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Hypo-Homocysteinemia Effect for Some Ethanolic Plant Extracts

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Abstract

Recently, elevated plasma homocysteine (Hcy) levels have been impilicated as a risk factor for atherosclerosis, resulting in coronary artery disease, cerebrovascular disease, and peripheral vascular disease. Elevation of plasma homocysteine has been gradually induced subcutenous s.c. for male albino rats, beginning from the 5th day, and up to three weeks. Oxidative stress is known as one of the mechanisms in injury for Hcy induction. Fruits and vegetables have been proved in lowering the Hcy effects. Fourty two male rats were divided individually into 7 groups as followed: control, Hcy group, Hcy administration with avocado, broccoli, lettuce, tomato and mixture ethanolic extracts (50 gm 100 ml^{-1}).

Hcy gradually administrated for groups 2-7 (0.3, 0.4, and 0.6 µmoL g^{-1} body weight), beginning from 5th day for one week, second and third week, respectively. In the first two weeks, groups 3-7 were fed on basal diet with 0.1 ml oral inj. (per rat weighted 150 gm) avocado, broccoli, lettuce, tomato and mixture extracts.

HPLC results showed remarkable Hcy concentrations with avocado, mixture and lettuce extracts; 1.8 and 2.8 and 2.9 µg mL^{-1}, respectively. By the end of the experiment, mixture and broccoli significant decreased total billirubin (0.42 mg dL^{-1} 35%, 0.44 mg dL^{-1} 32%, respectively), and GPT (29.55, 29.63 units mL^{-1}, respectively). LDL/HDL or risk factor has been declined significantly with broccoli extract (91%-0.04 mg dL^{-1}). Creatinine (0.29 mg dL^{-1}, 50%) and urea (29.63 mg dL^{-1}, 29%) showed significant improvement with tomato extract comparing to infected group. Avocado showed significant antioxidative activity or reduction in MDA (7.13 nmol mL^{-1}, 50%).

Keywords: Homocysteine (Hcy); Avocado; Cholesterol (CHL)

Introduction

Homocysteine (Hcy), discovered in 1932, is an intermediate amino acid formed during the metabolism of the essential amino acid methionine. Abnormal elevations of plasma Hcy levels up to 100-250 µM have been reported in patients with severe hyperhomocysteinemia, due to genetic defects of enzymes that are involved in Hcy metabolic pathways [1]. Recent research on homocysteine has implicated abnormal homocysteine metabolism in a wide range of important disease processes, including developmental birth defects, neurodegenerative diseases like Alzheimer's disease, autoimmune diseases like rheumatoid arthritis, hormonal imbalances, renal failure, cancer and degenerative diseases of aging.

Multivitamin therapy will be one of the therapeutic options for people at risk of atherothrombotic vascular disease [2]. So, high concentrations of plasma homocysteine are due to low serum concentrations of folate, vitamin B-12, and vitamin B-6. Antioxidant nutrients have important roles in cell function, and have been implicated in processes associated with ageing, including vascular, inflammatory and neurological damage. One of the mechanisms causing cell damage in vascular wall has been declared in producing free radicals with Hcy-induction [3].

Avocado (family *Lauraceae)* has some properties as antibacterial, antifungal, hypotensive, anti-Inflammatory and immune-enhancing effect [4]. It has high content of phenols and alkaloids, followed by reasonable amount of flavonoids, tanins and saponins [5]. Broccoli (family *Brassicaceae*) [6], which is rich in antioxidants, such as vitamin C, quercetin and kaempferol [7]. The antioxidative activity of broccoli methanol extract was measured through DPPH, ABTS, and B-Carotene methods [8].

On the other hand, lettuce (family *Asteraceae*) is the rich source of antioxidants like quercetin, caffeic acid and vitamin C [9]. It is used in the treatment of insomnia, anxiety, neurosis. It has acquired a folk reputation due to its medicinal value. Tomato fruit (family, *Solanaceae)* is consumed in diverse ways is rich in lycopene, which may have beneficial health effects [10]. Tomato fruit is an important source of lipid-soluble antioxidants in the human diet, because of their relatively high content of carotenoids. Tomato, the main dietary source of lycopene, is the most potent *in vitro* antioxidant.

Materials and Methods

Chemicals

L-homocysteine was bought from Sigma-Aldrich. All the analytical materials used were purchased from Biodiagnostic Company, except Cholesterol and HDL were brought from Beta Lab., Spectrum, Egypt.

Fruit extracts preparation

Tomato (*Solanum lycopersicum*), broccoli (*Brassica oleracea*), lettuce (*Lactuca sativa*) and avocado (*Persea americana*) were bought from Menoufia's local market in Egypt on Nov. 2011. Fruit plants were cleaned first by tap water and sliced the fleshy fruit or leaves (as in lettuce). Small pieces of whole the fruit or lettuce leaves were air dried or using oven temperature 55°C for 24 h, if needed. Well grinding was done for the dry pieces to produce the powder. Ethanolic extracts have been prepared individually (50 gm dried powder/100 ml), using

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magnetic stirrer for 3 h before filteration. Equal amounts of each dried fruit to form total 50 gm were extracted in 100 ml ethanol to produce the mixture. Concentrated samples (10 ml) were refrigerated, until the biological experiment.

Biological experiment

Biological experiments have been done in this research work up to 4 weeks to declare the role of Homocysteine as a risk factor. Fourty two male Sprague-Dawley rats were obtained from Research Institute of Ophthalmology, Giza in 2012. Rats were aged approximately 12-14 weeks. They were housed individually in healthy standard cages with metallic covers, under strict hygienic measures. Rats were divided into 7 groups, given two weeks acclimation period, during which they were fed a standard rat chow diet *ad libitum* containing 17% protein [11], with alternated 12 h dark/light cycle. The ambient temperature was held at constant temperature between 20-25°C. Six rats per group having mean weight 150 ± 5 gm were randomly assigned as the following below:

1) **Control group (c):** Rats were fed on the basal diet. Rats from groups 2-7 were injected s.c. by homocysteine (Hcy) for three weeks in the experiments. The Hcy concentration was gradually increased in these week.

2) **Hcy group (Hcy):** Rats were only injected s.c. by Hcy and were kept feeding on the normal basal diet, until the end of experiment. Hcy was injected beginning from the 5^{th} day for one week (0.3 µmoL gm^{-1} rat weight), week after (0.4 µmol gm^{-1} rat weight), and the third week (0.6 µmol gm^{-1} rat weight). Hcy was dissolved in 0.9% saline, pH 7.4

Groups 3-7 were tested for the potential protective effect for edible ethanolic extracts. The protection was carried out in the first two weeks.

(3) Group 3: Rats were fed on basal diet with oral injection of 0.1 ml concentrated for (rat weight 150 gm) tomato extract (50 gm 100 mL^{-1} EtOH) for the first two weeks.

(4) Group 4: Rats were fed on basal diet with oral injection of 0.1 ml concentrated for (rat weight 150 gm) broccoli extract (50 gm 100 mL^{-1} EtOH) for the first two weeks.

(5) Group 5: Rats were fed on basal diet with oral injection of 0.1 ml concentrated for (rat weight 150 gm) lettuce extract (50 gm 100 mL^{-1} EtOH) for the first two weeks.

(6) Group 6: Rats were fed on basal diet with oral injection of 0.1 ml concentrated for (rat weight 150 gm) avocado (50 gm 100 mL^{-1} EtOH) extract for the first two weeks.

(7) Group 7: Rats were fed on basal diet with oral injection of 0.1 ml concentrated for (rat weight 150 gm) the mixture extract (50 gm equally mixed from the four samples/100mL EtOH) for the first two weeks.

The animals were sacrificed at the end of the biological experiment (4 weeks), the blood was collected on days 7^{th}, 14^{th} and the end of experiment. Blood was collected from the orbital plexus under ether anesthesia. Blood was allowed to clot, and then centrifuged at 3000 rpm for 15 min, and serum kept at -20°C, until required. Food consumption was monitored daily, and body weight was determined twice a week. All procedures were performed in accordance with the guide to the care and use of experimental animals.

Plasma Homocysteine and Folic Acid Measurements

Total plasma Hcy concentration was measured at Al-Azhar University, Analytical lab, using high-performance liquid chromatography, according to the method described by Chmurzynska [12]. Compounds were separated by reversed-phase high-performance liquid chromatography, and then detected and quantified by UV absorbance detection at 355 nm.

Biochemical analysis

Tests are measured in serum, and all the absorbances have been measured using Milton Roy, Spectronic 1201 equipment. The serum was stored on freeze degree, until assaying through a month.

Lipid peroxidation was measured as Malondialdehyde MDA, and was measured with colorimetric method [13]. The thiobarbituric acid reactive product pink product was measured at 534 nm.

Lipid profile

Lipid profile was measured using enzymatically colorimetric method [14], at 500 nm. Triglycerides (TG), as well, were measured by enzymatically colorimetric method [15]. Low density lipoprotein-cholesterol (LDL-c) was calculated according to the equation mentioned by Friedewald [16] ($mg\ dL^{-1}$), as follows;

$$\mathrm{LDL} - c = Total\ CHL - HDL - c - (TG/5)$$

Liver function

Glutamic-Oxaloacetic Transaminase, GOT and Glutamic-Pyruvic Transaminase, GPT, were measured with colorimetric method [17], at 505 nm.

Kidney function

Using urease to form ammonium ions from urea, ions are measured by Berthelot reaction to form blue dye indophenols product [18]. Creatinine formed a colored complex with picrate in an alkaline medium [19].

Bilirubin

In the presence of DMSO, the total bilirubin participates in the reaction, and in the absence of DMSO, only conjugated bilirubin reacts [20].

Statistical analysis

Means of results were calculated among 6 replicates, with their Standard Deviations (SD) for each group. Analysis of variance was used to make statistical comparisons (ANOVA), with Dunnett's post hoc test. SPSS computer program [21], which was used to calculate the significance between groups at the same experiment and between time stages, at 1% and 5% probabilities.

Results and Discussion

The protective systems have been studied, especially those acting as defense in oxidative damages. Samman et al. [22] found that fruit and vegetable concentrate increased plasma β-carotene, retinol, α-tocopherol, ascorbic acid and folic acid levels; while they showed that plasma homocysteine was reduced and inversely related with serum folate concentrations.

Data mentioned are related to ours, where Hcy was increased, especially with tomato and broccoli (Table 1). High folic acid concentrations have been found with tomato and mixture extracts, up to 1.5 and 1.3 µg mL^{-1}, respectively. In tomato extract, high intake of folic acid increases plasma Hcy, which is completely correlated with Haghdoost-Yazdi et al. [23]; while Chmurzynska [12] found that mean serum Hcy levels for the dietary groups ranged from 5.6-7.6 nmoL mL^{-1}.

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Avocado showed that 1.8 μg mL^{-1} plasma Hcy was the best among the extracts in decreasing plasma Hcy (Table 1). The effect on Hcy concentration for avocado was followed by mixture and lettuce extracts. The relation between Hcy and folic acid concentrations was explained by Kale et al. [24]. They showed significant reduction of plasma folic acid, vit. B12, red blood cell folic acid, and increased plasma Hcy exist early in schizophrenia.

Effects on Billirubin

Billirubin is measured to diagnose, and/or monitor liver diseases, such as cirrhosis, hepatitis or gallstones. It is also used to evaluate people with hemolytic anemia, who may have episodes when excessive red blood cell destruction takes place, increasing bilirubin levels. Bilirubin can be measured as a total level, and/or as conjugated and unconjugated levels for these purposes.

Group	Homocysteine (μg/ml) R.time 5-5.6	Folic acid (μg/ml) R.time 7- 7.5
Control	0.9	0.1
Hcy	4.8	1.2
Hcy+tomato ext.	7.6	1.5
Hcy+broccoli ext.	6.9	-
Hcy+lettuce ext.	2.9	-
Hcy+avocado ext.	1.8	0.4
Hcy+mixture ext.	2.8	1.3

Table 1: Concentration of mean plasma homocysteine (μg/ml) at R.time 5-5.6 and mean plasma folic acid (μg/ml) at R.time 7-7.5 in serum of rat groups.

Significantly, lettuce ethanolic extract increased total billirubin value, comparing to that of Hcy by the end of the experiment (Table 2). While, insignificantly, avocado increased that value in the same period of time. Billirubin reduction was huge and significant in Hcy group in second week. Significantly, tomato, followed by mixture, then lettuce extracts, improved blood destruction in direct billirubin by the end of experiment ; while for total billirubin, tomato followed by mixture extracts, showed significant improvement in the first week.

Effects on liver functions (Aminotransferase Enzymes)

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered among the second class of enzyme classification (the transferases). These enzymes catalyze the transfer of alpha-amino group of aspartate and alanine to the alpha-keto group of ketoglutaric acid, resulting in the formation of oxaloacetic acid and pyruvic acid, respectively [25]. In the first two weeks, broccoli, followed by lettuce extract, showed the lowest GPT value; while broccoli, tomato and lettuce showed the highest improvement for GOT in the end of experiment (Table 3).

Groups	Total Bilirubin			F	Sig.	Direct Bilirubin			F	Sig.
	7 days	14 days	28 days			7 days	14 days	28 days		
control	0.85f ± 0.00	1.05a ± 0.03	0.71abc ± 0.26	7.612	0.005 * *	0.11g ± 0.00	0.17cd ± 0.03	0.27b ± 0.26	1.65	0.226 -
Hcy group	1.06c ± 0.00	0.79b ± 0.35	0.65abc ± 0.03	6.442	0.01 * *	0.29c ± 0.00	0.54a ± 0.13	0.26b ± 0.14	10.9	0.001 * *
Hcy+Tomato ext.	0.30i ± 0.00	0.43cd ± 0.20	0.54bc ± 0.31	1.811	0.197 -	0.24d ± 0.00	0.38abc ± 0.17	0.25b ± 0.12	2.56	0.111 -
Hcy+ Broccoli ext.	1.29b ± 0.00	0.67bc ± 0.12	0.44c ± 0.16	92.244	0 * *	0.67a ± 0.00	0.28bcd ± 0.25	0.23b ± 0.13	13.5	0 * *
Hcy+Lettuce ext.	0.88e ± 0.00	0.56bcd ± 0.26	1.01a ± 0.42	3.943	0.042*	0.04i ± 0.00	0.35abc ± 0.31	0.61b ± 0.49	4.25	0.035 *
Hcy+Avocado ext.	0.95d ± 0.00	0.66bc ± 0.34	0.72abc ± 0.46	1.31	0.299 -	0.07h ± 0.00	0.17cd ± 0.03	3.58a ± 5.38	2.478	0.118 -
Hcy+Mixture ext.	0.38h ± 0.00	0.62bc ± 0.15	0.42c ± 0.22	3.99	0.041*	0.04i ± 0.00	0.42ab ± 0.00	0.24b ± 0.24	11.085	0.001 * *
Total	0.83 ± 0.47	0.63 ± 0.28	0.65 ± 0.33			0.23 ± 0.20	0.29 ± 0.22	0.62 ± 1.87		
F	106479	5.716	2.474			18654.6	6.007	2.231		
Sig.	0	0	0.02			0	0	0.035		
Sig.	* *	* *	*			* *	* *	*		

Values are expressed as mean ± SD. Significance**at p<0.01; * at p<0.05.

Values which do notshare the same letter in each column are significantly different.

Table 2: Effect of Hcy on total and direct billirubin (mg/dl) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

Groups	GPT			F	Sig.	GOT			F	Sig.
	7 days	14 days	28 days			7 days	14 days	28 days		
control	32.8abc ± 3.69	32.21cd ± 1.53	30.94bc ± 2.89	0.66	0.531 -	16.97c ± 1.25	22.76b ± 7.83	21.16cd ± 3.69	23.65	0 * *
Hcy group	32.79ab ± 3.69	35.00abc ± 1.17	29.56c ± 0.67	8.728	0.003 * *	16.88c ± 0.94	22.30b ± 4.11	36.50a ± 3.69	4.695	0.026 *
Hcy+Tomato ext.	30.82b ± 3.69	36.15ab ± 2.78	30.49bc ± 2.87	6.129	0.011 *	20.92ab ± 0.47	18.50b ± 1.38	17.73de ± 3.69	3.178	0.071 -
Hcy+Broccoli ext.	24.10ab ± 3.69	33.52bcd ± 1.35	29.63c ± 5.45	8.953	0.003 * *	18.54bc ± 1.30	20.59b ± 2.74	18.02de ± 3.69	1.456	0.264 -
Hcy+Lettuce ext.	33.11c ± 3.69	32.79cd ± 0.00	33.07ab ± 0.91	0.04	0.961 -	21.16ab ± 4.38	18.52b ± 1.25	15.83e ± 3.69	3.726	0.049 *
Hcy+Avocado ext.	35.41ab ± 3.69	37.13a ± 4.22	32.13abc ± 1.05	3.573	0.054 -	17.64c ± 2.09	23.00b ± 6.83	20.69cd ± 3.69	2.01	0.169 -
Hcy+Mixture ext.	30.00a ± 3.69	29.18e ± 3.41	29.55c ± 1.61	0.109	0.897 -	17.40c ± 2.45	22.95b ± 6.24	27.83b ± 3.69	8.376	0.004 * *
Total	31.59 ± 4.54	33.82 ± 3.15	31.22 ± 2.85			19.01 ± 2.90	22.81 ± 5.70	25.22 ± 8.66		
F	4.392	7.087	2.603			3.294	2.645	30.576		
Sig.	0	0	0.015			0.003	0.014	0		
Sig.	* *	* *	*			* *	*	* *		

Values are expressed as mean ± SD. Significance**at p<0.01; * at p<0.05.

Values which do notshare the same letter in each column are significantly different.

Table 3: Effect of Hcy on GPT and GOT (units/ml) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

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Aminotransferase enzymes, GPT and GOT, increase for Hcy group significantly on second and fourth week, respectively, compared with control. Lettuce, followed by tomato, and then broccoli extracts, succedded in significantly reduced GOT values in the fourth week, comparing to Hcy group ; while slightly insignificant reduction for GPT in the fourth week using mixture plants extract comparing to Hcy. In the second week, mixture followed by lettuce, and then broccoli extracts, reduced GPT values significantly compared to Hcy group.

Effects on lipid profile

Data showed that after two weeks, TG has been significantly increased in Hcy group; while second and fourth weeks showed clear significant increases for cholesterol comparing to control (Table 4). The high risk factor or LDL/HDL ratio was obviously decreased by time increased with Hcy administration. On the other hand, broccoli and mixture extracts were the optimistic data in our study for their improvement, especially in the first two weeks.

Samman et al. [22] determined the effect of supplementation with dehydrated juice concentrates from mixed fruit and vegetables on selected plasma vitamins and antioxidant status. Triglyceride, TG and calculated vLDL (TG/5) was slightly increased with broccoli extract administration, while tomato and avocado extract increased cholesterol value as shown.

Broccoli extract has significant increase in TG levels during different time stages, compared to control and Hcy group. On the other hand, broccoli extract showed significant reduction in cholesterol compared to Hcy group. During time stages, there was a significant between TG and cholesterol levels. Broccoli extract also succedded to significantly decrease the bad cholesterol or LDL, compared to administrated Hcy, while broccoli showed significant increase in the good part of cholesterol HDL, compared to control in the first two weeks. Especially in the second week, Hcy showed the worst risk value, compared to others numbers. In a complete view, broccoli extract significantly improved or decreased the risk factor (LDL/HDL), during time stages compared to that for Hcy.

Lee et al. [26] studied a high-fat high-cholesterol diet supplemented with or without red-pigmented leafy lettuce for 4 weeks. Red-pigmented leafy lettuce-supplemented diet significantly decreased the level of total and LDL-cholesterol in the plasma of the mice. Consumption of tomato juice caused a significant elevation of plasma lycopene, as well as increased resistance of low-density lipoprotein (LDL) to oxidationin subjects, with type 2 diabetes mellitus [27].

HDL or good cholesterol concentration has been logically decreased for Hcy group, compared to control in the first two examined time stages; while LDL or bad cholesterol was significantly increased in Hcy group compared to control at 2nd, and 4th week (Table 5). Low folic with betaine has a remarkable improvement for HDL and LDL amounts.

Risk factor showed significant increase through Hcy injection

Groups	TG			F	Sig.	cholesterol			F	Sig.
	7 days	14 days	28 days			7 days	14 days	28 days		
Control	103.00d ± 3.69	92.00 cdef ± 18.62	153.75 ab ± 30.87	14.87	0 **	136.51a ± 3.69	58.73e ± 15.65	92.78abc ± 42.21	13.41	0 **
Hcy group	95.00e ± 3.69	103.00 abcd ± 4.38	147.67 ab ± 34.42	11.91	0.001 **	95.24f ± 3.69	81.75bcd ± 6.09	112.17ab ± 29.05	4.676	0.026 *
Hcy+Tomato ext.	126.00c ± 3.69	85.00 def ± 26.29	160.00 ab ± 29.13	16.34	0 **	138.10a ± 3.69	98.41ab ± 6.96	97.35abc ± 19.22	22.50	0 **
Hcy+Broccoli ext.	137.00b ± 3.69	119.00a ± 9.86	181.00a ± 44.77	8.659	0.003 **	47.62g ± 3.69	74.60 cde ± 5.22	79.24bc ± 20.11	11.79	0.001 **
Hcy+Lettuce ext.	107.00d ± 3.69	80.00ef ± 10.95	143.75 ab ± 32.02	15.91	0 **	123.81c ± 3.69	107.94a ± 1.74	89.67abc ± 40.50	3.17	0.071 -
Hcy+Avocado ext.	73.00g ± 3.69	76.00ef ± 6.57	126.00b ± 11.23	87.28	0 **	130.16b ± 3.69	75.40 cde ± 4.35	115.08a ± 14.36	60.37	0 **
Hcy+Mixture ext.	163.00a ± 3.69	96.50 bcde ± 18.08	133.75b ± 20.72	25.98	0 **	115.87d ± 3.69	66.67de ± 22.60	110.38ab ± 25.43	11.17	0.001 **
Total	109.00 ± 25.45	94.95 ± 22.99	142.48 ± 34.37			113.17 ± 25.56	79.60 ± 20.34	93.79 ± 27.73		
F	306.569	5.863	2.024			309.272	5.312	2.017		
Sig.	0	0	0.056			0	0	0.057		
Sig.	**	**	-			**	**	-		

Values are expressed as mean ± SD. Significance**at $p<0.01$; * at $p<0.05$.

Values which do not share the same letter in each column are significantly different.

Table 4: Effect of Hcy on TG (mg/dl) and cholesterol (mg/dl) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

Groups	HDL			F	Sig.	LDL			F	Sig.
	7 days	14 days	28 days			7 days	14 days	28 days		
Control	42.75ab ± 3.69	29.36abc ± 16.55	55.01a ± 18.33	4.751	0.025 *	73.16b ± 0.74	10.98c ± 2.83	7.03b ± 33.58	21.803	0 **
Hcy group	34.20de ± 3.69	17.10c ± 23.10	56.24a ± 12.56	9.828	0.002 **	42.04i ± 0.74	44.05ab ± 28.31	26.40ab ± 32.76	0.896	0.429 -
Hcy+Tomato ext.	43.89a ± 3.69	35.06abc ± 17.17	49.59ab ± 3.14	3.033	0.078 -	69.01c ± 0.74	46.36ab ± 29.38	15.76ab ± 26.09	8.32	0.004 **
Hcy+Broccoli ext.	43.89a ± 3.69	31.64abc ± 5.93	45.03ab ± 4.23	14.879	0 **	15.76 ± 26.09	19.17bc ± 13.12	-1.986c ± 23.36	9.226	0.002 **
Hcy+Lettuce ext.	41.61abc ± 3.69	41.33ab ± 26.54	50.16a ± 8.61	0.573	0.576 -	60.80f ± 0.74	50.61a ± 26.99	10.76b ± 46.73	4.322	0.033 *
Hcy+Avocado ext.	38.76bc ± 3.69	36.46abc ± 8.76	34.06b ± 19.40	0.213	0.81 -	76.80a ± 0.74	23.74abc ± 14.43	55.82a ± 33.55	9.635	0.002 **
Hcy+Mixture ext.	37.05cd ± 3.69	37.05abc ± 7.49	51.16a ± 19.85	2.576	0.109 -	46.22g ± 0.74	10.32c ± 18.73	32.47ab ± 39.51	3.089	0.075 -
Total	39.62 ± 5.32	33.46 ± 16.27	48.05 ± 12.81			51.76 ± 27.96	27.16 ± 24.13	17.25 ± 33.68		
F	8.099	1.795	1.643			9414.574	3.036	1.939		
Sig.	0	0.093	0.129			0	0.006	0.067		
Sig.	**	-	-			**	**	-		

Values are expressed as mean ± SD. Significance**at $p<0.01$; * at $p<0.05$.

Values which do not share the same letter in each column are significantly different.

Table 5: Effect of Hcy on HDL (mg/dl) and calculated LDL (mg/dl) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

compared with control, specially in second and fourth weeks. By the end of the experiment, hyperhomocysteinemia was almost cured through the treatment of low folic with betaine (Table 6). Generally, high folic, as well showed optimistic results, followed by low folic acid in protecting the hyperhomocysteinemia.

Effects on lipid peroxidation

Malondialdehyde level gives indicator for the antioxidant mechanism offered through the tested administrations. During the time elapsed, MDA level was getting higher for the relationship between Hcy and oxidative stress. In the same trend, Woo et al. [28] found positive correlation between serum Hcy concentrations and oxidative stress. Hyperhomocysteinemia in their research was induced in rats fed 0.8% methionine or 1.7% methionine.

Rao and Adinew [29] stated that anti-oxidative effects of *Persea americana* fruit extract were evaluated. Ethanol extract of *P. americana,* in the concentration of 300 mg/Kg body weight daily, was orally administered to rats introduced into Stz-induced hyperglycaemic state, for a period of 30 days. After the treatment with *Avocado* fruit extract, the elevated levels of blood glucose, glycosylated haemoglobin, blood urea and serum creatinine seen in the hyperglycaemic rats, reverted back to near normal.

Fourth week showed high MDA level for lettuce extract, with Hcy administration; while avocado extract showed the highest antioxidant availability in 4th week, and avocado extract in the 2nd week (Table 7), that might be in agreement with vitamins content in avocado which is shown in table 1. Generally, tomato and avocado extracts showed significant decrease in lipid peroxidation, compared to that for Hcy. In the same view, determination of thiobarbituric acid reactive cubstances (TBARS), hydroperoxides, and both enzymatic and non-enzymatic antioxidants [29], confirmed the anti-oxidative potential of *Avocado* fruit extract.

Group	7 days	14 days	28 days	F	Sig.
Control	1.71b ± 0.17	0.37b ± 0.16	0.128b ± 0.92	16.961	0 **
Hcy group	1.23d ± 0.16	2.60a ± 9.86	0.47ab ± 0.58	5.481	0.016 *
Hcy+Tomato ext.	1.57bc ± 0.15	1.32b ± 1.86	0.32ab ± 0.54	3.854	0.045 *
Hcy+Broccoli ext.	-0.54f ± 0.03	0.60b ± 0.54	-0.04b ± 0.61	10.371	0.001 **
Hcy+Lettuce ext.	1.46c ± 0.15	1.22b ± 2.19	0.21b ± 1.01	3.677	0.05 *
Hcy+Avocado ext.	1.98a ± 0.21	0.65b ± 0.58	1.64a ± 0.83	6.907	0.008 **
Hcy+Mixture ext.	1.25d ± 0.15	0.28b ± 0.46	0.63ab ± 1.12	3.46	0.058 -
Total	1.35 ± 0.73	0.11 ± 4.30	0.38 ± 0.81		
F	118.517	5.313	1.912		
Sig.	0	0	0.072		
Sig.	**	**	-		

Values are expressed as mean ± SD.

Values which do not share the same letter in each column are significantly different.

Table 6: Effect of Hcy on risk factor LDL/HDL in rats treated with low folic (without/with betaine), or high folic and plant extracts.

Group	7 days	14 days	28 days	F	Sig.
Control	4.46bc ± 1.64	8.30d ± 2.44	9.09f ± 1.03	16.961	0 **
Hcy group	5.56abc ± 2.25	12.51a ± 8.33	14.29b ± 1.03	5.481	0.016 *
Hcy+Tomato ext.	4.31c ± 1.12	3.42g ± 0.14	9.88ef ± 1.03	3.854	0.045 *
Hcy+Broccoli ext.	5.47abc ± 1.19	9.08c ± 2.53	11.25d ± 1.03	10.371	0.001 **
Hcy+Lettuce ext.	6.30abc ± 5.42	1.93h ± 3.49	16.45a ± 1.03	3.677	0.05 *
Hcy+Avocado ext.	4.85bc ± 0.57	8.90d ± 3.54	7.13g ± 1.03	6.907	0.008 **
Hcy+Mixture ext.	7.11abc ± 1.80	4.71ef ± 2.48	12.62c ± 1.03	3.46	0.058 -
Total	6.06 ± 2.43	6.77 ± 4.79	11.15 ± 2.79		
F	2.113	6.556	42.432		
Sig.	0.046	0	0		
Sig.	*	**	**		

Values are expressed as mean ± SD.

Values which do not share the same letter in each column are significantly different.

Table 7: Effect of Hcy on lipid peroxidation marker malondialdehyde MDA (nmol/ml) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

Serafini et al. [30] investigated that ingestion of fresh lettuce increased plasma total radical-trapping antioxidant potential (TRAP), than that for stored lettuce. They found that quercetin, p-coumaric acid, caffeic acid, plasma b-carotene and vitamin C were increased. In this trend, lettuce in second week showed highly significant reduction for MDA formation (Table 7).

The progress data of tomato extract correlated with Sarkar et al. [31], who stated that oxygen derived free radicals, the most reactive species, has been quenched by the antioxidant lycopene. Lycopene has a singlet oxygen quenching ability, twice as high as that of β-carotene, and 10 times higher that of α-tocoferol. Sarkar et al. [31], as well published that lycopene is a phytochemicafound in tomatoes having singlet oxygen quenching ability higher than other antioxidants, participates in most of chemical reactions to protect critical cellular biomolecules. They revealed decrease in lipid peroxidation (MDA), after various forms of lycopene supplementation.

The atherosclerotic index was calculated to be 46% lower in the mice fed with the lettuce diet, compared with the control diet. Lipid peroxidation measured by 2-thiobarbituric acid-reactive substances was markedly reduced in the plasma, liver, heart and kidney of the mice fed with the lettuce diet [26]. The content of antioxidants (total glutathione and B-carotene) was significantly increased by lettuce supplementation.

Sahin et al. [32] found that MDA levels in muscles, liver and serum (0.65 *vs.* 0.07; 2.5 *vs.* 1.15 and 1.79 *vs.* 0,55; P=0.001) linearly decreased in birds with dietary tomato powder supplementation in all birds. There were interaction effects of temperature and tomato powder levels on serum (P=0.02), breast and leg muscles and liver MDA concentrations (P=0.001). Alvares Delfino et al. [33] examined that folic acid treatment normalized plasma Hcy levels. Significantly increased total plasma antioxidant capacity levels, but had no significant effect on hydroperoxide levels.

Effects on urea and creatinine (Kidney Functions)

In renal failure, homocysteine is often elevated, but as Hcy is not primarily cleared by the kidney, the mechanism of elevation is uncertain in many renal failure patients. Our result showed relatively high significant amount of urea and creatinine in serum of Hcy group, compared with that for control. Data showed the dramatic reduction in creatinine levels, especially in the 2nd and 4th week. On the other hand, data has only obvious reduction in urea level during the experiment. Significant difference for creatinine and urea has been noticed in table 8, between time stages in Hcy group at p<0.01. Comparing to Hcy mixture extract showing significant creatinine reduction in the second week, and broccoli in the fourth week, while urea levels showed significant reduction by mixture, lettuce and tomato extracts in the 1st, 2nd and 4th week, respectively.

Rao and Adinew [29] stated that ethanol extract of *P. americana,* in the concentration of 300 mg/Kg body weight daily, after the treatment with *Avocado* fruit extract, the elevated levels of blood glucose, blood

Groups	Creatinine			F	Sig.	Urea			F	Sig.
	7 days	14 days	28 days			7 days	14 days	28 days		
Control	0.35a ± 0.00	0.30d ± 0.00	0.49ab ± 0.12	12.865	0.001 **	32.13c ± 3.69	40.80ab ± 4.03	28.33c ± 3.34	10.679	0.001 **
Hcy group	0.25f ± 0.00	1.90a ± 0.53	0.58a ± 0.12	46.588	0 **	43.92a ± 3.69	45.72a ± 12.18	42.21a ± 4.51	9.508	0.002 **
Hcy+Tomato ext.	0.31b ± 0.00	1.35b ± 0.35	0.29b ± 0.03	52.766	0 **	34.03c ± 3.69	30.99cde ± 1.87	29.94c ± 7.77	1.047	0.375 -
Hcy+Broccoli ext.	0.30c ± 0.00	1.08bc ± 0.01	0.28b ± 0.04	2160.1	0 **	32.32c ± 3.69	25.67def ± 9.58	31.04bc ± 2.25	2.031	0.166 -
Hcy+Lettuce ext.	0.26e ± 0.00	0.99bc ± 0.59	0.44ab ± 0.13	7.099	0.007 **	40.87ab ± 3.69	23.38ef ± 6.04	31.61bc ± 2.99	23.354	0 **
Hcy+Avocado ext.	0.24g ± 0.00	1.00bc ± 0.09	0.38ab ± 0.12	137.224	0 **	40.49ab ± 3.69	33.46bcd ± 3.54	37.17ab ± 5.15	4.233	0.035 *
Hcy+Mixture ext.	0.26e ± 0.00	0.80c ± 0.43	0.58a ± 0.38	3.998	0.041 *	23.76d ± 3.69	38.31abc ± 8.23	30.18c ± 3.30	10.377	0.001 **
Total	0.28 ± 0.04	1.02 ± 0.55	0.42 ± 0.18			33.37 ± 8.23	31.11 ± 9.95	32.85 ± 6.62		
F	678.144	6.463	2.702			27.08	9.646	5.234		
Sig.	0	0	0.012			0	0	0		
Sig.	**	**	*			**	**	**		

Values are expressed as mean ± SD. Significance**at p<0.01; * at p<0.05.

Values which do not share the same letter in each column are significantly different.

Table 8: Effect of Hcy on creatinine (mg/dl) and urea (mg/dl) in rats treated with low folic (without/with betaine), or high folic and plant extracts.

urea and serum creatinine seen in the hyperglycaemic rats, reverted back to near normal. Creatinine and urea showed in table 8 significantly increased in Hcy group, compared to that for control.

As will be seen in histograms, chronic renal failure increase plasma Hcy levels (hyperhomocysteinemia), when glomerular filtration rate is reduced [34], that illustrated the reason of increased creatinine and urea with injection of rats with Hcy.

Finally, this work with many others in homocysteinemia is spotting light on the bad role of Hcy, discovered by the beginning of elapsed decade. The bad effects for Hcy the intermediate amino acid concentrates on renal failure, cholesterolemia, cancer, and ageing parameters due to oxidative degeneration. Our research proved the improvement role of some plant extracts, which showed optimistic results, especially shown by avocado and broccoli extracts that had been clearly proved in chemical composition for these fruits or serum Hcy, or serum folic content. Cell pathological or chemical results were in agreement in our work, which we hope that it can be one step with others, to protect humanity from the risk of homocysteinemia.

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