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# Methanolic extract of *Lupinus Termis* ameliorates DNA damage in alloxan-induced diabetic mice

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**Abstract. – Diet therapy is showing a bright future in the therapy of diabetes mellitus (DM). The seeds of *Lupinus termis* are used in the Middle East and Africa as food and in folklore medicine. In traditional medicine, the seeds are reputed to be effective for diabetes. The aim of this work was to evaluate the antigenotoxic effect of *Lupinus termis* methanolic extract (LTE) against DM oxidative stress.**

**MATERIAL AND METHODS, The analysis of micronuclei (MN) and chromosomal aberrations are accurate cytogenetic techniques used to show chromosomal damage caused by clastogenic affects. The present study was designed to evaluate: (1) the effects of DM on bone marrow MN frequency and chromosomal aberrations, (2) the effect of oral treatment by gavage of LTE on MN frequency and chromosomal aberrations produced by DM.**

**RESULTS, Frequencies of MN and chromosomal aberrations have been significantly increased in diabetic mice compared with the normal mice ($p < 0.05$). LTE at a dose 25, 50 and 100 mg/kg b.wt. for 15 days groups treatment in diabetic mice were significantly decreased MN frequency and chromosomal aberrations in a dose dependent manner.**

**CONCLUSIONS, Our results suggest that LTE is a suitable agent for preventing DM-induced DNA damage. To the best of our knowledge, this is the first report on LTE having a potential diabetes-associated DNA damage-protecting activity *in vivo*.**

*Key Words:*

Diabetes mellitus, *Lupinus termis* methanolic extract, Antigenotoxicity.

## Introduction

Diabetes mellitus (DM) is a major worldwide health problem predisposing to markedly increased cardiovascular mortality. Other serious morbidities and mortalities are related to development of nephropathy (kidney damage), neuropathy (nerve damage), and retinopathy (blindness)[1-3].

Oxidative stress can be associated to an increased rate of reactive oxygen species (ROS) generation, a decrease of antioxidant defense or a combination of both. ROS-mediated alterations include damage to cells, tissues or organs and are proposed as the major factors in the mechanism of several diseases[4]. An increased production of oxygen-derived free radicals and the decrease in the activity of free radical scavenger system have been reported in diabetes[5]. Exposure of the genetic material to ROS could cause DNA damage[6]. Genetic instability is suggested as a biomarker for cancer risk[7]. Many Authors reported that DM has the ability to induced DNA damage[8-10].

In recent years, search for novel type of antioxidants from several plant materials has achieved considerable attention. Management of diabetes with minimized side effects is still a complicated medical challenge. There is an increasing demand by patients to use the natural products with antidiabetic activity, because both insulin and oral hypoglycemic drugs possess undesirable side effects[11]. Naturally occurring antioxidant, antimutagens and anticarcinogens can be found in fresh fruits and vegetables, including legumes[12].

Legumes contain a rich variety of phytochemicals, including phytosterols, natural antioxidants and bioactive carbohydrates[13], which if consumed in sufficient quantities may help to reduce tumour risk[14]. Epidemiological and intervention studies indicated that legume consumption is inversely associated with the risk of coronary heart disease[15], type 2 diabetes mellitus[16] and obesity[17], and results in lower LDL cholesterol and higher HDL cholesterol[18,19].



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Lupins are protein-containing legumes that have been present in Andean and Mediterranean diets since ancient times. Some lupin species exhibit antioxidant capacity related mainly with the presence of phenolic compounds[20].Because lupins belong to the Leguminosae family; they may also have potential for their content of phytoestrogens such as isoflavones. Isoflavones belong to the group of flavonoids. Currently, isoflavones have been associated with beneficial effects in humans, such as prevention of cancer, cardiovascular diseases, osteoporosis and menopausal symptoms[21].

The aim of this study is to evaluate the chemopreventive activity of LTE using micronucleus (MN) and chromosomal aberrations on DNA damage induced in diabetic mouse bone marrow cells *in vivo*. Among short-term mutagenicity/genotoxicity assays, the MN and the chromosomal aberrations have been widely used for identifying chemopreventive agents. These two tests are sensitive, easy to perform, and can be carried out either *in vivo* or *in vitro*[22,23]. This study represents the first data about the chemopreventive effect of LTE on the DNA damage of experimental diabetic mice.

## Materials and Methods

### Animals

Male Swiss mice (20 g to 25 g) were procured from the Animal House, National Research Center, Egypt. Mice were maintained in an air-conditioned room (25±1°C) with a 12 h light: 12 h dark cycle. A standard pellet diet and tap water were supplied *ad libitum*.

### Chemicals

Alloxan was obtained from Sigma-Aldrich Inc. (St. Louis, Mo, USA). All other chemicals used were of analytical grade.

### Plant Material and Extract Preparation.

*Lupinus termis* used in this study was procured from Agricultural Research Center Giza, Egypt.

Half kilogram batch of the coarsely powdered seeds was macerated for 2 h, with 95% methyl alcohol USP, prior to packing in a glass percolator. Following 48 h maceration, percolation was carried out. The process was repeated 5 times, and the alcohol removed by evaporation under reduced pressure (average yield was 10% w/w of extract)[24].

### Experimental Design

In this experiment total of 60 mice were used. Mice were divided into six groups of ten animals in each group. They were treated as follows:

**Group 1:** Normal control (vehicle treated).
**Group 2:** Normal mice received oral LTE (100 mg/kg b.wt) dissolved in1ml of corn oil for 15 days.
**Group 3:** Alloxan induced diabetic mice (a single i.p injection at 120 mg/kg b.wt, in citrate buffer 0.1 M, pH 4.5[25]. After 72 h, animals with serum glucose levels higher than 250 mg/dl were considered diabetic and were included in the study[26].
**Groups 4, 5 and 6:** Diabetic mice received oral LTE (25, 50 and 100 mg/kg b.wt) dissolved in1ml of corn oil for 15 days.

## Cytogenetic Analysis

### Micronucleus Test

The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum[27]. The cell suspension was centrifuged at 1000 rpm for 10 min and the supernatant was discarded. A small drop of the resuspended cell pellet was spread on to clean glass slides and air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 minutes and stained with May-Grünwald/Giemsa at pH 6.8[28]. Scoring the polychromatic erythrocytes and the percentage of micronucleated polychromatic erythrocytes (MNPCEs) was determined by analyzing the number of MN cells from 1000 PCEs per animal. Cytotoxicity was assessed by scoring the relative proportion of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). This ratio was determined by counting a total of 1000 erythrocytes for each animal.

### Chromosomal Aberrations

Bone-marrow metaphases were prepared following the method of Yosida and Amano[29]. 100 well spread metaphases/animal were analyzed for the different types of chromosome aberrations including gaps, fragments, breaks, deletions, centric fusion and polyploidy under 100 × magnification with a light microscope (Olympus, Saitama, Japan). The mitotic index was also recorded by count the cell division in 1000 cell/animal. Five animals were taken for each treatment.

The percent reduction in the frequency of MNPCEs and chromosomal aberrations was calculated according to Waters et al [30], using the following formula: % Reduction = frequency of DNA damage in A – frequency of DNA damage in B/frequency of DNA damage in A – frequency of DNA damage in C × 100, where A corresponds to the group with diabetic mice (positive control), B to the group treated with Diabetes plus LTE and C corresponds to the negative control (vehicle).

### Statistical Analysis

Results are presented as means ± S.E. and the statistically significant difference between the control and treated groups was determined using the Student's *t*-test.

## Results

### Micronucleus Test

The elevation of MN was observed in diabetic mice ($p < 0.05$) and the positive effect of LTE in decreasing the frequency of MN ($p < 0.05$) in comparison with diabetic mice level was reflected in Table I.

The PCE/NCE ratio was significantly suppressed with diabetes as compared to control ($p < 0.05$, Table I). The treatment with LTE succeeded in preventing the DM-induced bone marrow depression after treatments with 25, 50 and 100 mg/kg b.wt. for 15 days when compared to alloxan group (positive control). The PCE/NCE ratio was significantly ($p < 0.05$) when compared to depression induced by DM only after median and high dose of treatment. The percentage of reduction of MNPCE increased with increasing the dose of treatment with LTE (Table I).

### Chromosomal Aberrations

The effect of LTE on DNA damage induced by DM in mouse bone marrow cells was determined by chromosomal aberrations as shown from the results presented in Table II. The percentage of chromosomal aberrations elevated ($p < 0.05$) in diabetic mice compared with negative control. Oral administration of LTE at different doses for 15 days reduced DNA damage ($p < 0.05$) induced by DM in a dose dependent matter. The percentage of reduction of aberrations reached to 38.23, 48.52 and 63.23 after treatment with 25, 50 and 100 mg LTE/Kg b.wt. respectively (Table II).

Table II also showed that DM decreased the mitotic index (MI) and induced mitotic delay in mouse bone marrow cells. The mitotic index increased with increasing treatment concentrations of LTE with diabetic mice group and showed a statistically significance ($p < 0.05$) compared with the DM group.

## Discussion

DM is a metabolic disorder affecting millions of people worldwide. In 2000, it affected 171 million people around the world and it is expected that in 2030, the number of DM sufferers will reach more than 366 million people corresponding to 4.4% of the world's population[31].

Increasing evidences in both experimental and clinical studies suggest that oxidative stress caused by hyperglycemia plays a major role in

**Table I.** The effect of LTE on DM-induced the frequency of micronuclei.

| Treatments Mg/kg b.wt | Time of treatment (day) | NO of MN | MNPCE Mean ± S.E. | PCE/NCE ratio | Reduction % |
|---|---|---|---|---|---|
| I. Control (vehicle) | – | 31 | 0.62 ± 0.58 | 1.78 ± 0.64 | |
| II. DM | – | 419 | 8.38 ± 0.57[a] | 0.50 ± 0.51[a] | |
| III. LTE | | | | | |
| 100 | 15 | 32 | 0.64 ± 0.45 | 1.62 ± 0.40 | |
| IV. DM/LTE | | | | | |
| 25 | 15 | 301 | 6.02 ± 0.41[ab] | 0.69 ± 0.34[a] | 30.41 |
| 50 | 15 | 225 | 4.50 ± 0.50[ab] | 0.81 ± 0.55[ab] | 50.0 |
| 100 | 15 | 118 | 2.36 ± 0.57[ab] | 0.89 ± 0.3[ab] | 77.57 |

The total No of scored cells is 5000 (5 animals/group). [a]Significant compared to vehicle control ($p < 0.05$); [b]Significant compared to DM treatment ($p < 0.05$) (*t*-test).

**Table I.** The effect of LTE on DM-induced chromosomal aberrations in the mouse bone marrow cells *in vivo*.

| Treatments mg/kg b.wt | No. | Abnormal metaphases | | No. of different types of metaphases | | | | | | Mean (%) ± SE | Reduction % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean (%) ± SE | | G. | Frag. and/or Br. | Del. | C.F. | M.A. | Polyp | | |
| | | Including gaps | Excluding gaps | | | | | | | | |
| I. Control | 23 | 4.60 ± 0.41 | 2.80 ± 0.48 | 9 | 8 | 6 | 0 | 0 | 0 | 3.95 ± 0.40 | |
| II. DM | 91 | 18.20 ± 0.52[a] | 14.60 ± 0.42[a] | 18 | 37 | 10 | 3 | 20 | 3 | 1.70 ± 0.47[a] | |
| III. LTE 100 | 26 | 5.20 ± 0.58 | 3.20 ± 0.50 | 10 | 10 | 6 | 0 | 0 | 0 | 3.92 ± 0.43 | |
| IV. DM/LTE | | | | | | | | | | | |
| 25 | 65 | 13. 0 ± 0.45[ab] | 9.0 ± 0.63[ab] | 20 | 20 | 7 | 2 | 15 | 1 | 2.35 ± 0.33[ab] | 38.23 |
| 50 | 58 | 11.60 ± 0.50[ab] | 7.0 ± 0.45[ab] | 23 | 15 | 6 | 1 | 11 | 2 | 2.61 ± 0.51[ab] | 48.52 |
| 100 | 48 | 9.60 ± 0.48[ab] | 6.0 ± 0.60[ab] | 18 | 13 | 6 | 1 | 10 | 0 | 3.02 ± 0.28[ab] | 63.23 |

Total number of examined metaphases 500 (5 animals/group). [a]Significant compared to vehicle control ($p < 0.05$); [b]Significant compared to DM treatment ($p < 0.05$) (*t*-test); G.: Gap; Frag.: Fragments; Br. Breaks; Del.: Deletions; C.F.: Centric Fusions; M.A.: Multiple Aberrations; Polyp: Polyploidy.

pathogenesis of DM. Diabetes is usually accompanied by increased production of the molecules of ROS and/or impaired antioxidant defense systems, which result in producing oxidative damage to bio-molecules[32,33]. Exposure of the genetic material to ROS could cause DNA strand breaks[6] and MN[34]. These types of damages could lead potentially serious consequences for the cell[7,35]. Impaired DNA repair mechanisms, genetic instability and subsequent risks to health complications such as cancer are found in T2DM patients[36,37]. Reduced antioxidant defenses in diabetes could be an important factor underlying this association[38].

Our results showed an elevation in DNA damage induced by DM using micronucleus and chromosomal aberrations. These results are in the same line with the previous data reported by the Authors[8,9,10,39]. The elevation in the frequency of micronuclei and chromosomal aberrations could be influenced by oxidative stress and glutathione levels in DM subject[38]. Gene polymorphisms of antioxidant and DNA repairing genes are also reported to influence the DNA damage[40].

The use of natural products or their active components for the prevention and/or treatment of chronic diseases are based primarily on the traditional medicine of various ethnic societies and on epidemiological data[41]. In present time, searching for safe and efficacious medicinal plants, possessing antidiabetic, antigenotoxic and antioxidant activities, are very important for therapy of complications of chronic diabetes.

The results obtained through this study clearly demonstrate that the LTE seeds at 25, 50 and 100 mg/kg b.wt. for 15 days is not genotoxic, and not clastogenic. In addition, these results support previous results obtained from genetic toxicological studies performed by ethanolic extract of the *Lupinus termis* seeds[24] and on other lupin species such as *Lupinus angustifolius*[42].

The antigenotoxic activity of LTE was performed using micronucleus and chromosomal aberrations, a sensitive protocol for detection of DNA damage[22,23]. Our results showed that LTE have the ability to reduce the frequency of MN and chromosomal aberrations induced by DM in mouse bone marrow cells in a dose dependent manner. No available data about antigenotoxic activity of *lupinus termis* on DNA damage induced by DM was observed. Other literature reported that some medicinal plants such as Kafta, Somma, Araar and Doum have the ability to re-

duce the DNA damage induced by DM in rat somatic and germ cells[10]. Celikler et al[9] observed that the seaweed *Ulva rigida* effective in reducing the chromosome damage induced by DM, in the rat micronucleus assay.

The lupinus genus is widely distributed approximately 300 species are found in the Mediterranean countries, Africa, North and South America[43]. Lupin is a legume with a rich source of plant protein and amino acid[44] Similar to other legumes, lupine contains phenolic compounds and carbohydrates that may affect human health or results in a reduced risk of disease[44,45].

Flavonoids are the major group of phenolic compounds, thus biologically active and may be potent antioxidants, scavengers of active oxygen species and electrophiles, blockers of nitration, or chelators of metals. They can undergo autoxidation to produce hydrogen peroxide in the presence of metals and are capable of modulating the activity of certain cellular enzymes[46].

Alkaloids can be pharmacologically active and may have narcotic, analgesic, antitussive, chemotherapeutic, antiarrythmic (cardiotonic), diuretic, hypoglycemic or uterotonic properties[47]. It has been shown that oral administration of *Lupinus termis* reduces high blood pressure and hyperglycemia in rabbits, rats and mice[48]. Indeed, the addition of lupin seeds to the food of diabetic-hypercholesterolemia rabbits decreases cholesterol levels and postprandial hyperglycemia[49]. On the other hand, alkaloids sparteine sulfate administered by intravenous in fusion to normal men increases either basal or glucose-induced insulin secretion[50].

Moreover, Pereira et al[51] have demonstrated that aqueous lupin extract enhances insulin release from isolated rat pancreatic islets, and sparteine has been shown to increase insulin secretion *in vitro*[52]. However, a number of health benefits results from ingestion of oligosaccharides such as reduction of serum cholesterol, reduction of blood pressure and they may have anticancer effects[53]. Recently it has been investigated phytochemical representing the minor compounds like tocopherols and phytosterols in lupin extract[54]. This interest is connected with the activity of such compounds against cardiovascular diseases[55]. Most of these beneficial effects are due to antioxidant activity especially when the presence of phenolic compounds and tocopherols are involved in the stability of oils and thus its toxicological safety[56].

Damage to DNA may cause mutations that potentially lead to cancer[57,58]. Therefore, protection against DNA damage and induction of DNA repair enzymes represent important mechanisms of anticarcinogenic activity of natural compounds. The ability of LTE to ameliorate the DNA damage induced by DM may be due to 1- Scavengers of active oxygen species and electrophiles, blockers of nitration, or chelators of metals[46]. 2- Antihyperglycemia[48], hyperglycemia- induced protein glycation generates superoxide free radicals[1-3]. 3- Enhancement of antioxidant defense enzymes[46].

In conclusion, the present investigation showed that LTE possess antigenotoxic effect against DNA damage induced in alloxan-diabetic animals. Thus, *lupinus terms* may be implicated as a preventive agent against diabetes mellitus. However, more work is warranted to elucidate its myriad mechanisms of action.

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