



## Potentiality of Vitamin C and / or *Lupinus termis* to Modulate Blood Glucose level and Oxidative Stress Status of Alloxan-induced Diabetic Rats

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### Abstract

*Lupinus termis* (*termis*) has long been utilized as integral food in developing countries prior to being recognized as a hypoglycemic agent and vitamin C is known as a potent antioxidant. Here we tested the hypothesis that the administration of vitamin C and *termis* solely or in combination modulates the adverse effects on blood glucose and vitamin C levels, liver and kidney functions as well as oxidative stress biomarkers of diabetes induced by alloxan in Wistar rats. Fifty male Wistar albino rats (110.0 ± 15.0g) were fed on a standard experimental diet and allotted to five groups; non diabetic control group (C), diabetic control (D) and three diabetic groups each of which were given oral vitamin C (100mg/kg body weight, DVC) or *termis* (75mg/100g body weight, DT) or their combination at the same levels (DVCT) for four weeks. Induction of diabetes significantly ( $P < 0.05$ ) increased the blood glucose and decreased the vitamin C levels as compared to the non diabetic controls, associated with a significant depression in the activity of the kidney and liver function and in the oxidative stress biomarkers. All treatments had positive effects on the measured parameters; however, the combined treatment surpassed the sole treatments in such modulating effects. In conclusion, the combination of vitamin C and *termis* appears to be a beneficial strategy not only to control hyperglycemia but also to modulate the negative effects of diabetes on kidney and liver function and oxidative stress status.

**Keywords:** Diabetes, Albino rats, vitamin C, *L. termis*, oxidative stress biomarkers.

### Introduction

The number of patients suffering from diabetes mellitus (DM) has significantly increased mainly in developing countries and it is becoming a serious threat to mankind<sup>1</sup>. DM is a chronic metabolic disease accompanied by high glucose levels as a consequence of lack endogenous insulin secretion. Moreover, there is a progressive lipid peroxidation concomitant with defects in antioxidant defense mechanisms determinants oxidative stress status<sup>2,3</sup>. Thus, in diabetic patients, the increase of oxidative stress has been implicated in different pathological complications such as neuropathy, kidney and liver damage<sup>4</sup>. The World Health Organization (WHO), declared that about 80% of the world population is essentially dependent on herbal traditional drugs which came from the virtue of pragmatic knowledge<sup>5,6</sup>. In Egypt, traditional medicine recommends a different herbal recipes, either to exert a hypoglycemic effect such as *Trigonella foenum*<sup>7</sup> or to increase the serum insulin level such as, *Zizyphus*<sup>8</sup>. *Termis* (*Lupinus termis*) exhibited noticeable elevation on the level of serum insulin in alloxan-diabetic animals as well as in diabetic subjects<sup>9,10</sup>. Vitamin C is recognized as potent natural antioxidant that can afford primary as well as secondary protection against the oxidative damage of lipids and lipoproteins<sup>11</sup>. The increase of the oxidative stress biomarkers has been associated with the decrease of plasma vitamin C levels that can occur in several conditions such as diabetes mellitus<sup>12</sup>. Therefore, the current study is adopted to test the hypothesis that the administration of an antioxidant

agent like vitamin C or one of the Egyptian traditional remedies for diabetes like *termis* and their combination modulates the adverse effects of hyperglycemia and oxidative stress in alloxan-induced diabetes in Wistar albino rats.

### Material and Methods

**Chemicals and drugs:** The chemicals and drugs utilized in this study were of analytical grade. Alloxan was supplied by Sigma chemical Company (St. Louis U.S.A.). Vitamin C (ascorbic acid 100 mg/ tablet) obtained from the Epico Company, Egypt.

**Experimental animals:** All animals were humanely treated and the study design was approved by the Animal Use in Scientific Research Ethical Committee, Cairo University. Fifty adult male Wistar Albino rats, with average weight of 110.0 ± 15.0 g were obtained from Ophthalmology Institute, Giza, Egypt and used in this study. Animals were allotted to 5 homogenous groups and housed individually in suitable plastic cages in a room maintained at 22 ± 3 °C with a 12 h light: dark cycle. Food and water were provided ad-libitum. Before the commencement of a four weeks experimental period, the animals were subjected to one week acclimatization period.

**Herbal preparation:** The seeds of *termis* (*lupinus termis*) were obtained from a local herbal store identified and authenticated by Dr. M. M. Saad Assistant Professor, Department of Agricultural Botany, Cairo University, Giza, Egypt. The seeds were washed, dried at 37°C for 24 h, and finely grinded. Five

grams of the T powder is suspended in 100ml of double-distilled water and 1.5 ml/100g body weight (75mg/ 100g BW) was orally given to the treated groups<sup>9</sup>.

**Experimental Diet and Design:** All animals were fed on a standard experimental diet which was prepared according to the requirement of laboratory animals 13 and composed (g/Kg) of corn starch 150, casein (>85% protein) 200, sucrose 500, corn oil 50, cellulose 50, mineral premix 35, vitamin remix 10, Dl-methionine 3 and choline chloride 2.2. Experimental animals were randomly allotted into 5 treatments each of 10 rats as follow: i. Group (C): fed on a standard basal diet (control), ii. Group (D): alloxan-diabetic group and was given 1.5 ml of distilled water orally daily. Group (DVC): alloxan-diabetic group and was given vitamin C orally at the dose of 100 mg / kg body weight suspended in 1.5ml of distilled water<sup>14</sup>, iii. Group (DT): alloxan-diabetic group and was given T orally at the dose of 75mg /100 g body weight suspended in 1.5ml distilled water daily<sup>9</sup>, iv. Group (DVCT): Diabetic treated and was given vitamin C and T orally at the dose of 100mg /kg body weight and 75mg / 100g body weight suspended in 1.5 ml distilled water daily.

**Induction of diabetes:** For induction of diabetes, alloxan was injected subcutaneously at the dose of 120 mg/kg body weight for the first 3 d only to induce hyperglycemia<sup>9</sup>, while the control groups received a similar injection with normal saline. DM was verified by measuring fasting blood glucose samples from tail using a digital glucometer. The rats that have a blood glucose level of  $\geq 20$  mmol/L and symptoms of polyuria and polyphagia were considered diabetic<sup>9</sup> and enrolled for the experimental treatments. At the end of the different treatments (4wk), the animals were fasted for 12 h, then sacrificed under light anesthesia and fasting blood samples were collected from the hepatic portal vein in EDTA tube for plasma separation while serum samples were collected in another tube and stored at -200c. Blood serum glucose and vitamin C was determined spectrophotometrically<sup>15,16</sup>.

**Biochemical assays for kidney and liver functions:** The activities of plasma aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed<sup>17</sup>. Serum creatinine and urea were determined<sup>18,19</sup>. Plasma total protein (TP) and albumin (A) were estimated<sup>20,21</sup> while globulin (G) was determined by difference between TP and A.

**Oxidative stress biomarkers assessment:** Blood reduced glutathione content (GSH), plasma catalase activity (CAT) and malondialdehyde (MDA) levels were determined<sup>22-24</sup>. The activity of superoxidase dismutase (SOD) in erythrocyte was determined calorimetrically<sup>25</sup>.

**Statistical analysis:** The data was subjected to statistical analysis using SPSS version 18-statistical software (one-way ANOVA) to compare the mean values among the groups and Duncan's multiple range tests was put in function to identify the significance between means among the groups<sup>26</sup>.

## Results and Discussion

The results of the impact of daily oral dose of vitamin C (DVC), dried termis powder suspension (DT) and their combination on serum glucose and vitamin C levels are presented in figure 1 and 2 whereas, kidney and liver functions and oxidative stress biomarkers of diabetic rats are presented in tables 1 and 2, respectively. The alloxan induction for diabetes significantly ( $P < 0.05$ ) increased the serum glucose level of diabetic rats as compared to healthy non diabetic control rats (268.25 vs. 89.54 mg/dl). The data revealed that both DVCT and DT treatments were able to decrease blood glucose levels by 166.68 and 156.64 % at the end of 4 weeks experimental period as compared to control diabetic rats (figure-1). In that order, serum vitamin C level (figure-2) showed a significant ( $P < 0.05$ ) decrement due to diabetic induction (44.71 vs. 69.2 mg/dl) compared to non diabetic control rats and the DVCT – treatment was able to minimize this decrement to 5.72% of the C group (data not presented).

From table-1, It was observed that diabetic group (D) demonstrated significant ( $P < 0.05$ ) increment in the urea, creatinine levels and AST and ALT activities, on the contrary, the levels of total protein and Albumin showed significant ( $P < 0.05$ ) decrement. The administration of T (DT) and the combined treatment (DVCT) in diabetic rats had significantly ( $P < 0.05$ ) re-modulated the levels of urea, creatinine, AST and ALT as compared to diabetic control rats. The results of plasma CAT, erythrocytes SOD activities and blood GSH (Table 2) were significantly ( $P < 0.05$ ) decreased due to diabetic induction and their levels decreased by 44.32%, 44.49 and 46.7%, respectively (data not presented) meanwhile, the level of MAD was significantly increased by 95.4% as compared to non diabetic control group (C). All treatments were relatively able to regain the normal values of the control healthy rats; however the combined treatment (DVCT) surpassed the sole treatments in the degree of improvement.

DM is a chronic metabolic disease accompanied by high glucose levels as a consequence of lack endogenous insulin secretion. Moreover, there is a progressive lipid peroxidation concomitant with defects in antioxidant defense mechanisms determinants oxidative stress status<sup>2</sup>. Moreover, there is alteration in intermediary metabolism of carbohydrates, proteins and lipids<sup>3</sup>. Despite, the tremendous development of diabetic medication, these drugs are costly and may not be able to maintain long control of hyperglycemia. The wide diversity of the native herbal recipes used by diabetic patients in the developing countries encourages more researches in the area of unconventional hypoglycemic drugs. Traditional practitioners in Egypt have prescribed several kinds of Egyptian herbs and plant, belonging to various families as a treatment of DM<sup>6</sup>. Rats treated with alloxan displayed most signs of the DM such as hyperglycemia, polyuria, polyphagia, and polydipsia, which imitate those symptoms in human hypoinsulinaemia or type 1 diabetes and insulin resistance<sup>7</sup>. The present study showed that

vitamin C, termis and their combination significantly ( $P < 0.05$ ) alleviated most signs of DM including hyperglycemia, and impairment of liver and kidney function indices resulting from experimentally induced type 1 diabetes (figure 1, table 1 and 2). The ability of termis (L.termis) to modulate the glucose level in alloxan – induced diabetic rats (figure 1) and to alleviate the adverse effects of DM on kidney and liver functions as well as on the oxidative stress biomarkers is steady with the findings of other studies<sup>7,10</sup>. Previous studies have shown that termis exhibited anti-hyperglycemic effect and also helped to repair the kidney and liver damage in alloxan induced diabetic rats due to the presence of active components like saponins, alkaloids, tannins and quinovic acid<sup>7</sup>. It is well established that some saponins have hypoglycemic activity, which may be due to the inhibition of liver gluconeogenesis or glycogenolysis<sup>27,28</sup>. Therefore, the presence of these constituents may explain the hypoglycemic activity of these herbs. Furthermore, the hypoglycemic effect of these herbs may be due to the increased level of serum insulin, and also may be due to the enhancement of peripheral metabolism of glucose<sup>27</sup>. Moreover, the

histopathological finding of diabetic pancreas treated with L-Termis confirmed a marked improvement as compared with diabetic non treated rats suggesting that activities of L-Termis has acted in a way in preventing the death of beta cells and /or helped in the recovery of partially destroyed beta cells<sup>28</sup> or even have triggered the beta cells to increase insulin production which promotes glucose uptake and utilization. Another hypoglycemic mechanism attributed such results to the higher protein and fiber content of L- Termis stimulating higher insulin response, in addition to several photochemical found in L-Termis<sup>29</sup>. The results of plasma CAT, blood erythrocytes SOD and blood GSH (table 2) showed marked decrease by diabetic induction as their levels decreased by 44.32%, 44.49 and 46.7%, respectively, while, the levels of MAD was severely increased by 95.4% as compared by non diabetic control group (C). All treatments were relatively able to regain the normal values of the control healthy rats; however the combined treatment (DVCT) surpassed the either sole treatment in the degree of improvement.

**Table-1**  
**Effect of vitamin C and /or Termis on the kidney and liver function indices of alloxan- induced diabetic Wistar rats**

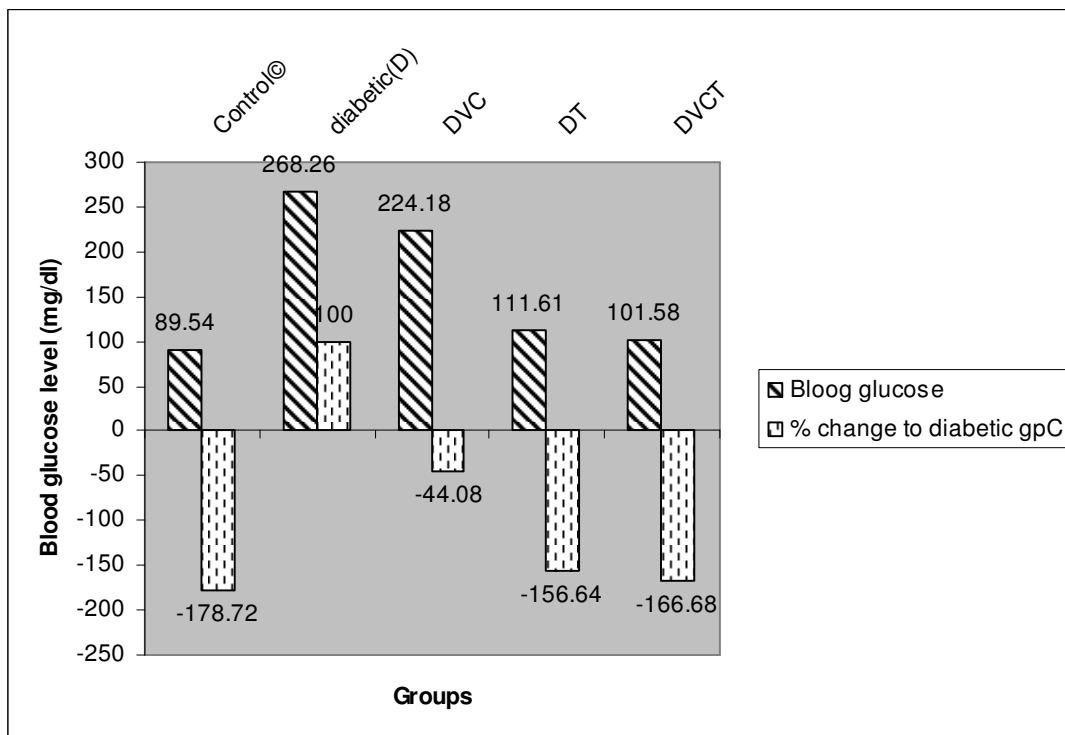
Parameters	Groups				
	C	D	DVC	DT	DVCT
Urea(mg/dl)	33.4± 4.13b	61.28± 7.33a	54.67±6.34a	38.24±5.22b	32.67±3.87b
Creatinine(mg/dl)	0.36±0.03b	0.64± 0.05a	0.52± 0.04a	0.39± 0.04b	0.42± 0.02b
TP(g/dl)	6.67± 0.68a	4.76±0.54b	5.51±0.41ab	6.35± 0.71ab	7.18± 0.65a
A(g/dl)	3.77±0.29 a	2.55±0.21 b	2.78 ±0.28 ab	3.32 ± 0.34ab	3.65 ± 0.37 a
G(g/dl)	1.72 ± 0.18a	1.87± 0.21a	1.85 ± 0.19a	1.79 ± 0.20a	1.68 ± 0.13a
A/G ratio	2.23± 0.25a	1.53± 0.17a	1.89 ± 0.22a	1.97± 0.28a	2.26± 0.27a
AST(U/L)	24.61± 3.32d	65.24±6.78a	45.68± 5.38b	33.39± 4.12c	34.96± 2.16c
ALT(U/L)	26.28± 2.56d	70.56± 8.11a	47.72± 6.21bc	36.54± 3.16c	30.38± 4.10cd

The values are mean ± SE, Values with different superscripts are significant differed  $P < 0.05$

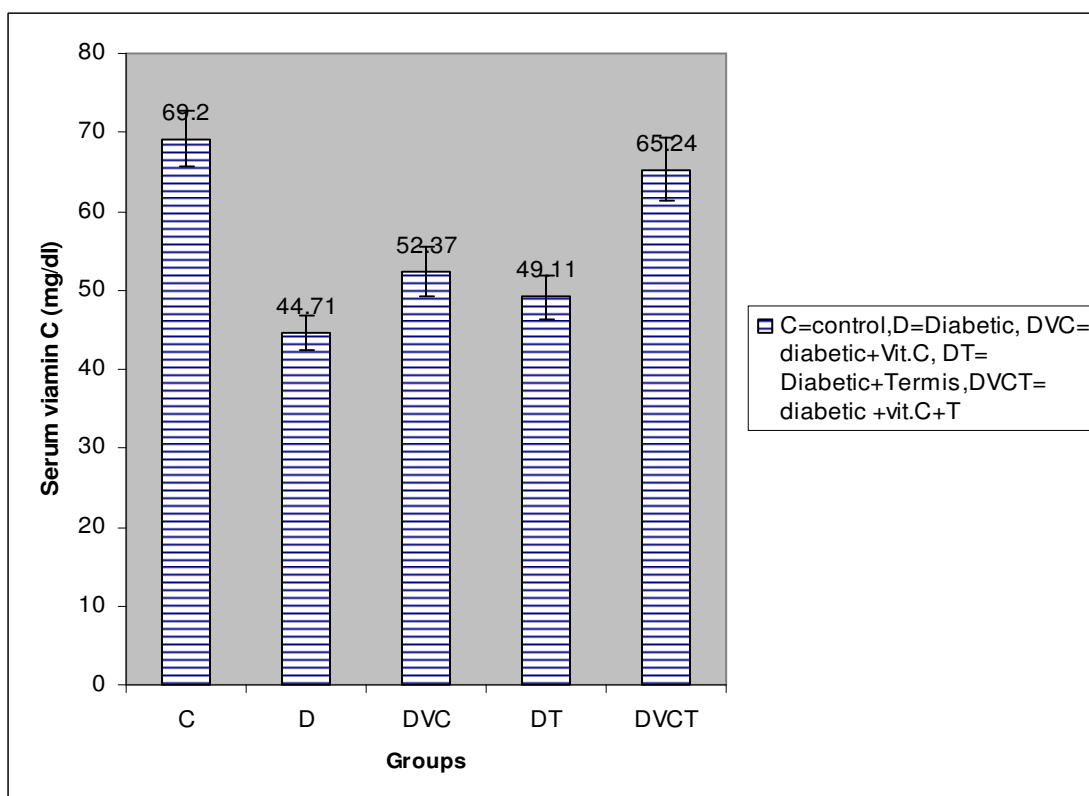
**Table-2**  
**Effect of vitamin C and /or Termis on some oxidative stress biomarkers of alloxan –induced diabetic Wistar rats**

Parameters	Groups				
	C	D	DVC	DT	DVCT
Plasma CAT (U/ml protein)	62.22± 2.40a	34.64± 1.96b	57.64± 2.83a	52.51± 3.49a	61.02± 3.58a
Plasma MAD (nmol/ml)	1.97±0.20b	3.85±0.27a	1.94±0.28b	2.59± 0.27b	2.19± 0.34b
Erthrocytes SOD(U/ml Hb)	222.57± 16.00a	118.61± 9.188 c	187.09± 21.55 b	176.25±17.33 b	230.13±21.22 a
Blood GSH (mg/dl)	31.65± 2.32a	17.57± 1.49c	28.69± 2.56ab	23.41± 2.07b	32.83± 2.50a

The values are mean ± SE, Values with different superscripts are significant differed  $P < 0.05$



**Figure-1**  
 Effect of vitamin C and/ or termis on blood glucose level of diabetic Wistar rats



**Figure-2**  
 Effect of vitamin C and / or termis on serum Vitamin C level of diabetic Wistar rats

Systemic antioxidant defense relies on SOD the antioxidant enzyme that catalyzes the conversion of the highly reactive superoxide anion radical, to less reactive species, and CAT that converts H<sub>2</sub>O<sub>2</sub> to water and molecular oxygen preventing the oxidative damage<sup>2</sup>. Also, GSH plays important biomolecule in antioxidant defense against chemical toxicity. In a state of oxidative stress and impaired ecological oxidative balance, biological organisms are not protected from the reactive oxygen species (ROS) toxic effects and thus are prone to cell damage and diseases<sup>20</sup>. In diabetic rats, there were significant decrease in antioxidant enzymes like catalase (CAT) and (GSH) which represent increased consumption due to oxidative stress. In the current study, MAD which is a biomarker of intense lipid peroxidation and also indirect evidence of high free radical production in diabetes showed significant (P<0.05) increase in diabetic alloxan – induced rats 2 . Whereas, the daily intake of vitamin C (100mg/ kg BW) as potent antioxidant was able to ameliorate the negative effect of diabetes on the antioxidant biomarkers was previously observed<sup>20</sup>. Moreover, it was suggested that L-Termis participates in preventing the death of beta cells and /or helped in recovery of partially destroyed beta cells as demonstrated in histopathological findings of pancreas and the photochemical activities of termis may have triggered the beta cells to increase insulin production which promotes glucose uptake and utilization<sup>28</sup>.

The combined daily oral administration of vitamin C and termis exhibited the best results and was able to restore the normal values of the blood glucose and vitamin C, liver and kidney function indices as well as antioxidant biomarkers in regard to non diabetic control values.

Vitamin C is recognized as potent natural antioxidant that can afford primary as well as secondary protection against the oxidative damage of lipids and lipoproteins 11. The daily intake of vitamin C (100mg/ kg BW) as powerful antioxidant was able to ameliorate the negative effect of diabetes on the antioxidant biomarkers which in consistent with others<sup>14,30</sup>.

## Conclusion

In conclusion, the combination between vitamin C and termis is suggested to be a beneficial strategy not only to control hyperglycemia but also to modulate the negative effects of diabetes on kidney and liver functions as well as oxidative stress status. This combination containing both vitamin C and Termis has been tested as a possible future nutraceutical to ameliorate health complications in diabetes.

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