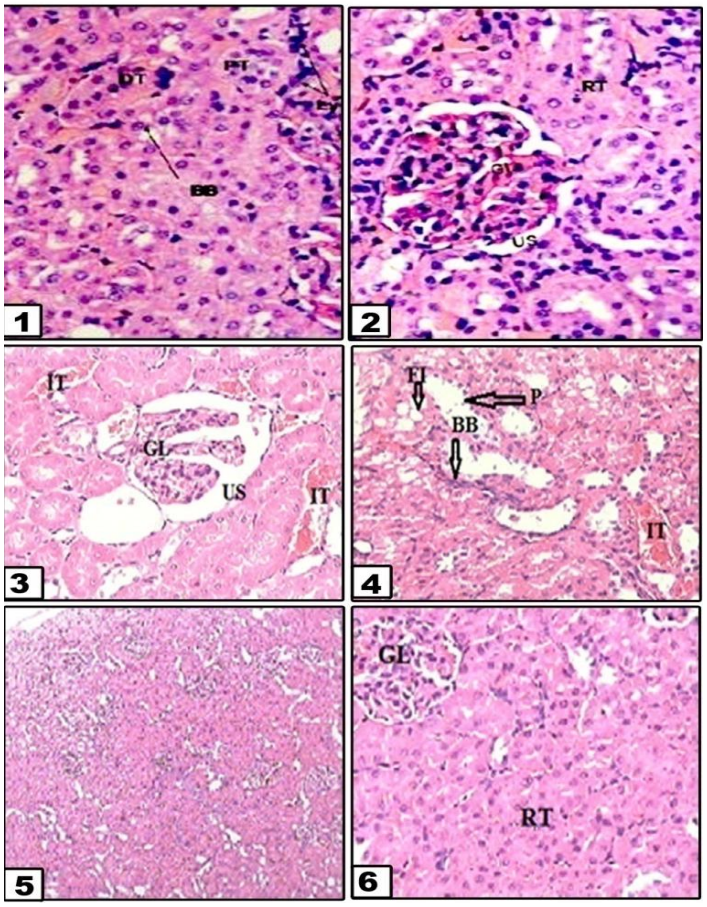

Figure 2. Photomicrographs of histological sections of renal tissue of Wistar rat.
Sections 1 and 2 is the control showing normal glomeruli and renal tubules; sections 3 and 4, Chocolate brown HT-treatment showing lobulated glomeruli, mild interstitial leukocytes infiltration and deposition of red deposits within lumina of renal tubules; sections 5 and 6, Chocolate brown HT and olive oil-treatment showing amelioration of renal picture.

Osawa et al. (1990) reported that free radical-induced oxidative damage can be effectively protected by use of various dietary antioxidants. However, not many studies focused on neuroprotective effect of dietary oils have reported that coenzyme Q (CoQ) concentration in mitochondria depends on various situations, both endogenous and exogenous, both physiological and pathological. CoQ concentration depends on a balance between 'inputs' and 'outputs'. With regard to 'inputs', CoQ levels are determined by the endogenous synthesis of CoQ and by the supply of this component through the usual diet; regarding the 'output', the most relevant ones are those caused by oxidative stress and by cellular metabolism itself with particular regard to energy production (Lenaz et al., 1990). Experimental studies in animals have shown that diets lacking olive oil might lead to substantial disturbances in neural function, which in most circumstances can be restored by the inclusion of olive oil in the diet. Olive oil may potentially be safe for use as a sedative drug to improve the central nervous system and also led to the reductions in the risk of Alzheimer's and Parkinson's diseases. The olive oil could be used as a protector against Alzheimer's and Albarkson a result of the different characteristics.

The results indicate that there are significant elevations in the level of aspartate aminotransferase (AST), alkaline phosphatase and creatinine while decrease in the level of alanine aminotransferase (ALT) and urea in serum of male albino rat treated with Chocolate brown HT (50 mg/kg b.wt.) for 4 weeks. This agree with the findings of Aboel-Zahab et al. (1997) of significant decrease in rat body weight, serum cholesterol and HDL - cholesterol fraction, while, significant elevations inT4 hormone, liver RNA content, liver enzymes (SGOT, SGPT and alkaline pbosphatase), total protein and globulin fractions in serum of male albino rat.

The results of the present study indicated that there are

significant elevations in the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and urea while creatinine was significantly decrease in treated rat in the serum of male albino rat treated with olive oil and Chocolate brown HT (50 mg/kg b.wt.) for 4 weeks. The aminotransaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] represent an important link between carbohydrate and amino acid metabolic pathways (Christic and Michelson, 1975). Also, these enzymes are considered good sensitive tools for detection of any variations in the physiological process of living organisms (Tolba et al., 1997). The concentrations of these enzymes (AST and ALT) in the liver of treated rats were significantly increased than that in the control rat. The elevation in the activities of these enzymes could be due to a variety of conditions including muscle damage, intestinal and hepatic injury and toxic hepatitis (Farkaset et al., 2004). Also, the increase in serum alkaline phosphatase (ALP) activity in patients could be attributed to hepatocytes injury and interruptions in their natural activities (Farkaset et al., 2004).

The results of this study also showed that histological analysis in the liver treated with food chocolate brown stain show that moderate histology changes was the rupture of hepatic tissue in the central vein (CV) and the region casement (PA) while in the liver treated with virgin olive oil with colored food chocolate brown showed an improvement in the tissue of liver, while there were some liver cells from the organizational almost with the appearance of pool simple cells, inflammatory. Also, the kidney cortex of rat treated with food chocolate brown stain shows the change in the natural structure of the fabric in some of the renal glomeruli (GL) and intertubular urinary (RT) with blood stagnation in the tissues interface (IT) while in the kidney cortex of rat treated with virgin olive oil with the chocolate brown colored food, a clear improvement in urinary intertubular (RT) and glomeruli (GL) was seen where he regained normal kidney tissue composition.

Aboel-Zahab et al. (1997) found that histopathological studies showed food chocolate brown pigment deposition in the portal tracts and Van Kupffer cell of the liver as well as in the interstitial tissue and renal tubular cells of the kidney mainly induced by colourant A. Congested blood vessels and areas of hemorrhage in both liver and renal sections were revealed in those rats given colourants B and C. There were no - untoward - effects recorded in the stomach tissue. Scientists attributed the destruction of liver tissue as a result of increased free radical and LPO and high enzymes GOT, GPT and the accumulation of triglycerides and release enzymes into the bloodstream, which have a clear indication of the emergence of cellular necrosis of the liver tissue. Extra virgin olive oil is beneficial to health and protects from damage by free radical oxidation. It contains the highest concentration of polyphenols compared to other types of virgin olive oils (that is Extra Virgin, Virgin, Ordinary Virgin and Lampante Virgin). Moreover, olive oil has been reported to elevate the activities of hepatic antioxidant enzymes in rat such as catalase, superoxide dismutase and glutathione peroxidase (Ruiz-Gutiérrez et al., 2001). Experimental studies in animals have shown that diets lacking olive oil lead to substantial disturbances in neural function, which in most circumstances can be restored by the inclusion of olive oil in the diet. In the past 10 years there has been an emerging interest in treating neuropsychological disorders (depression and schizophrenia) with omega 3 polyunsaturated fatty acids. It is clear from the literature that DHA is involved in a variety of processes in neural cells and that its role is far more complex than simply influencing cell membrane properties (Schaffer, 2007).

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