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ESTIMATION INFLUENCES OF GREEN TEA AS A MEDICAL HERB FOR TREATING DIABETES MELLITUS

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ABSTRACT: Hyperglycemia means glucose toxicity and increases the ability of glucose to glycate proteins .The present study is an attempt to confirm that diabetes is a member of oxidative stress and to show the effect of green tea as therapeutic herbal on such group. This study enrolled twelve male rabbits divided into two groups, G (1,n=6) as control group ,G(2,n=6) as diabetic group under treatment with green tea.The result reveled that trace element such as copper and zinc shows no change in there levels in both groups while the biological level of (GSH, MDA,Vit.C, and Vit. E) were increased scientifically after treatment while decrease levels uric acid and allontion were noticed .

Key words: Diabetic, Antioxidants, Green tea , Glutathione , Trace elements , Lipid peroxidation

INTRODUCTION

Diabetes Mellitus (DM) 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 (Champe & Harvey , 1994) .

Oxidative damage results from oxidative stress when an imbalance in oxidant-antioxidant equilibrium develops via impairment in the regeneration of antioxidant capacity or in association with increased production of ROS (Sies , 1991) . The cause of diabetes mellitus is not fully understood ,Recently increasing evidence suggest that free radicals formation are involved in the pathogenesis of diabetes and development of diabetic complications. (Coleman et al., 1989) In type 1, β- cells are tending to be destroyed by free radicals because of the low antioxidant enzyme nature. Immune-effect cells such as macrophages, T-cells, natural killer cells and β- cells are believed to produce free radical that causes damage to β- cells.

Defense of the body using non-enzymatic antioxidants: such as Glutathione (GSH), ascorbic acid vitamin C, α- tocopherol vitamin E , uric acid ,fatty acids, Some of trace and ultra trace elements such as zinc, copper, and iron and the following schematic show the relation ship between these antioxidants at scavenging free radical species while vitamin C exhibits its antioxidant action by donating hydrogen atom to free radical. (Atalay & Laaksones , 2002)

The antioxidant defense of the body using enzymatic antioxidants such as catabolism of purines by uricase in animals and oxidation by reactive oxygen species to formation allantoin and other products. (Fig.2).

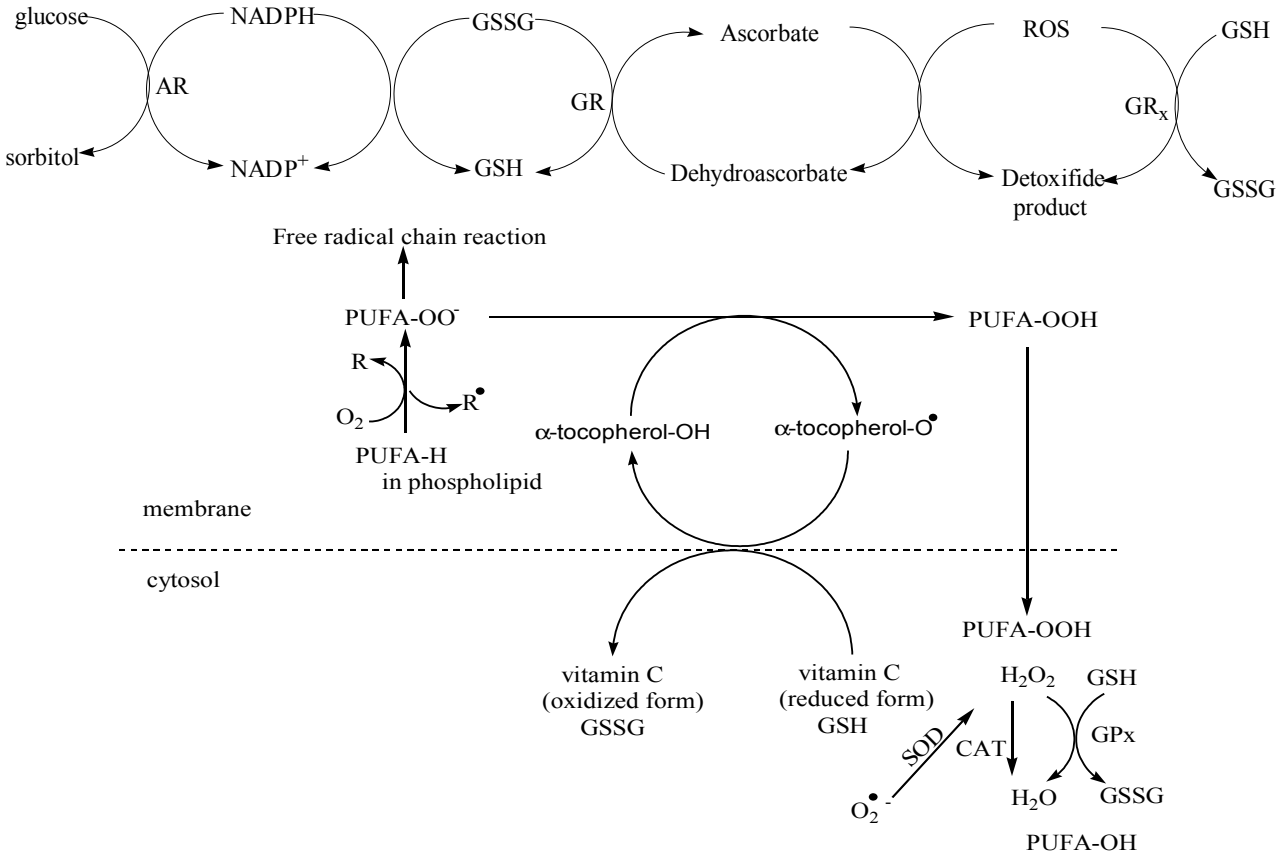

Fig (1): The reactions between ascorbate and α-tocopherol in trapping free radicals (Murray etal., 2000)

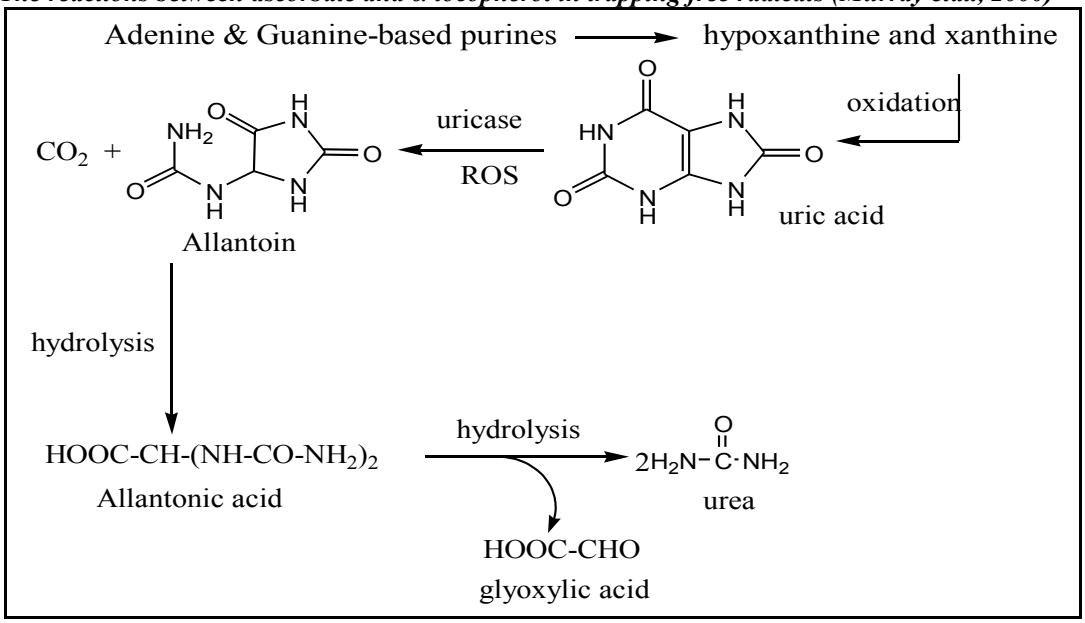

Fig (2):Catabolism of purines (Zitnanova et al.,2004)

The Polyol Pathway

Several studies have shown that glucose at abnormally high intracellular concentration is preferentially metabolized via the polyol pathway. Glucose is reduced by the aldose reductase into sorbitol which is oxidized to fructose by the sorbitol dehydrogenase. NADPH is required for the activity of aldose reductase, therefore, an enhancement of polyol pathway result in an intracellular depletion of NADPH, which is a cofactor of glutathione reductase for regenerates reduced glutathione . Thus, an intracellular depletion of this cofactor, by decreasing the activity of glutathione reductase, decreases reduced glutathione which protects towards oxygenated free radical-induced damage (Greene & Stevens, 1996) . Depletion of NADPH also leads to decreased $NO^{\bullet}$.Synthesis because NADPH is cofactor of NO-synthase, which synthetize $NO^{\bullet}$ from L-arginine. An increase in NAD^{+} / NADH ratio is linked to $O_2^{\bullet -}$ formation via the reduction of prostaglandin G2(PGG2) to PGH2 by prostaglandin hydroperoxidase that uses NADH or NADPH as reducing co substrate. (Bonnefont et al., 2000).

Auto Oxidation Reaction (Lipid Peroxidation)

All biomolecules can attack by free radicals, but lipids more susceptible by attacking free radical react with polyunsaturated fatty acid by the formation of radical chain reaction which is known as lipid peroxidation. (Murray et al., 2000)

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. (Ahmed & Mukhtar, 2001).

Constituents of green tea

Catechins – the polyphenol are generally considered to be the most important elements of green tea.Catechins found in green tea include Epicatechin (EC), Epicatechin.3-gallate (ECG), Epigallocatechin(EGC) and epigallocatechin -3-gallate (EGCG). Other polyphenols include flavanols, carotenoids, lignin, protein, minerals (aluminium and manganese are particularly prominent), Caffeine and very small amounts of other methylxanthine such as theophylline, theobromine and L-theanine (Ahmed & Hasam, 1999) . Flavonids (polyphenols) proven medicinal properties include antioxidant, antiallergic antibacterial and antiviral effects. They also have ability to strengthen veins and decrease their permeability (Chopra & David, 2000) . Tea tannins called catechins (polyphenols) have antiseptic and antioxidant properties, they are able to form complexes with other molecules thereby detoxifying the system (Van Wyk et al., 1997) . A recent study showed that black, green and Oolong tea are all extremely good sources of vitamin C (Du Toit et al., 2001).

Diabetes with green tea

Since blood sugar tends to increase with age, the ability to significantly lower blood glucose has been confirmed also in studies using diabetic rats (Wu Ly, et al., 2004) . An aqueous solution of green tea polyphenols was found to inhibit lipid peroxidation (LP), scavenge hydroxyl and superoxide radical in vitro by increased antioxidant potential as seen from improvement in superoxide dismutase and glutathione level (Kuttan , 2002).

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

1. Determination of malondialdehyde (MDA), an end product of lipid peroxidation.
2. To demonstrate the role of scavengers of free radical, the study included assessment of glutathione (GSH), vitamins (E&C).
3. Determination of uric acid and allantoin as scavengers of free radicals.
4. Determination of the serum copper (Cu) and zinc (Zn) as trace elements.
5. Estimate the influences of green tea as a medical herb for treating diabetes mellitus investigate the correlation between oxidant MDA and antioxidants also between uric acid and other oxidants and antioxidants.

MATERIALS AND METHODS

Experimental Animals:

The experimental animals (male domestic rabbits *Oryctalagus Cuniculus*) were treated as following:

Rabbits housing:

Twelve male rabbits (1.9-2.2) Kg body weight and (4-6) months of age were purchased from local market/Hilla. The rabbits (2 rabbits/cage) housed under controlled animal conditions of temperature (25±3 C˚) and relative humidity (50±5)%.Upon arrival, animals were adapted for two weeks and were maintained on a regular feed (control diet) consist of alfalfa and concentrate pullet (Crude protein 10%, ground soybean 20%, wheat flour 35%, corn 35%, mineral & vitamins 1 gm/Kg). Total energy was 13.6 Kj/Kg protein.

Experimental design

The rabbits were divided into two groups:

1. *The control group*: (n=6) was fed the control diet.
2. *Diabetes Mellitus group DM*: (n=6), 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 & 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, leave it for two weeks before treatment with green tea 5% dissolved in double distilled water for 1 week. (Kim & Miller , 2005)

1. *Blood sampling*: Fasting blood samples were collected from marginal ear vein by using syringe (5ml) G23 every two weeks (for each experiment). Blood was allowed to clot 20 minutes then centrifuged at (4000 rpm=0.894Xg) for 10 minutes the serum sample were tested for biochemical measurements.
2. *Biochemical measurements:* Some biochemical measurements to make out by using special enzymatic kits were performed as in the following:

- Total cholesterol Determination
- Determination of serum triglyceride (TG)
- Measurement of serum lipoprotein cholesterol
    - High-density lipoprotein (HDL)
    - Very low density lipoprotein (VLDL).
    - Low-density lipoprotein (LDL).

- Determination of serum Malondialdehyde & reduced glutathione (Burtis CA., Ashwood ER., 1999)
- Determination of ascorbic acid (vitamin C)& Blood-Glucose (Tietz , 1995)
- Determination of vitamin E (Hashim & Schuttringer, 1966)
- Determination of serum iron & total iron binding capacity (TIBC)by using a linear chemical kit (France).
- Determination of serum-copper & zinc by Atomic Absorption Technique.
- Determination of serum uric acid & allantoin by HPLC Technique (Benzie et al , 1999)

RESULTS AND DISCUSSION

1. 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 & Nuttal , 2004) . 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, fig (3).

The depletion of total cholesterol when compared with control supported the hypothesis that considers glucose increasing was due to increase in the cholesterol synthesis (Hadwan, 2002) . 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)

2. Glutathione (GSH) Levels

In diabetes mellitus group 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, Tab. (1).

3. Malondialdehyde (MDA)

Dien conjugation (DC) and thiobarbituric acid reactive species (TBARS) are widely used as indicators of lipid peroxidation .DC is a measure of early events of lipid peroxidation reactions whereas TBARS measure end product of lipid peroxidation, (MDA), that is a goal marker of cell membrane damage following ROS production during stress. (Vasankari et al., 1995) 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.(1). The plasma MDA concentration is increased in patients on hemodialysis and can be explained by oxidative stress due to uremia , hemodialysis treatment and impaired antioxidant status (Cristol et al ., 1997) (Roselaar et al., 1995) (Hassel & Yong, 1998) (Peuchant et al., 1994).

In other study illustrate that MDA concentration did not increased in the absence of iron application but showed a significant decrease at an approximate rate of 0.03 (µmole/L/h) within 180 min, this decreases did not result from plasma volume changes but rather from elimination of MDA by hemodialysis treatment because of its relatively low molecular mass (Roob et al., 2000) . Antioxidant vitamins act as quencher for free radicals, thus, increased levels of serum antioxidant vitamins could be reduce the level of MDA, the end product of lipid peroxidation, however the new trend in the management of diabetes and decrease lipid peroxidation degree is to use therapeutic antioxidant and scavengers like vitamin E and vitamin C (Tutuncu et al., 1998).

Elevated levels of lipid peroxidation products in serum of diabetic subjects and rats have been shown in several studies, higher levels of MDA is associated with decreased of antioxidnt activity and increased oxidative stress (Domingues et al., 1998) (Marr et al., 2002) (Sahin et al., 2001) . Elevated level of MDA might increase susceptibility of diabetic patients to cardiovascular complications. The treatment with taurine (2-amino ethane sulfonic acid) reduces iron –mediated myocardial oxidative stress, preserves cardiovascular function improves survival in iron overloaded mice, protects reduced glutathione levels and can also react directly with a variety of cytotoxic aldehydes including MDA (Oudit et al., 2004) .

The results of this study reported different correlations between serum MDA and other parameters in three groups. In the present 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 ($r=0.99$, $p=0.057$).

Antioxidant vitamins act as quencher for free radicals, thus, increased levels of serum antioxidant vitamins could be reduce the level of MDA, the end product of lipid peroxidation ,and it is worth to mention that the new trend in the management of diabetes and decrease lipid peroxidation degree is to use therapeutic antioxidant and scavengers like vitamin E and vitamin C (Tutuncu et al., 1998) .

4. **Trace Elements (Copper and Zinc)**

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 ($p>0.05$) Tab (1) .There has been many studies reported that serum Zn and Cu levels are decreased in chronic renal failure (CRT) undergoing hemodialysis. In their study serum, Zn levels were significantly lower than in the control, whereas serum Cu levels were significantly higher in the control than in the CRF patients. (Kaminska et al ., 1994) Plasma Zn was significantly lower in the hemodialysis (HD) patients while plasma Cu was within normal range. (Ongajooth et al., 1996) In another study indicated that both Zn and Cu levels were significantly lower in HD patients than in the control (Yilmaz et al., 2000) .

Decreased levels of serum zinc in diabetic patients may be due to reduced levels of antioxidant enzymes or due to excessive urinary out put especially in patients with diabetic nephropathy , gastrointestinal malabsorption or genetic factors or signs of infection during which Zn will act as a defense mechanism ,while Zinc administration, which inhibits lipid peroxidation and increases glutathione availability by preventing the oxidation of glutathione also has ability to inhibit the uptake of iron. (Farinati et al ., 2003).

5. **Vitamin C (Ascorbic acid) Levels**

Serum vitamin C is a free radical scavenger by interacts with free radicals in the water compartment of cells as well as in the fluid between cells .It is considered to be one of the most important antioxidants in extra cellular fluids. 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. (1). Several studies have been showed that patients with diabetes have lower serum levels of vitamin than non-diabetic subjects which 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 has been proposed.

Ascorbic acid depletion results from irreversible oxidation of ascorbic acid by iron. In ascorbic acid synthesizing species such as the rat, the decreased plasma levels of ascorbic acid may be that iron overload affected the rate of ascorbic acid synthesized in the liver (Tousoulis et al., 2003) (Wang et al., 1994) (Dabbagh et al., 1994).

6. **Vitamin E (α -tocopherol) Levels**

Serum vitamin E is a principal modular of free radicals activity, it is a potent antioxidant acting as scavenger of reactive oxygen species (ROS) and reduces oxidative stress. In diabetes mellitus 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. (1).

Reduced level of serum vitamin E possible is due to increased utilization of this antioxidant in neutralizing free radicals in diabetic patients, thus supplementation of vitamin E to diabetic patients could reduce level of oxidative and diabetic complications by increases glutathione and lowers lipid peroxidation. (Jain et al., 2000) The present study showed that dietary Iron overload (IO) led to a marked decreased in serum α-tocopherol levels which is agree with previous data (Hoeldtke et al., 2003) .

Vitamin E supplements could fully prevent iron –induced lipid peroxidation. Also correction of impaired vitamin C status may protentiate the vitamin E effect through regeneration of vitamin E from the vitamin E radical formed during the antioxidation action of vitamin E (Roselaar et al., 1995) .

7. **Uric Acid and Allantoin**

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. (1) .

Table (1);- 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 Lipid Peroxidation

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
MDA 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

In DM group, the results showed positive correlation between HDL-C (r=0.84, p<0.002), LDL-C (r=0.874, p<0.000), Vit.E (r=0.612, p<0.000), blood sugar (r=0.899, p<0.000), copper (r=0.730, p=0.002) and allantoin (r=0.24, p<0.001) levels and negative correlation with GSH (r=-0.669, p<0.002), Vit. C(r=-0.78, p<0.000) and MDA (r=-0.99, p<0.001) levels , Table.(2).

Table. (2): The correlation between the levels of uric acid µmole/L with other variables in Diabetes mellitus group.

Correlation of uric acid	Diabetes mellitus Correlation coefficient (r)	
	r	P value
MDA µmole/L	-0.99	0.0013
Copper ppm	0.37	0.002
Allantoin µmole/L	0.24	0.0011
Vitamin C mg/dL	-0.785	0.0004
GSH µmole/L	-0.669	0.0029
LDL mg/dL	0.874	<0.000
Vitamin E mg/dL	0.612	<0.000
HDL mg/dL	0.842	0.0024
Blood sugar	0.889	<0.000

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. Nitric oxide over production may be occurred in patients with poorly controlled type 1 diabetes and led to increased peroxynitrite and lipid peroxidation and suppressed uric acid. (Marr et al., 2002) There was a significant increase of uric acid and allantoin levels in plasma of children with down syndrome (DS) , during increased oxidative stress – (ROS) can be able to contribute the formation of allantoin from uric acid and the oxidation of urate to allantoin implied that urate was scavenger of ROS (Zitnanov et al., 2004).

No significant correlation was found between allantoin and urate while urate concentration have been reported to correlate negatively with vitamin E concentration and positively with lipoperoxides as (TBARS) (Benzie et al., 1999) .The allantoin – uric acid pathway play a critical control point in the reaction of nitric oxide and superoxide anion in cells production uric acid – derived free radical which is possibly prevented by uricase (Vasquez-Vivar et al ., 2004) . The activity of uricase enzyme is less than other antioxidant enzymes (Cu-Zn,SOD, Mn-SOD, catalase) in oxidative stress. The alteration in lipid profiles showed a remarkable correlation with uricase activity which expressed as allantoin to uric acid ratio.There was a positive correlation between uricase activity and HDL and a negative correlation with LDL , Tab.(2).

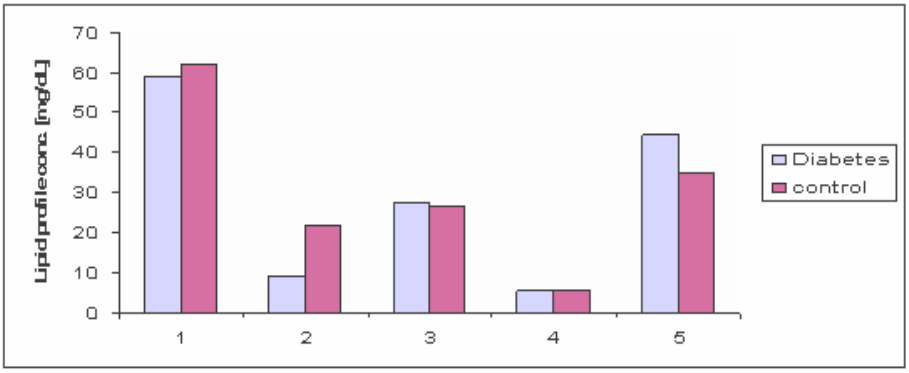

Figure (3): Level of Lipid Profile in the Serum of Healthy Control and Experimental Rabbits with Diabetes Mellitus (DM).

The values are the mean ± SD.
Column (1) Total cholesterol (TC) ;
Column (2) High density lipoprotein (HDL) ;
Column (3) Triglyceride (TG) ;
Column (4) Very low density lipoprotein (VLDL)
Column (5) Low density lipoprotein (LDL).

CONCLUSION

The observation in diabetic rabbits increment in (MDA ,LDL, serum iron , B.sugar, uric acid & allantoin), and decrement in (GSH, HDL, zinc and Copper), while the vitamins (C and E) were unaffected .Decreased levels of GSH, HDL and zinc are the powerful indicators to evaluate the oxidative stress syndrome in diabetic than non-diabetic.

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