Effect of Different Low Protein Diets Supplemented with Vitamin E, Nigella Sativa Seeds, and Green Tea on Renal Functions among Rats

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Abstract: The present study was conducted to investigate the influence of vitamin E, Nigella Sativa seeds, green tea leaves and their combinations on renal functions of rats fed L-arginine diet. The two main experimental groups; include Group 1 (n=6 rats) used as a negative control group fed on basal diet and group 2 (n= 54 rats) fed on L-arginine diet (2% L-arginine) along the study period. After two week of feeding L-arginine diet, this group was divided into nine subgroups (6 rats each). Subgroup (1) was fed on L-arginine diet, as a control positive group; Subgroups (2 and 3) were fed on low protein diets (10% protein) supplemented with 100 and 200 mg vitamin E/ 100g diet, respectively. Subgroups (4 and 5) were fed on low protein diets supplemented with 2.5% and 5% Nigella Sativa seed/100g diet, respectively. Subgroups (6 and 7) were fed on low protein diets supplemented with 2.5% and 5% green tea/100g diet, respectively. Subgroups (8 and 9) were fed on low protein diets supplemented with 100 mg vitamin E, 2.5% Nigella Sativa seed and 2.5% green tea & 200 mg vitamin E, 5% Nigella Sativa seed and 5% green tea/100g diet, respectively. Food intake increased significantly among experimental groups fed 5% Nigella Sativa seeds and the group fed the higher concentration of mixture supplements, on the other hand body weight gain% increased significantly in all treated groups, except for group fed 5% green tea. All experimental groups with the two concentration from (vitamin E, Nigella Sativa seeds, green tea and their combinations) resulted in improvement in all parameters including (kidney functions, liver enzymes, lipid profile, glutathione peroxidase, superoxide dismutase and catalase), especially the group which fed the higher concentration of supplements. In conclusion, vitamin E, Nigella Sativa seeds, green tea leaves and their combination improved the nutritional and biological status of rats renal insufficiency

Key words: Vitamin E, Nigella Sativa seeds, green tea leaves, L-arginine, kidney functions.

INTRODUCTION

Chronic Renal failure is a gradual loss of the kidney's ability to excrete wastage, the hall mark of renal failure is progressive azotaemia caused by the accumulation of the nitrogenous end products of metabolism, accompanied by a metabolic derangements such as metabolic acidosis and hyperkalemia, disturbances of body fluid balance and effects on many other organ systems (Thadhani et al., 1996 and Agrawal and Swartz, 2000). Renal failure is very serious medical condition and even deadly if left untreated (National Kidney Foundation, 2002).

Low-protein diets ameliorate uremic symptoms and some of its metabolic complications. These diets can be used successfully to treat patients with chronic renal failure (CRF) because they are able to activate normal compensatory responses when protein intake is restricted and their protein and energy requirements are similar to healthy subject (Mitch and Maroni, 1998).

Renal failure is accompanied by oxidative stress, which is caused by enhanced production of reactive oxygen species and impaired antioxidant defense. The therapeutical interventions aimed at reducing oxidative stress in chronic renal failure patients.

Administration of antioxidants such as (alpha-tecopherol) is important in those patients (Ben et al., 2006 and Wesen, 2011).

Nigella Sativa seed is a widely grown herb in different parts of the world. In south Asia it is known as Kalonji, in the English literature it is known as black cumin and its Arabic name is Habat–ul-Sauda (Duke, 1992 and Khan et al., 2003). Nigella sativa L. from the ranunculaceae family have long been used in Egyptian folk medicine for a variety of complaints (Atta, 2003). Recently, black seeds (Nigella sativa) have been reported to be analgesic (Al-Ghamdi, 2001), anti-inflammatory (Mutabagani & El-Mahdy, 1997), antihypertensive (El-Tahir et al., 1993), antidiabetic, anticancer, and antioxidant and have been proposed to lower serum levels of cholesterol and triglycerides, balance enzyme activities, increase glutathione in the kidney, and reconstruct kidney tissue after nephrotoxicity (Burits& Bucar, 2000, Sattar et al., 2002 and Khan et al., 2003).

Nigella seeds contain 40% fixed oil, a saponin (melantin) and up to 1.4% volatile oil (Chevallier, 1996). Fixed oil of seeds is rich in linoleic (56%), oleic (24.6%) and plamitic (12%) acids (Venskutonis and Sivik 2002).

The chemical composition of green tea GT (Camellia sinensis), with respect to its main constituents is similar to that of plant´s fresh leaves. Up to 30% of GT´s dry leaves is polyphenols (Ahmad and Mukhtar, 1999). Epigallocatechin gallate which occurred naturally in green tea EGCG is the most abundant catechin, accounting for about 65% of GT´s catechin content, and is also the component with the highest antioxidant properties (Guo et al., 1996). Many studies have found beneficial effects associated with the consumption of GT. Scientists have cited these beneficial effects in fighting obesity, liver, stomach, breast, prostate, lung and skin cancers (Zaveri, 2006), decreasing the risk of atherosclerosis and heart disease (Cooper et al., 2005) and protecting against the development of neurodegenerative diseases as Parkinson´s disease and Alzheimer´s disease (Crespy and Williamson, 2004).

The main objective of the present study is to investigate the influence of vitamin E, Nigella Sativa seeds, green tea and their combination on nutritional and biological parameters of rats renal dysfunction.

MATERIALS AND METHODS

Materials

- Casein, vitamins, minerals, cellulose, choline chloride, and L-arginine were purchased from El-Gomhoreya Company, Cairo Egypt.
- Sixty male albino rats (Sprague Dawley Strain) were obtained from Helwan farm, Ministry of Health, Egypt.
- Vitamin E was purchased from Pharco pharmacy in Cairo-Egypt.
- Nigella Sativa seeds and green tea were purchased from local market, Cairo Egypt.

Methods

Sixty male albino rats (180 - 190g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet for adaptation. The basal diet in the preliminary experiment consists of 20% casein (protein > 80%), soybean oil4%, cellulose

5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the remainder is corn starch (Reeves et al., 1993). Vitamin composition of diets prepared according to (A.O.A.C. 1975).

After a period of adaptation, the rats were divided into two main groups, the first group (n = 6 rats) fed on basal diet, as a control negative group, while the second group (n = 54 rats) fed on basal diet containing 2% (W/W) L-arginine for two weeks to induce renal dysfunction according to the method described by (Yokozawa et al., 2003).

The second main group was divided into nine subgroups (6 rats esch). The first one of the subgroup was fed on basal diet containing 2% arginine as a positive control group. Subgroup (2 and 3) were fed on low protein diets (10% protein) containing 2% arginine and (100 and 200 mg vitamin E/ 100g diet), respectively. Subgroups (4 and 5) were fed on low protein diets containing 2% arginine and (2.5% and 5% Nigella Sativa seed)/100g diet, respectively. Subgroups (6 and 7) were fed on low protein diets containing 2% arginine and (2.5% and 5% green tea)/100g diet, respectively. Subgroups (8 and 9) were fed on low protein diets containing 2% arginine and (100 mg vitamin E, 2.5% Nigella Sativa seed and 2.5% green tea) and (200 mg vitamin E, 5% Nigella Sativa seed and 5% green tea) /100g diet, respectively.

During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly. At the end of the experiment, the animals were fasted overnight, then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum uric acid (Fossati et al., 1980), serum urea nitrogen (Patton and Crouch 1977), creatinine by (Bohmer, 1971), aspartate amino transferase (AST) and alanine amino transferase (ALT) (Reitman and Frankel, 1957), serum alkaline phosphates (Bergmeyer and Brent 1974), total cholesterol (TC) (Allain et al, 1974), triglycerides (TG) (Fossati and prenape, 1982), high-density lipoprotein cholesterol (HDL-C) (Lopes-Virella et al., 1977). While serum low-density lipoprotein cholesterol (LDL-C) and very low- density lipoprotein cholesterol (VLDL-C) were calculated according to the equation of Friedwald et al. (1972), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were determined according to the methods described by Hissin and Hilf, (1976); Kakkar et al., (1984) and Sinha, (1972), respectively. Kidney was separated from each rat and weighted to calculate kidney weight to body weight %. Statistical analysis was carried out using the program of statistical package for the social sciences (SPSS), PC statistical software (version 20; untitled-SPSS Data Editor). Data were expressed as mean ± Standard deviation (mean ± SD). Differences between controls and treated groups were tested for significance using a one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant differences (LSD) test at $p < 0.05$ (Steel and Torri, 1980).

RESULTS AND DISCUSSION

The data in Table (1) showed that, the mean value of food intake by control positive group was significant lower (P<0.05) than control negative group. Meanwhile statistical analysis showed non-significant changes in food intake among all experimental groups.

Table (1): Effect of low protein diets containing vitamin E, Nigella Sativa seeds and green tea on food intake, body weight gain% and kidney weight/body weight%.

Parameters / Groups	Feed intake g/day/each rat	Body weight gain %	Kidney weight / body weight %
Control (-)	15.800 ^{a} ± 1.303	36.800 ^{a} ± 1.923	0.610 ^{f} ± 0.030
Control (+)	13.600 ^{d} ± 1.140	10.400 ^{f} ± 1.949	1.115 ^{a} ± 0.159
LPD containing 100 mg Vit. E /100g diet	14.016 $^{c\ d}$ ± 1.060	13.400 $^{c\ d\ e}$ ± 1.140	1.008 ^{b} ± 0.100
LPD containing 200 mg Vit. E /100g diet	14.340 $^{b\ c\ d}$ ± 1.275	16.00 ^{b} ± 1.581	0.889 $^{c\ d}$ ± 0.047
LPD containing 2.5 g Nigella Sativa seed/100g diet	14.886 $^{a\ b\ c\ d}$ ± 0.677	14.198 $^{c\ d}$ ± 0.815	0.931 $^{b\ c}$ ± 0.039
LPD containing 5 g Nigella Sativa seed/100g diet	15.592 $^{a\ b}$ ± 0.678	15.003 $^{b\ c}$ ± 1.190	0.865 $^{c\ d}$ ± 0.041
LPD containing 2.5 g green tea/100g diet	13.720 ^{d} ± 1.018	12.566 $^{d\ e}$ ± 0.959	0.939 $^{b\ c}$ ± 0.064
LPD containing 5 g green tea/100g diet	13.800 ^{d} ± 1.151	11.838 $^{e\ f}$ ± 0.920	0.849 $^{c\ d\ e}$ ± 0.042
LPD containing low concentrations of combination of all treatments.	14.544 $^{a\ b\ c\ d}$ ± 0.634	13.848 $^{c\ d}$ ± 0.694	0.825 $^{d\ e}$ ± 0.029
LPD containing high concentrations of combination of all treatments	15.376 $^{a\ b\ c}$ ± 0.736	15.171 $^{b\ c}$ ± 0.194	0.754 ^{e} ± 0.045

LPD: *Low protein diet. Values are expressed as mean ± SD.*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05

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The main value of BWG% of the positive control group decreased significantly (P≤0.05), as compared the negative control group. All experimental groups, except for the group which fed with (5% green tea) showed significant increase (p<0.05) in BWG%, as compared to the positive control group. On the other hand, all experimental groups showed significant decrease p≤0.05, as compared to the negative control group.

Statistical analysis showed a significant increase $p \leq 0.05$ in kidney weight/ body weight% for control positive group compared with control negative group at ($p<0.05$). All treated groups showed significant decrease in kidney weight / body weight%, as compared to the positive control group. Feeding groups of rats on diets supplemented with the two concentrations of the combination and high concentration of green tea revealed the highest decrees in kidney weight/ body weight%.

In concordance with our findings, Christiane et al. (1999) found that chronic renal failure rats fed on low protein diet, gained weight more than rats fed high protein diet. Also, and in agreement with obtained results, Kanter et al. (2005) found that feeding Nigella Sativa oil to rats suffered from hepatoprotoxicity for 60 day increased weight gain significantly.

Klaus et al. (2005) reported that, dietary supplementation with (-) epigallocatechin gallate EGCG purified from green tea GT for 1 month attenuated diet- induced obesity and decreased body weight of mice by decreasing energy absorption and increasing fat oxidation. On the other hand, (Murase et al., 2002) reported also, supplementation with GT catechins resulted in a significant reduction of high- fat diet induced body weight gain, visceral and liver fat accumulation and the development of hyperlipidemia in mice.

Lin and Lin- Shiau, (2006) found that, body weights of rats have been significantly reduced by feeding green tea GT leaves. Wolfram et al. (2005) reported that, feeding mice on diets supplemented with GT at levels from 1% to 4% had significantly decreased food intake, body weight gain and fat mass. Serum leptin levels were also lower and that decreases appetite. On the other hand Sayama et al. (2000) reported also, Addition of 2% GT powder to the diet suppressed fat accumulation and body weight by reduction of food.

The data in Table (2) showed that, Feeding rats on L-arginine diet increased the mean value of serum uric acid, urea nitrogen and creatinine significantly $p \leq 0.05$, as compared to the rats fed on basal diet. The mean values of serum uric acid, urea nitrogen and creatinine increased by about 443.07%, 200.718% and 615.89% in the (control positive group), than healthy rats fed on basal diet (control negative group). All treated groups showed significant decrease $p \leq 0.05$ in serum uric acid, urea nitrogen and creatinine, as compared to the positive control group.

The mean values of serum (uric acid, urea nitrogen and creatinine) decreased gradually with increasing the amounts of vitamin E, Nigella Sativa seeds, green tea and their combinations. The best results for serum uric acid, urea nitrogen and creatinine was noticed in the chronic renal failure group which treated with the high levels of (vitamin E, Nigella Sativa seed and green tea), followed by the group which treated with containing 5g Nigella Sativa seeds/100g diet. Using the high levels from the combination decreased the mean values of serum uric acid, urea nitrogen and creatinine by about 50.82%, 42.99% and 51.30%, than that of the positive control group.

Table (2): Effect of low protein diets containing vitamin E, Nigella Sativa seed and green tea on kidney functions of rats suffering from chronic renal failure.

Parameters / Groups	Uric acid	Urea nitrogen	Creatinine
	mg/dl		
Control (-)	0.722 ^{h} ± 0.31	27.710 ^{g} ± 1.852	0.516 ^{i} ± 0.027
Control (+)	3.921 ^{a} ± 0.186	83.329 ^{a} ± 2.970	3.694 ^{a} ± 0.169
LPD containing 100 mg Vit. E /100g diet	3.297 ^{b} ± 0.148	72.575 ^{b} ± 2.646	3.113 ^{b} ± 0.139
LPD containing 200 mg Vit. E /100g diet	2.736 ^{d} ± 0.157	61.863 $^{c\,d}$ ± 3.658	2.527 $^{d\,e}$ ± 0.089
LPD containing 2.5 g Nigella Sativa seed/100g diet	2.560 $^{d\,e}$ ± 0.135	64.522 ^{c} ± 2.077	2.677 ^{d} ± 0.174
LPD containing 5 g Nigella Sativa seed/100g diet	2.176 ^{f} ± 0.120	54.145 ^{e} ± 3.866	2.116 ^{g} ± 0.080
LPD containing 2.5 g green tea/100g diet	2.936 ^{c} ± 0.154	69.202 ^{b} ± 2.770	2.900 ^{c} ± 0.147
LPD containing 5 g green tea/100g diet	2.534 ^{e} ± 0.109	58.838 ^{d} ± 2.957	2.324 ^{f} ± 0.086
LPD containing low levels of combination of all treatments.	2.316 ^{f} ± 0.211	59.008 ^{d} ± 2.087	2.374 $^{e\,f}$ ± 0.180
LPD containing high levels of combination of all treatments	1.928 ^{g} ± 0.083	47.505 ^{f} ± 2.683	1.799 ^{h} ± 0.080

LPD: *Low protein diet. Values are expressed as mean ± SD.*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05

In agreement with our findings Christiane et al. (1999**)** reported that, serum urea nitrogen and creatinine values were higher in all of the chronic renal failure CRF groups compared to the respective control groups. On the other side (Teplan et al., 2003) found that, serum urea nitrogen and proteinuria levels declined in groups of patients fed on low protein diet.

Ocak et al. (2007) reported that, vitamin E, vitamin C, and N-acetylcysteine decreased the blood urea nitrogen levels increased in rats suffering from chronic renal failure, although significant differences were detected only in the vitamins E or C groups. On the other hand, significantly (Badary et al., 2000) found that, treatment of rats with the main constituents of Nigella sativa (thymoquinone 10 mg/kg per day) for 5 days lowered serum urea, TG, and TC. Ali and Blunden (2003) reported that, the pharmacological actions of the crude extracts of the seeds and some of its active constituents; e.g. volatile

oil and thymoquinone have been include protection against nephrotoxicity induced by either disease or chemicals.

Ali (2004) reported that, Nigella sativa seeds or oil are characterized with possess strong antioxidant properties and was effective against disease and chemically-induced hepatotoxicity and nephrotoxicity. Treatments of rats with N. sativa did not cause any overt toxicity, and it decreased concentrations of urea and creatinine, while increased glutathione and total antioxidant status concentrations in renal cortex and enhanced growth when compared with the control.

Khan et al. (2003) found that, Nigella Sativa reconstruct kidney tissue after nephrotoxicity. Also (Al-Kubaisy and Al-Noaemi, 2007) found that, N. sativa oil may have affinity to repair liver tissue from free radicals and can be used as adjutant therapy. On the other hand, Hadjzadeh et al. (2007) found also, treatment of rats with ethanolic extract of NS reduced the number and size of calcium oxalate deposits; it could also lower the urine concentration of calcium oxalate.

Yokozawa et al. (2003) reported that, green tea GT polyphenol is effective against renal failure in rats. GT polyphenol administered to rats at a daily dose of 50 or 100 mg/kg body weight for 30 days with 2% L-arginine diet decreased serum levels of creatinine and urea nitrogen. Takako et al. (2003) confirmed that, green tea polyphenol would ameliorate renal failure induced by excessive dietary arginine by decreasing uremic toxin, and NO production and increasing radical-scavenging enzyme activity.

Table (3) shows the effect of low protein diets containing two levels of (vitamin E, Nigella Sativa seeds, green tea and their combination) on liver enzymes including Aspartate Amino Transferase AST, Alanine Amino Transferase ALT and Alkaline Phosphatase ALP (u/l) of rats suffering from chronic renal failure. Feeding rats on arginine diet (basal diet containing 2% arginine) increased the mean value of serum AST, ALT and ALP significantly $p \leq 0.05$, as compared to the rats fed on basal diet only.

The mean value of serum AST, ALT and ALP of the chronic renal failure group (control positive) increased by about 84.177%, 137.065% and 104.835%, than that of the negative control group. Serum AST, ALT and ALP decreased gradually with increasing the levels of vitamin E, Nigella Sativa seed, green tea and their combination.

All treated groups recorded significant decrease $p \leq 0.05$ in AST, ALT and ALP, as compared to the positive control group. On the other hand these treated groups showed significant increase in these parameters, as compared to the negative control group. The highest decrease in the mean values of AST, ALT and ALP recorded for the group which treated with the high level of the combination (200 mg vitamin E, 5g Nigella Sativa seed and 5g green tea)/100g diet, followed by the group which treated with 5g Nigella Sativa seed/100g diet.

In concordance with our findings, (Shaw et al., 1993) reported that, a number of endogenous protective factors such as catalase, superoxide dismutase, and glutathione peroxidase are active in the defense against oxidative cell injury by means of being free-radical scavengers or lipid peroxidation inhibitor. (Burton and Ingold, 1989) reported also, vitamin E is thought to be the major antioxidant found in membrane. Moreover, (Maurizio et al., 1992) shown that dietary supplementation with high doses of oral vitamin E for 3

weeks before the injury (as well as concurrent with the injury) can protect against the liver disease induced by chronic CCl4, administration.

Table (3): Effect of low protein diets containing vitamin E, Nigella Sativa seed and green tea on liver enzymes of rats suffering from chronic renal failure.

Parameters / Groups	AST	ALT	ALP
	U/l		
Control (-)	52.155 ^{h} ± 2.710	26.715 ^{h} ± 2.497	80.236 ^{h} ± 2.446
Control (+)	96.058 ^{a} ± 4.637	63.332 ^{a} ± 2.066	164.352 ^{a} ± 4.060
LPD containing 100 mg Vit. E /100g diet	84.528 ^{b} ± 3.792	54.153 ^{b} ± 2.500	143.767 ^{b} ± 4.373
LPD containing 200 mg Vit. E /100g diet	69.549 ^{e} ± 2.190	44.084 ^{d} ± 3.227	124.020 $^{d\ e}$ ± 4.608
LPD containing 2.5 g Nigella Sativa seed/100g diet	75.453 ^{d} ± 3.208	44.527 ^{d} ± 2.419	133.322 ^{c} ± 4.253
LPD containing 5 g Nigella Sativa seed/100g diet	63.985 ^{f} ± 1.428	37.172 ^{f} ± 2.310	115.694 ^{f} ± 4.702
LPD containing 2.5 g green tea/100g diet	79.705 ^{c} ± 3.059	49.215 ^{c} ± 2.675	139.569 ^{b} ± 4.542
LPD containing 5 g green tea/100g diet	67.898 ^{e} ± 1.548	41.147 $^{d\ e}$ ± 3.420	119.839 $^{e\ f}$ ± 4.662
LPD containing low levels of combination of all treatments.	71.098 ^{e} ± 3.255	39.971 $^{e\ f}$ ± 2.192	126.550 ^{d} ± 3.816
LPD containing high levels of combination of all treatments	59.771 ^{g} ± 1.956	33.480 ^{g} ± 2.189	106.883 ^{g} ± 3.295

LPD: *Low protein diet. Values are expressed as mean ± SD.*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05

Chalasani et al. (2008) showed that vitamin E produced tremendous improvement when administered to patients with non alcoholic steatohepatitis and in obese children with fatty non alcoholic liver disease. This can be seen from the report of Clinical Research Network who found that 96 weeks treatment with vitamin E in non diabetic and nonalcoholic steatohepatitis patients resulted in significant improvement.

Sanyal et al. (2010) reported that, in a randomly assigned 247 adults with non alcoholic steato hepatitis without diabetes, 84 received vitamin E at a dose of 800IU once daily for 9 weeks. It was discovered that vitamin E therapy was associated with a

significant higher rate of improvement than placebo. Serum alanine, aspartate aminotransferase and hepatic steatosis, lobular inflammation were reduced.

Kanter et al. (2005) found that NS treatment for 60 day decreased the elevated lipid peroxidation and liver enzyme levels. On the other hand, Burits & Bucer (2000) reported that the constituents of the essential oil of N. sativa possessed variable antioxidant activity and were also found to have an effective free radical scavenging activity, when tested for lipid peroxidation in liposomes, these results were evident from the significant reduction in serum (AST) and (ALT).

Al-Gharably et al. (1997) & Nagi et al. (1999) suggested that the black seed oil exhibited hepatoprotective effect against liver damage. Also, El-Mady et al. (2000) reported that Nigella sativa L. oil protects against induced hepatotoxicity; serum levels of (AST) and (ALT) were decreased when compared to the control group.

Abe et al. (2005) suggest that the drinking of green tea with a high catechin content may help to prevent and/or attenuate the development of a certain type of hepatitis. Also, Almurshed (2006) suggest that both black and green tea possess preventive effects against carbon tetrachloride CCl4 induced liver damage in rats.

El-Beshbishy et al. (2005) observed that the antioxidant property of flavonoidal compounds of GT extract contributes to decrease the oxidative stress in liver and increase the levels of antioxidant enzymes, superoxide dismutase, catalase and glutathione.

Sugiyama et al. (1999) who reported that feeding rats on diet supplemented with the powder of GT extract showed a significant decrease in serum AST and ALT compared with control. Studies by Arteel et al., (2002) also noted that there was a significant lowering in the activities of AST and ALT in rats treated with GT extract in the diet.

The results in Table (4) showed that, feeding rats on L-arginine diet increased the mean values of serum cholesterol and triglycerides significantly ($p \leq 0.05$), as compared to healthy rats fed on basal diet (145.460±4.808 and 82.590±6.022 mg/dl) vs. (89.689±4.413 and 45.283±4.488 mg/dl), respectively. All experimental groups showed significant decrease in serum cholesterol and triglycerides $p \leq 0.05$, as compared to the positive control group, except triglyceride of the group which treated with 100mg vitamin E/100g diet.

Treating rat with diet containing 2% L-arginine and the combination of high level of (vitamin E, Nigella Sativa seed and green tea) recorded the best results in decreasing the levels of serum cholesterol and triglyceride. This treatment decreased the mean values of serum cholesterol and triglyceride by about 32.233% and 29.357%, than that of the positive control group, respectively.

Table (5) showed that, the mean value of serum HDL-c decreased significantly $p \leq 0.05$, while LDL-c and VLDL-c increased in the positive control group, as compared to the negative control group. The data in this table showed significant increase in HDL-c of all treated groups, while LDL-c and VLDL-c decreased significantly, as compared to the positive control group.

Table (4): Effect of low protein diets containing vitamin E, Nigella Sativa seed and green tea on serum cholesterol and triglycerides of rats suffering from chronic renal failure.

Parameters / Groups	Cholesterol	Triglycerides
	mg/dl	
Control (-)	89.689 ^{g} ± 4.413	45.283 ^{f} ± 4.488
Control (+)	145.460 ^{a} ± 4.808	82.590 ^{a} ± 6.022
LPD containing 100 mg Vit. E /100g diet	124.640 ^{b} ± 4.128	75.659 $^{a\ b}$ ± 5.850
LPD containing 200 mg Vit. E /100g diet	112.081 ^{d} ± 4.058	67.438 $^{c\ d}$ ± 6.291
LPD containing 2.5 g Nigella Sativa seed/100g diet	118.533 ^{c} ± 5.399	70.601 $^{b\ c}$ ± 5.586
LPD containing 5 g Nigella Sativa seed/100g diet	104.434 ^{e} ± 3.414	62.603 ^{de} ± 6.358
LPD containing 2.5 g green tea/100g diet	121.059 $^{b\ c}$ ± 4.622	72.680 $^{b\ c}$ ± 6.704
LPD containing 5 g green tea/100g diet	107.031 $^{d\ e}$ ± 3.394	65.590 $^{c\ d\ e}$ ± 5.402
LPD containing low levels of combination of all treatments.	110.667 ^{d} ± 3.296	65.643 $^{c\ d\ e}$ ± 5.959
LPD containing high levels of combination of all treatments	98.566 ^{f} ± 2.053	58.344 ^{e} ± 2.474

LPD: *Low protein diet. Values are expressed as mean ± SD.*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05.

Treating rats with combination of (200mg vitamin E, 5g Nigella Sativa seed and 5g green tea/100g diet) recorded the best results in improving serum lipoproteins. This treatment decreased the mean value of serum LDL-c and VLDL-c by about 58.505% and 27.020%, respectively, while HDL-c increased by about 70.334%, than that of the positive control group.

In this respect, Mydlik et al. (2002) reported that, oxidative stress, increased lipid peroxidation and decreased activity of antioxidant system may contribute to the accelerated development of atherosclerosis in chronic renal failure patient during renal replacement therapy.

Parker et al. (1995) and Williams et al. (1992) reported that, vitamin E, the most effective lipid-soluble and chain-breaking antioxidant in nature, is known to lower the

plasma LDL-C concentration, have an inhibitory effect on early atherosclerosis by decreasing the LDL oxidation in hypercholesterolemic hamsters and rabbits, respectively. (Pryor, 2000) and Gey et al. (1991) confirmed that, correlates dietary vitamin E levels with cardiovascular disease incidence and mortality. A protective effect of vitamin E has been reported in 16 European study populations, in which a strong inverse correlation was observed between vitamin E levels and risk of CVD mortality.

Table (5): Effect of low protein diets containing vitamin E, Nigella Sativa seed and green tea on serum lipoproteins of rats suffering from chronic renal failure.

Parameters / Groups	HDL-c	LDL-c	VLDL-c
	mg/dl		
Control (-)	49.828 ^{a} ± 3.319	30.804 ^{h} ± 0.637	9.056 ^{f} ± 0.897
Control (+)	26.074 ^{f} ± 2.747	102.867 ^{a} ± 1.482	16.517 ^{a} ± 1.204
LPD containing 100 mg Vit. E /100g diet	31.703 ^{e} ± 3.287	77.805 ^{b} ± 1.595	15.131 $^{a\ b}$ ± 1.169
LPD containing 200 mg Vit. E /100g diet	39.311 ^{c} ± 1.514	59.282 ^{e} ± 3.080	13.487 $^{c\ d}$ ± 1.258
LPD containing 2.5 g Nigella Sativa seed/100g diet	35.997 ^{d} ± 2.030	68.416 ^{d} ± 2.701	14.120 $^{b\ c}$ ± 1.117
LPD containing 5 g Nigella Sativa seed/100g diet	40.573 ^{c} ± 1.582	51.280 ^{f} ± 2.885	12.520 $^{d\ e}$ ± 1.271
LPD containing 2.5 g green tea/100g diet	33.975 $^{d\ e}$ ± 2.752	72.548 ^{c} ± 1.928	14.535 $^{b\ c}$ ± 1.340
LPD containing 5 g green tea/100g diet	40.391 ^{c} ± 1.761	53.521 ^{f} ± 2.394	13.118 $^{c\ d\ e}$ ± 1.080
LPD containing low levels of combination of all treatments.	40.591 ^{c} ± 1.819	56.948 ^{e} ± 1.283	13.128 $^{c\ d\ e}$ ± 1.191
LPD containing high levels of combination of all treatments	44.413 ^{b} ± 0.961	42.684 ^{g} ± 1.121	11.668 ^{e} ± 0.495

LPD: *Low protein diet. Values are expressed as mean ± SD..*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05

On the other hand, (Giugliano, 2000) reported that, the cardioprotective effects of vitamin E are attributed to its antioxidant properties. Specifically, vitamin E is able to extinguish single oxygen species as well as to terminate free radical chain reactions. Upston et al. (1999) reported also, alpha-tocopherol acts as an antioxidant either by donating a hydrogen radical to remove the free lipid radical, reacting with it to form

nonradical products, or simply trapping the lipid radical. It is thought to exert its primary protective effects via the protection of LDL from oxidation.

Islam et al. (2000) decided that, the increased oxidative stress and attendant increased oxidizability of lipoprotein, such as LDL could contribute to the accelerated atherosclerosis in dialysis patients, since alpha-tocopherol in the major antioxidation LDL. Alpha-tocopherol supplementation may also provide a measure of protein against CVD in patient with chronic kidney failure on dialysis therapy.

With regard to N. sativa Al-Logmani and Zar (2011) reported that, N. sativa oil had favorably modified serum lipid profile in rats with significant decreases in total cholesterol, LDL-cholesterol, triglycerides and increased HDL. Moreover, Al-Logmani and Zari (2009) reported also, the effects of N. sativa oil on the tested physiological parameters in streptozotocin-diabetic rats are more beneficial after 7 weeks than after 3 weeks.

Zahida et al. (2011) reported that N. sativa has ability to reduce lipid profile which is a major risk factor for coronary artery disease in cardiac patients. The exact mechanism of action of N. sativa is not known, however, it has been proved that volatile oil of N. sativa has two main constituents i.e. nigellone and thymoquinone which play a key role in heart disease prevention (Abdel-Aal and Attia, 1993).

Lin et al. (1998), Yokozawa et al. (2002), Murakami and Oshato (2003) and Lin and Lin-Shiau (2006) who stated that green tea GT leaves in diet was associated with lower serum levels of TC, LDL-C and TG but higher serum levels of HDL-C. Hasegawa et al. (2003) reported that, this effect is attributed to a reduction in cholesterol absorption and to an increased excretion of biliary acids and cholesterol, another proposed action is the inhibition of cholesterol synthesis in the liver. Some in vitro studies, Coimbra et al. (2006) and Feng et al. (2002) reported that, green tea GT is particularly rich in epigallocatechin gallate EGCG, a powerful antioxidant. The EGCG exerts a protective effect against lipoprotein oxidation, namely, against LDL oxidation

Graham, (1992) and (Peterson, et al., 2005) stated that, fresh tea leaves are rich in flavanol monomers known as catechins such as epicatechins, which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weight in black tea. On the other hand, Wan et al. (2001) stated also, catechins have beneficial effects in prevention of cardiovascular diseases including LDL oxidative susceptibility, serum lipids and lipoprotein concentrations. While, Lee et al. (2008) found that, (-) gallocatechin gallate (GCG)-rich tea catechins have strong effects on lowering TC and TG concentrations in hyperlipidemic rats.

Table (6) shows the effect of low protein diets containing two levels of vitamin E, Nigella Sativa seeds, green tea and their combination on glutathione peroxidase, superoxide dismutase and catalase of rats suffering from chronic renal failure.

Feeding rats on diet containing 2% L-arginine decreased the mean values of serum glutathione peroxidase, superoxide dismutase and catalase significantly $p \leq 0.05$, as compared to the rats fed on basal diet. The mean values of glutathione peroxidase, superoxide dismutase and catalase increased gradually with increasing the levels of vitamin E, Nigella Sativa seed, green tea and their combination. The highest increase in these parameters recorded for the group treated with the combination of (200mg vitamin E,

5g Nigella Sativa seed and 5g green tea/100g diet), followed by the groups which were treated with diet containing 5g green tea/100g diet and 5g Nigella Sativa seed/100g diet, respectively.

Valko et al. (2007) reported that, antioxidants play a crucial role in providing defense against oxidative stress, an imbalance between the generation of reactive oxygen species and the endogenous antioxidant status. The antioxidant defense system can be broadly classified as enzymatic (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), and nonenzymatic (vitamins, enzyme constituents such as zinc and selenium, and other biomolecules, such as, albumin, ceruloplasmin, uric acid, and bilirubin) present in both intracellular and extracellular fluids.

Table (6): Effect of low protein diets containing vitamin E, Nigella Sativa seed and green tea on glutathione peroxidase, superoxide dismutase and catalase of rats suffering from chronic renal failure.

Parameters / Groups	Glutathione peroxidase (GPX) (mmol/dl)	Superoxide dismutase (SOD) (U/dl)	Catalase (CAT) (mmol/dl)
Control (-)	18.880 ^{a} ± 1.128	96.550 ^{a} ± 1.909	75.000 ^{a} ± 2.858
Control (+)	10.087 ^{g} ± 0.917	66.765 ^{f} ± 4.489	48.000 ^{g} ± 4.052
LPD containing 100 mg Vit. E /100g diet	12.850 ^{f} ± 0.552	73.945 ^{e} ± 5.479	53.350 ^{f} ± 4.168
LPD containing 200 mg Vit. E /100g diet	15.356 ^{d} ± 0.520	78.884 $^{c\,d\,e}$ ± 3.812	60.720 $^{c\,d}$ ± 3.177
LPD containing 2.5 g Nigella Sativa seed/100g diet	14.343 ^{e} ± 0.477	76.856 $^{d\,e}$ ± 5.932	57.680 $^{d\,e}$ ± 2.501
LPD containing 5 g Nigella Sativa seed/100g diet	16.704 $^{b\,c}$ ± 0.264	83.653 $^{b\,c}$ ± 2.997	63.678 $^{b\,c}$ ± 2.435
LPD containing 2.5 g green tea/100g diet	13.110 ^{f} ± 0.786	74.103 ^{e} ± 6.024	54.842 $^{e\,f}$ ± 4.292
LPD containing 5 g green tea/100g diet	15.916 $^{c\,d}$ ± 0.702	81.545 $^{b\,c\,d}$ ± 2.809	61.636 $^{c\,d}$ ± 2.149
LPD containing low levels of combination of all treatments.	15.683 ^{d} ± 0.559	80.525 $^{c\,d}$ ± 5.529	61.911 $^{c\,d}$ ± 2.109
LPD containing high levels of combination of all treatments	17.456 ^{b} ± 0.345	87.001 ^{b} ± 8.751	66.858 ^{b} ± 2.382

LPD: *Low protein diet. Values are expressed as mean ± SD.*
Values in the same column subscribed by different letters showed significant differences as calculated by ANOVA and LSD at P≤0.05.

Giray et al. (2003) confirmed that, the mechanisms of vitamin E supplementation caused an increase in glutathione peroxidase and super oxide mutase activities and decrease in thiobarbituric acid reactive substances.

Gavras and Salerno (1996) found that, black cumin causes a significant increase in CAT, GSH-Px and SOD activities in comparison with ischemia/reperfusion I/R group, suggesting that it might have an antioxidant effect through the increase in SOD, GSH-Px and CAT enzyme activities. Altogether, the mechanism of the protective effect of Black cumin on renal I/R injury can be explained by its antioxidant activity.

Moskaug et al. (2005) reported that, dietary polyphenols have been identified as potent antioxidants, and have also been shown to up- regulate the synthesis of intracellular glutathione and glutathione peroxidase activity, and attenuate mitochondrial oxidative stress. Babu et al. (2006) found that, green tea polyphenol supplementation in animal models of oxidative stress has also been shown to increase activities of antioxidant enzymes, specifically glutathione peroxidase, and increase concentrations of glutathione.

El-Beshbishy et al. (2005) observed that the antioxidant property of flavonoidal compounds of green tea GT extract contributes to decrease the oxidative stress in liver and increase the levels of antioxidant enzymes, superoxide dismutase, catalase and glutathione.

From these results, it was concluded that, vitamin E, Nigella Sativa seed, green tea leaves and the combination of them improved the nutritional and biological status of rats suffering from chronic renal failure.

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تأثير وجبات غذائية مختلفة منخفضة البروتين مدعمة بفيتامين هـ، بذور حبة البركة والشاي الأخضر على وظائف الكلى في الجرذان

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المستخلص: أجريت هذه الدراسة لبحث تأثير فيتامين هـ، بذور حبة البركة، أوراق الشاي الأخضر و خليطهم علي وظائف الكلي للفئران المغذاه علي وجبات تحتوى علي L أرجينين. شملت المجموعات التجريبية الرئيسة مجموعتين: المجموعة (1) "وعددها 6 جرذ" تم تغذيتها علي غذاء أساسي واستخدمت كمجموعة ضابطة سلبية، والمجموعة (2) "عددها 54 جرذ" تم تغذيتها على غذاء يحتوي على 2٪ أرجينين طوال فترة التجربة. بعد مرور أسبوعين من التغذية على الغذاء المحتوى على الأرجينين، تم تقسيم هذه المجموعة الي تسع مجموعات فرعية (6 جرذان لكل منهم). المجموعة الفرعية (1) تم تغذيتها على غذاء يحتوى على الارجينين، واستخدمت كمجموعة ضابطة إيجابية "مصابة"، المجموعات الفرعية (2 و 3) تم تغذيتها على غذاء منخفض البروتين (10٪ بروتين) مدعم بفيتامين هـ "100 و 200 ملجم / 100 جرام غذاء، على التوالي. المجموعات الفرعية (4 و 5) تم تغذيتها على غذاء منخفض البروتين مدعم بنسب "2.5٪ و 5٪ بذور حبة البركة/100جرام غذاء، على التوالي. المجموعات الفرعية (6 و 7) تم تغذيتها على غذاء منخفض البروتين مدعم بنسب 2.5٪ و 5٪ شاي أخضر/100 جرام غذاء، على التوالي. المجموعات الفرعية (8 و 9) تم تغذيتها على غذاء منخفض البروتين مدعم بنسب " 100 ملجم فيتامين هـ، 2.5٪ بذور حبة البركة و 2.5٪ شاي أخضر/100 جرام غذاء" و " 200 ملجم فيتامين هـ، 5٪ بذور حبة البركة و 5٪ شاي أخضر/100 جرام غذاء" على التوالي. إزداد المتناول من الطعام زيادة ذات دلالة إحصائية في المجموعات التجريبية المغذاة على 5٪ بذور حبة البركة والمجموعة المغذاة على التركيز المرتفع من خليط التدعيمات، ومن ناحية أخري إزدادت النسبة المئوية للزيادة في الوزن معنوياً في كل المجموعات المعاملة، باستثناء المجموعة المغذاة على 5٪ شاي أخضر. كل المجموعات المختبرة بتركيزين من (فيتامين هـ، بذور حبة البركة، شاي أخضر وخليطها) أدت إلى تحسن في كل معايير الاختبارات (وظائف الكلى، إنزيمات الكبد، صورة الدهون، الجلوتاثيون بيروكسيديز، سوبر أكسيد ديسميوتيز والكاتاليز)، وخاصة المجموعة التي تم تغذيتها على التركيز المرتفع من التدعيمات. تم استنتاج أن كل من فيتامين هـ، بذور حبة البركة، الشاي الأخضر وخليطهم يحسن الحالة الغذائية والبيولوجية في الفئران المصابة بقصور كلوي.

الكلمات المفتاحية: فيتامين هـ، بذور حبة البركة، أوراق الشاي الأخضر، أرجينين ، وظائف الكلي.