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Effect of treatments with green tea on the levels of serum iron, total iron binding capacity and transferring saturation percentage in sera of rabbits with hyperprotenemia with induced diabetes mellitus

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In the present study the serum iron level (SI) and transferring saturated (Tsat%) were significantly increased($p<0.05$) in rabbits with hyperproteinemia than control, while the total iron binding Capacity (TIBC) value was significantly decreased ($p<0.05$). In the second group diabetes mellitus (DM) also the SI level and Tsat% were significantly increased ($p<0.05$) than control before and after treatment with Green tea, while the (TIBC) values were significantly decreased ($p<0.05$) before and after treatment with Green tea. The (iron, TIBC and Tsat%) were significantly decreased after treatment with green tea ($p<0.000$). In the hyperproteinemia group, the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were insignificant differences. While the low density lipoprotein cholesterol (LDL-C) was significantly lower comparing with control. In the Diabetes Mellitus group, the TC, TG and vLDL, changes were insignificant, while LDL-C level was significantly increased and HDL –C level were significantly decreased in comparison with control. Malondialdehyde (MDA) levels in serum of rabbits with (DM) group before and after treated with green tea was significantly increased while changes in levels of MDA in hyperproteiemia and iron overload were insignificantly. The results indicated positive correlation between the levels of MDA and (HDL-C, LDL-C, Vit C, reduced serum glutathione (GSH), and uric acid (UA) and negative correlation in hyperproteinemia group. This study showed that (GSH) before and after 2 weeks treatment with green tea was significantly increased. While the rabbits left 1 month without treatment, the reduced glutathione was significantly decreased in hyperproteinemia group (Hp).

Key word: Diabetes mellitus, Hyperproteinemia, Trace element, Uric acid, Vitamines, Reduced glutathione, Malondialdehyde, Green tea.

Diabetes is not one disease but rather is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a reactive or absolute deficiency in insulin. Metabolic alterations caused by inadequate release in insulin are aggravated by an excess of glucagons. (Champ and Harvey, 1994).

The damage of cellular molecules such as proteins, lipids and DNA by oxidation has been linked to chronic pathologies, like diabetes, cardiovascular disease, chronic inflammations (arthritis), obesity and cancer. Oxidation occurs when harmful modified molecules "free radicals" attack cellular constituents. Free radicals are usually reactive species bearing one or more

unpaired electrons (e-) . Diet containing 20% or more of their total energy as protein have been labeled "high protein diets" and diets with (30-40) % as protein have been labeled "very high protein diet" (Jear *et al*., 2001). The mean maximal protein intake for the average weight U.S. male contain 125 to 186 g/day and for average female between 89 to 133 g/day consequently, very high protein diets for average U.S. male could range from 187 to 270 g/day and for female, 134 to 246 g/day(Ogden *et al*., 2004).

At this point, it should be noted that there is a physiological limit to the amount of protein that can be ingested before it comes toxic. A by product of dietary protein metabolism is nitrogen which in turn is converted into urea by the liver and then excreted by the kidneys into the urine. The upper limit of protein ingestion is determined by the liver's ability to synthesize urea. When nitrogen intake from dietary protein exceeds, the ability of the liver to synthesize urea, excessive nitrogen (as ammonia) spills into bloodstream causing hyperammonemia and toxicity. Additionally excess amino acids from the metabolism of high amounts of dietary protein may become toxic by entering the circulation causing hyper aminoacidemia (Cordain *et al*., 2000).

High protein diet may be ergogenic and facilitate improved performance because of the stimulatory effect of dietary branch chain amino acid (BCAA) upon muscle protein synthesis (Anthony *et al*., 2002). In addition to facilitating muscle synthesis during the post exercise, BCAA may also improve endurance performance by reducing mental fatigue by reducing the synthesis of brain 5-hydroxytryptamine: a substance that may promote central fatigue (Blomstrand, 2001).

Green Tea

Green tea is made from dried leaves of *Camellia Sinensis*, and along history of use, dating back to china approximately 5000 years ago. The three forms of teas, which are differentiated by processing method, are green, Oolong, and black tea. Of these, green tea undergoes the least amount of processing, and it has been used for medical purposes for thousands of years. Green tea has become well known for its antioxidant, antimutagenic and anticarcinogenic effects. Other possible benefits include treatment of cardiovascular disease (CVD), diabetes, dermatological problems, obesity and oral health problem (Ahmad and Muktar, 2001).

The present study was designated to determine hyperproteinemia via oxidative hypothesis through rabbits with induced diabetes mellitus and detection the following parameters for these states and study other relations.

- Determination of malondialdehyde (MDA).
- To demonstrate the role of scavengers of free radical.
- Determination of the serum iron, total Iron binding capacity (TIBC) and transferrin saturation percent (Tsat %), also copper (Cu) and zinc (Zn) as trace elements.
- Determination of uric acid and allantoin as scavengers of free radicals
- Estimate the influences of green tea as a medical herb for treating the two studies groups, diabetes mellitus and hyperproteinemia.

MATERIALS & METHODS

Animals and experimental design

The rabbits were divided into three groups.

1. ***The control group*** (n=5) was fed the control diet.
2. ***Hyperproteinemia*** (n=9) was fed the control diet in addition to increase soya bean by one third with total energy less than 13 Kj/Kg. This diet was chosen because diet rich in protein and soya bean has been used to produce hyperproteinemia in animals. More fiber was added (Wheat bran) to reduce the

difference in energy between this diet and control diet (Machin *et al*., 2004).

3. ***Diabetes Mellitus group*** DM (n=5), the rabbits were injected subcutaneous with alloxan [(2,4,5, 6) tetraoxyhexa hydro pyrimidine] to induce the diabetes after fasted 12 hrs. The compound was freshly prepared (100 mg/Kg) and administer for three days respectively so at the total dose was 300 mg/Kg body weight (Farjou and Al-Lami, 1988).The rabbits were injected intraperitonially with 15% glucose solution after (4-6) hrs each dose of alloxan and the animals had been taken 5% glucose with tap water for the first day only. Then left to relief and to eat enough after 7 days latter the rabbits had diabetes indicated by the positive glucose test in urine and the blood glucose had more than 200 mg/dL.

Treatment

To ensure that rabbits had diabetes infection, left it for two weeks before treatment with green tea 5% dissolved in double distilled water for 1 week (Kim and Miller, 2005). Treatment for hyperproteinemia nine rabbits were allocated randomly into two groups:-

Group I (five rabbits) fed orally 3% green tea dissolved double distilled water daily for 2 weeks and the volume was ranged between (1-4) ml according to body weight. (Grespy and Williamson 2004)

Group II: (four rabbits) left it without treatment for 1 month

Biochemical measurements

Some biochemical measurements to make out by using special enzymatic kits were performed as in the following:

1. Total cholesterol and serum triglyceride Determination : measured enzymatically (Young, 1995).
2. Determination of blood-glucose, ascorbic acid (vitamin C) and High-density lipoprotein (HDL) (Tietz, 1995).
3. Very low density lipoprotein (vLDL) vLDL concentration was calculated by divided triglycerceride by five (Fried wald *et al*., 1972)
4. Low-density lipoprotein (LDL) LDL concentration was calculated by the following equation (Ram, 1996).
   LDL = TC – (HDL+TG/5)
5. Determination of serum Malondialdehyde and reduced glutathione (Burtis & Ashwood, 1999) (Tietz, 1995).
6. Determination of Urea (Fawcett and Scott, 1960).
7. Determination of vitamin E (Hashim & schuttringer, 1966)
8. Determination of Total Iron Binding Capacity (TIBC) and serum iron:- measured by using a linear chemical kit (France)
9. Determination of serum-copper & zinc by Atomic Absorption Technique.
10. Determination of serum uric acid and allantoin by high performance liquid chromatography (Benzie *et al*., 1999).

RESULTS & DISCUSSION

Lipid profile

Increasing protein content in diet for 15-30% of the total energy, while carbohydrate content decreases, this can results in no significant differences of the total cholesterol, low density lipoprotein, and high density lipoprotein levels. A lowered levels of triglyceride has been formed to be significant ($P<0.05$) (Gannon and Nuttall, 2004). In the present study for Hyperproteinemia group, the total cholesterol, HDL, TG and vLDL were insignificant. While the LDL concentration was significantly lower ($P < 0.007$) in comparison with control. In the second group of animals Diabetes Milletus (DM) the total cholesterol, TG and vLDL did not significantly changes and LDL concentration was significantly increased ($P<0.002$), while the HDL concentration was significantly decreased ($p<0.000$) in comparison with control, Table 1.

Table 1. Comparison (Mean ±SD&P-test) of lipid profile in healthy rabbits control and hyperproteinemia & diabetes

Parameter	Control	Hyperproteinemia	Diabetes
TC mg/dL	62.32 ± 13.45	55.94 ± 6.13 , p =0.23	58.85±2.42, p = 0.4
LDL mg/dL	34.99±5.39	29.127±1.21 , p =0.007	44.277±3.61 , p<0.002
HDL mg/dL	22.03±6.22	20.82±4.34, p=0.67	9.026±4.05, p<0.000
TG mg/dL	5.68±1.13	29.96±9.71 , p=0.575	27.75±8.06, p=0.823
vLDL mg/dL	5.30±2.54	5.99±1.93 , p=0.573	5.55±1.61 , p=0.822

Table 2. Level of several non-enzymatic antioxidants such as (GSH, vit C, vit E & uric acid), urea and allantoin in serum of rabbit's control and hyperproteinemia group

Parameter	Control	Before treatment with green tea	After treatment with green tea for 2 weeks	Without treatment with green tea for 1 month
GSH µmole/dL	11.3±2.92	21.52±9.95 p=0.042	21.0±1.69 p=0.007	8.87±2.01 p=0.004
Vit.C mg/dL	0.01±0.0038	0.022±0.0052 p<0.000	0.042±0.0057, p< 0.000	-
Vit.E mg/dL	0.458±0.042	0.365±0.0196, p =0.002	0.521±0.117, p=0.05	-
Uric acid µmole/L	3.335±0.685	85.390±7.43, p<0.000	3.866±0.182, p<0.000	40.05±9.45, p<0.000
Urea g/L	0.445±0.086	0.75±0.270, p=0.029	0.288±0.068, p=0.001	0.4±0.072, p=0.0042
Allantoin µmole/L	325± 87.2	16580.5±1690.5, p<0.000	254.2±35.3, p<0.000	355.4±45.3, p<0.000

In diabetes mellitus , the levels of reduced serum glutathione (GSH) were significantly decreased (P< 0.004) before treatment comparing with control and significantly increased (P<0.0033) after treatment with green tea.

Decreased HDL and an elevated TG were recognized to be independent risk factors in dyslipidemic patients (Okopien *et al,* 2006). In a number of studies, an increase in LDL cholesterol levels has been found to be a risk factor for nephropathy and higher HDL cholesterol level may be protective against the development of albuminuria in the patients with type 1 diabetes (Molitch *et al*, 2006).

Glutathione (GSH) levels

The study showed that reduced serum glutathione (GSH) before and after two weeks treatment with green tea was significantly increased (P<0.042, 0.007). When the rabbits left one month without treatment, the reduced glutathione was significantly decreased (P<0.004) in hyperproteinemia group. This is may be due to the type of feeding based on the presence of soya was approximately 30% than that of control, Table 2.

Lipid Peroxidation

Malondialdehyde (MDA) levels in serum of rabbits with diabetes mellitus (DM) group before and after treated with green tea was significantly increased (p=0.0023, 0.030) respectively, Tab.4. While changes in levels of MDA in hyperproteinemia (p=0.26), Table 3.

The plasma MDA concentration is increased in patients on hemodialysis (Cristol *et al*., 1997) and can be explained by oxidative stress due to uremia (Roselaar *et al*., 1995). Elevated levels of lipid peroxidation products in serum of diabetic subjects and rats have been shown in several studies (Marra *et al*; 2002), higher levels of MDA is associated with decreased of antioxidnt activity and increased oxidative stress (Al-Zamely, 2001). In the first group hyperproteinemia (HP), MDA levels were positively correlated with GSH (r=0.63, p=0.0014), uric acid (r=0.929, p<0.000), allantoin (r=0.948, p<0.000), vit C (r=0.822, p<0.000), HDL (r=0.836, p<0.000) & LDL (r=0.875, p<0.000) and negatively correlated with vit.E (r=-0.655, p=0.001) & copper (r=-0.99, p<0.000). In the second group diabetes mellitus (DM), MDA levels were positively correlated with HDL (r=0.99, p=0.01), GSH (r=0.93, p=0.002), vit. C (r=0.92, p<0.000), vit. E (r=0.94, p=0.0027), uric acid (r=0.96, p=0.008), allantoin (r=0.92, p=0.003), zinc (r=0.83, p<0.000), LDL (r=0.88, p<0.000), TG(r=0.99, p=0.003), B. sugar (r=0.97, p<0.000) and copper (r=0.99, p=0.057).

Table 3. level of several trace elements (Iron, copper and zinc) and Malondialdehyde in the serum of rabbits control and hyperproteinemia group

Parameter	Control	Before treatment with green tea	After treatment with green tea
MAD µmole/L	1.26±0.255	1.41±0.185, p=0.26	1.38±0.11, p=0.31
Serum Iron(SI) µg/dL	21.59±3.88	37.87±4.72, p<0.000	-
Total Iron Binding Capacity (TIBC)	920±32.89	820±59.59, p=0.005	-
Transferrin Saturation% (Tsat%)	2.33±0.344	4.6±0.30, p<0.000	-
Copper (Cu) ppm	1.18±0.057	0.58±0.058, p<0.000	0.89±0.12, p<0.000
Zinc (Zn) ppm	0.64±0.13	0.41±0.105, p=0.004	0.67±0.103, p=0.001

Iron and other indices of Iron Metabolism
Total iron binding capacity and transferrin saturation%

In the present study the serum iron level and transferrin saturated (Tsat%) were significantly (p<0.05) increased in rabbits with Hyperproteinemia than control, while the total iron binding Capacity (TIBC) value was significantly (p<0.05) decreased Table 3.

In the second group diabetes mellitus (DM) also the SI level and Tsat% were significantly (p<0.05) increased than control before and after treatment with Green tea, while the TIBC values were significantly (p<0.05) decreased before and after treatment with Green tea, Table 4.

Trace elements (Copper and Zinc)

The results of trace elements indicated that the levels of copper and zinc before treatment were significantly decreased (p<0.000, p=0.004) comparing with control and after treatment with green tea were significantly increased (p<0.001, p<0.000), in the hyperproteinemia group. Table 3. In the diabetes mellitus group the level of copper was significantly decreased

($p<0.05$) before treatment but the changes after treatment with green tea was insignificant differences, while the levels of zinc before and after treatment with green tea were insignificant differences ($p>0.05$). Table 4.

Vitamin C (Ascorbic acid) levels

The result of Vit.C level was significantly increased ($p<0.000$, $p<0.000$) before and after treatment with green tea in hyperproteinemia group, Table 2. In DM group the level of Vit.C before treatment was not changed but after treatment with green tea was significantly increased ($p=0.005$) Table 4. Several studies have been showed that patients with diabetes have lower serum levels of vitamin than non-diabetic subjects (Tousoulis *et al*., 2003).

Reduced levels of serum vitamin C in diabetic patients may be due to:-

1. Reduce renal reabsorption of vitamin C induced by hyperglycemia.
2. The competition between glucose and vitamin C for the uptake into certain cells and tissues.
3. The activity of polyol pathway that inhibited affectively by utilizing high level of vitamin C to avoid hyperglycemia.
4. And possible secondary depletion due to increased oxidative stress have been proposed (Will and Byers, 1996).

Vitamin E (α -tocopherol) levels

In hyperproteinemia group, the level of Vit. E was significantly decreased ($p=0.002$) before treatment comparing with control and significantly increased ($p=0.05$) after treatment with green tea. Table 2.

In DM group, the level of Vit. E had insignificant differences before treatment comparing with control but it was significantly increased ($p=0.00117$) after treatment by green tea, Table 4.

Table 4. Level of several non-enzymatic antioxidants such as (GSH, vit.C, vit.E, uric acid), blood sugar, allantoin, (Iron, TIBC, Tsat%), malondialdehyde, copper and zinc in serum of control and Diabetes mellitus

Parameter	Control	Before treatment with green tea	After treatment with green tea
GSH μmole/L	11.3±2.92	5.6±1.516, p=0.004	17.2±4.65, p=0.0033
Vit.C mg/dL	0.0104±0.003847	0.010±0.0038, p=1.0	0.017±0.0034, p=0.005
Vit.E mg/dL	0.458±0.042	0.436±0.023, p=0.36	0.53±0.037, p=0.00117
Uric acid μmole/L	3.335±0.685	4.05±1.329, p=0.31	2.36±0.8, p=0.041
B. sugar mg/dL	104.98±6.785	221.23±12.86, p<0.000	138.22±27.76, p=0.0017
Allantoin μmole/L	325± 87.2	638.97±280.14, p=0.044	339.33±117.66, p=0.05
MAD μmole/L	1.26±0.255	3.55±1.14, p=0.0023	1.93±0.174, p=0.030
Serum Iron(SI) μg/dL	21.59±3.88	56.21±3.78, p<0.000	42.26±6.92, p<0.000
Total Iron Binding Capacity μg/dL (TIBC)	920±32.89	823.12±45.31, p=0.004	760.62±49.88, p=0.004
Transferrin Saturation% (Tsat%)	2.33±0.344	6.82±0.254, p<0.000	5.55±0.579, p=0.001
Copper (Cu) ppm	1.18±0.057	0.93±0.21, p=0.037	1.1±0.41, p=0.420
Zinc (Zn) ppm	0.64±0.13	0.56±0.26, p=0.55	0.50±0.14, p=0.37

Uric acid and Allantoin

In hyperproteinemia group, the results of (uric acid, allantoin and urea) were significantly increased ($p<0.000$, $p < 0.05$ and $p < 0.000$) respectively before treatment with green tea comparing with control and significantly decreased $p<0.000$, $p < 0.001$ and $p < 0.000$ respectively after 2 weeks of treatment. After 1 month without treatment, the levels of (urea, uric acid and allantoin) were significantly decreased ($p=0.0042$, $p<0.000$ and $p<0.000$) respectively. Table 2.

In the DM group, the levels of (allantoin and blood sugar) were significantly increased ($p=0.044$, $p<0.000$) and uric acid was insignificantly before treatment with green tea in comparison with control and the levels of (uric acid, allantoin and blood sugar) were significantly decreased ($p=0.014$, $p=0.05$, $p=0.0017$) after treatment with green tea, Table 4.

The results for this study reported different direct correlation between serum uric acid and serum of several parameters such as : MDA, allantoin, GSH and vitamin C, whereas there was direct correlation but not significant with copper, while the others were significant negative correlation between serum uric acid levels and vitamin E, HDL and LDL – cholesterol in the first group (HP). In the second group (DM) : the direct correlation between serum uric acid and copper, HDL, LDL, vitamin E, blood sugar, whereas there was direct correlation but not significant with allantoin, while there were significant negative correlation between uric acid levels and MDA , vitamin C and GSH.

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