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The Effect of *Lupinus albus* and *Hyphaene thebaica* on Chromosomal Aberrations and Histopathological Changes of Liver and Pancreas in Streptozotocin-induced Diabetic Rats

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Abstract

Background: The folk medicine in Egypt has described several kinds of Egyptian herbs and plant prescriptions, belonging to various families to be concerned with the treatment of diabetes mellitus**.** The present study focuses on evaluating the chromosomal aberration in somatic cells of STZ-diabetic rats treated with *Lupinus albus* (termis) and *Hyphaene thebaica* (doum) as well as their effect on liver and pancreas histopathology.

Material and methods: STZ was injected intraperitoneally at a single dose of 50 mg/kg to induce diabetes. Termis seeds suspension (75 mg/100 g b.wt.) was daily orally administered, doum fruit suspension was daily orally administered (1g/kg b.wt.) and also gliclazide, reference drug, was administered at a dose equivalent to the therapeutic dose of rat. After four weeks of administration, bone-marrow metaphases were prepared for examining chromosomal aberration. These were followed by statistical analysis. Liver and pancreas were dissected, processed and stained with H&E for investigating histopathological changes.

Results: The results of chromosomal analysis showed that, diabetic rats had a highly significant increase of chromosomal aberrations compared to that of normal healthy control. Animals which were treated with *Lupinus albus* and *Hyphaene thebaica* showed significant improvements in the frequencies of chromosomal aberrations. Also, treatment of diabetic rats with termis and doum revealed marked improvement in liver and pancreas histopathology.

Conclusion: It is concluded that diabetes is much harmful on the histological picture of liver and pancreas and on chromosomal aberration frequencies in the animal body, whilst the administration of termis or doum reduced these harmful effects of diabetes.

Key words: *Lupinus albus*, *Hyphaene thebaica*, gliclazide, streptozotocin, diabetic rat.

Introduction

The prevalence of diabetes is increasing at an alarming rate, and its incidence is nearing epidemic levels across a variety of populations **(1)**. Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy,neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications **(2**). Diabetes mellitus is classified into type I, type II, other specific types, and gestational diabetes (**3)**.

Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants have been described in the scientific and popular literature as having hypoglycemic activity **(4)**.

Lupinus albus is a member of the Leguminosae family **(5)**. Lupine is the one that has the highest protein content in its composition apart from being a good source of fibres. However, lupin seed is one of the legumes with the lowest levels of non-nutritional compounds (trypsin inhibitors, phytic acid, saponins and lectins) **(6)**.

Doum palm fruit (*Hyphaene thebaia*) is a desert palm tree with edible oval fruit, originally native to the Nile valley. It is a member of the palm family, Arecaceae **(7)**.

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Doum palm fruit is also a source of potent antioxidants **(8)**.

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea **(9)**. It causes hyperglycemia by its direct cytotoxic action on pancreatic beta cells **(10)**.

Aim of the study:

The present study was done to elucidate the effect of *Lupinus albus* and *Hyphaene thebaica* (as antidiabetic agents) on the chromosomal aberration and histopathology of liver and pancreas in STZ-diabetic adult male albino rats.

Material and methods:

Material:

1- The drugs:

Streptozotocin (STZ): powder, supplied by *Sigma*, used for induction of diabetes.

Gliclazide**:** Diamicron® tablets, 80 mg supplied by *Servier*, used as a reference drug.

2- Plant preparation:

The seeds of Lupinus termis were washed, dried at 37 °C for 24 h, and meld well into fine powders. The herb powder was suspended in double-distilled water (5g/100ml**) (11)**.

The pulp of *Hyphaene thebaica* was dried and ground into powder. Aqueous suspension was made by suspending 10 gm of the powdered pulp in 100 ml of distilled water **(12)**.

3- The animals and induction of diabetes:

Adult male albino rats (*Rattus norvegicus*), weighing 100g $\pm$20gm were obtained from the Egyptian Institution of Serum and Vaccine (Helwan, Cairo, Egypt). Animals were kept under normal conditions throughout the experiment. All rats had access free to food and water *ad libitum* throughout the experimental period. The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ(50 mg/kg body weight) in cold 0.9% citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Diabetes was confirmed in STZ rats by measuring glucose concentration 48 h after injection of STZ. The rats with blood glucose level>200 mg/dl were considered to be diabetic. The treatment was started on the 7th day after STZ injection.

The animals of this study were divided into the following groups:

A- Normal group (control healthy group).

B- STZ-diabetic group: (Group 2) animals were injected with STZ (50 mg/kg b.wt. interperitoneally).

C- Diabetic rats treated with Lupinus albus: animals were administered plant suspensions, daily at a dose of ≈75 mg/100 g b.wt. by intragastric tube.

D- Diabetic rats treated with *Hyphaene thebaica*: animals were administered plant suspensions, daily at a dose of (1g/kg b.wt.) by intragastric tube.

E- Diabetic rats treated with gliclazide: animals were administered drug suspended in distilled water, daily at a dose equivalent to therapeutic dose of rat.

The treatment continued for 4 weeks.

Methods:

1. Histopathological assay

Specimens from liver and pancreas were collected from all experimental groups and fixed in 10% neutral buffer formalin, dehydrated in ethyl alcohol and cleared in xylene. 4-5µ thick sections were prepared and stained with Haematoxylin and Eosin **(13).**

2. Cytogenetic assay

Slides for chromosomal aberration from bone marrow were prepared by 1mg/kg colchicine injection 2 hours before killing. Then stained with Giemsa and examined under light microscope.

3. Statistical analysis:

The statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by Duncan's test (1955). The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 15.

Results:

Histopathological results:

Microscopically examined sections (fig.1) showed the histological architecture of the control rat liver. Examined sections of diabetic rat (fig.2) showed kupffer cells activation, cytoplasmic vacuolization of hepatocytes and loss of hepatic archeticture. Liver of rat treated with gliclazide (fig.3) revealed cytoplasmic vacuolizations of centrolobular hepatocytes.

Examined sections from diabetic rat treated with termis(fig.4) showed slight activation of kupffer cells. Liver of diabetic rat treated with doum (fig.5) revealed vacuolations of sporadic hepatocytes. Examined sections (fig. 6) showed the histological architecture of the control rat pancreas. Sections of Pancreas of diabetic rat (fig.,7, 8) showed necrosis and vacuolations in some cells of islets of Langerhans and epithelium lining of pancreatic acini. Pancreas of diabetic rat treated with gliclazide(fig.9) revealed vacuolations of some cells of islets of Langerhans. Examined sections of diabetic rat treated with termis(fig.10) showed normal histological structure of pancreatic tissue. sections of diabetic rat treated with doum(fig.11) showed normal histological structure of pancreatic tissue.

Cytogenetic results:

The cytogenetic changes after injection of STZ and oral adminstration of *L. termis* and gliclazide were studied in the form of frequencies of chromosomal aberrations and mitotic indices in rat bone marrow cells. In all groups, 50 metaphases /animal were examined for scoring both the structural and numerical chromosomal aberrations (CAs) and 1000 cells for mitotic indices (MI). Figure (12) represents normal metaphase.structural chromosomal aberrations were observed in the present study in form of centric fusion (fig.13) and centromeric attenuation (fig.14). A cell was considered centromerically attenuated when methaphase contains at least three chromosomes with centromeric spilt. While numerical chromosomal aberrations were represented by polyploidy (fig.15). The effects of termis and doum suspensions on chromosomal aberration are represented in table (1). *Lupinus termis* administration decreased the levels of centromeric attenuation and total structural aberration significantly when compared with diabetic value. It also restored level of centric fusion and polyploidy to its normal level. *Hyphaene thebaica* decreased centromeric attenuation and centric fusion significantly ($p<0.05$) as compared to diabetic value and reduced the level of polyploidy to its normal level.

Gliclazide also was able to decrease the level of cetromeric attenuation, centric fusion and total structural aberration but they were still significantly higher than normal value. It also restored polyploidy to its normal level. The pronounced decrease in aberration was in termis and doum treated groups.

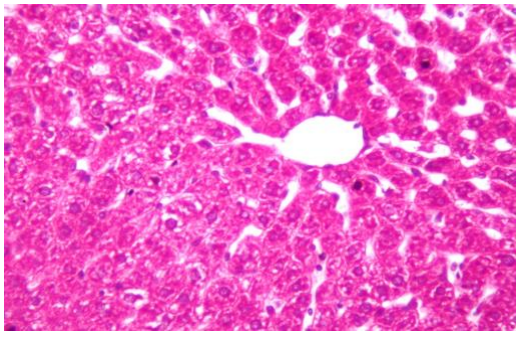

Fig.1 A photomicrograph of control liver showing the normal hepatic architecture from central vein and polyhedral hepatocytes (x400).

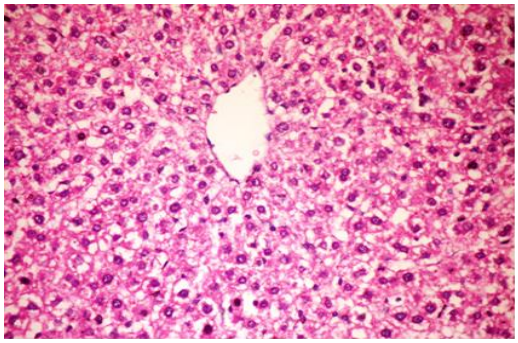

Fig.2 A photomicrograph of liver of diabetic rat Kupffer cells activation and cytoplasmic vacuolization of hepatocytes(x400).

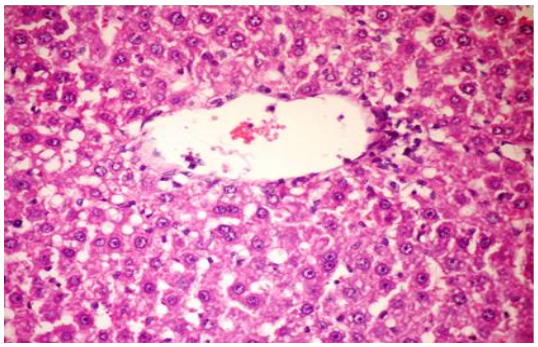

Fig.3 A photomicrograph of liver of diabetic rat treated with diamicron showing vacuolation of centrolobular hepatocytes(x400)

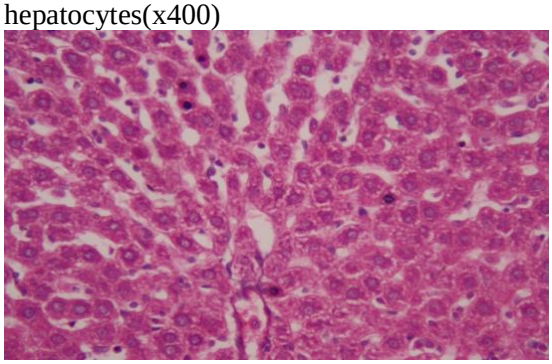

Fig.4 A photomicrograph of liver of diabetic treated with termis showing slight activation of kupffer cells (x400)

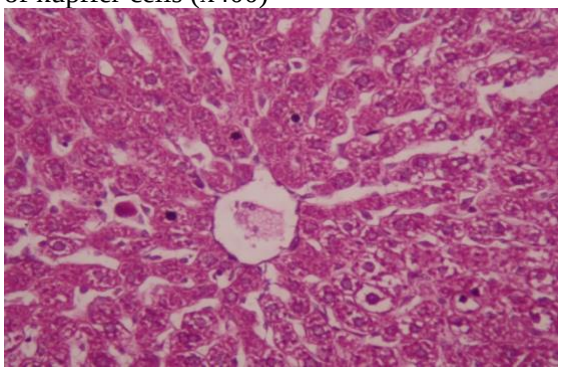

Fig.5 A photomicrograph of liver of diabetic rat treated with doum showing vacuolation of sporadic hepatocytes(x400).

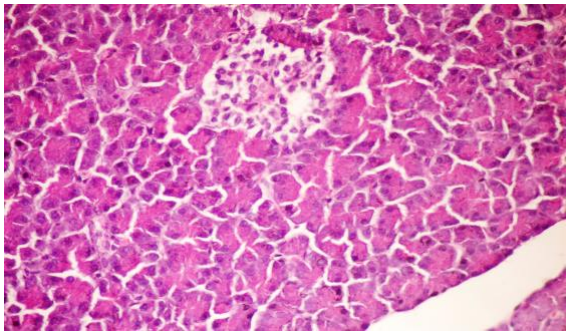

Fig. 7: A photomicrograph of Pancreas of diabetic rat showing necrosis of some cells of islets of Langerhans (H&E X 400)

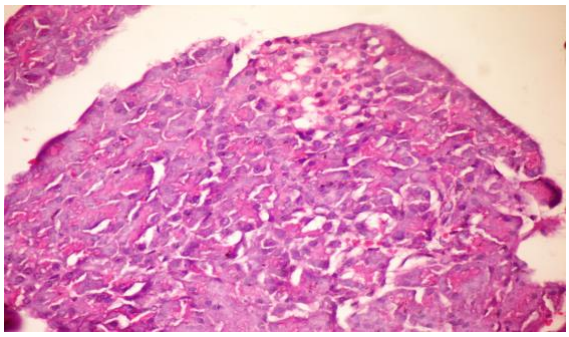

Fig.8: A photomicrograph of Pancreas of diabetic rat showing vacuolations in some cells of islets of Langerhans and epithelium lining of pancreatic acini (H&E X 400)

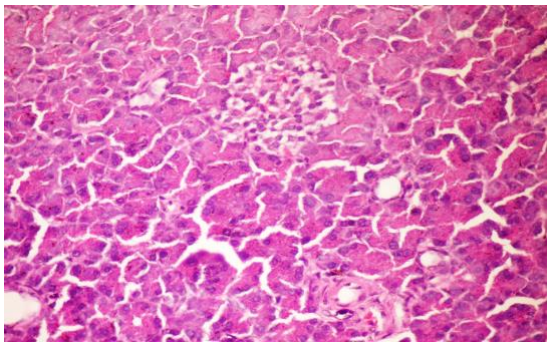

Fig.9 A photomicrograph of Pancreas of diabetic rat treated with gliclazide showing vacuolizations of some cells of islets of langerhans (H&E X400)

Fig. 6: A photomicrograph showing the normal histological architecture of the control rat pancreas. (x400).

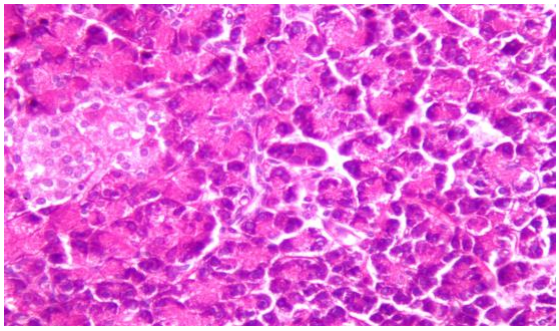

Fig.10: A photomicrograph of Pancreas of diabetic rat treated with termis showing normal histological structure of pancreatic tissue (H&E X400).

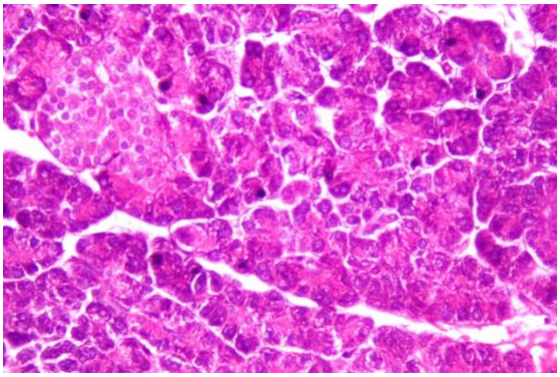

Fig.11: A photomicrograph of Pancreas of diabetic rat treated with doum showing no histopathological changes (H&E X 400)

Fig. (12): A photomicrograph of normal metaphase spreading from rat bone marrow cells.

Fig. (13): A photomicrograph of metaphase spreading from rat bone marrow cells showing centricfusion

(C.F.).

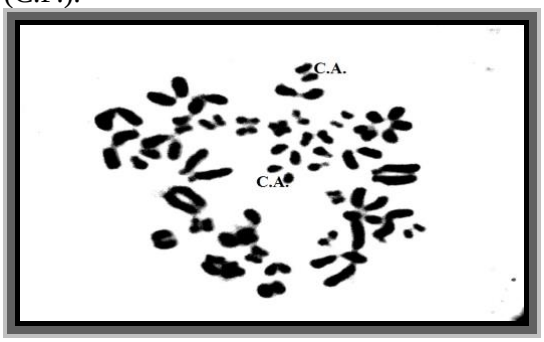

Fig. (14): A photomicrograph of metaphase spreading from rat bone marrow cells showing centromeric attenuation (C.A.).

Fig. (15): A photomicrograph of metaphase spreading from rat bone marrow cells showing polyploidy.

Table 1: Chromosomal aberration induced in rat bone-marrow cells in control and treated groups after four weeks of experiment.

	Normal	Diabetic	Diabetic+L.	Diabetic+H.	Diabetic+G
Centromeric attenuation	3.4+0.24	21+0.32[a]	4.6+0.4[ab]	8.6+0.51[ab]	10+0.55[ab]
Centric fusion	2.6±0.24	11.4±0.24[a]	3.4±0.24[b]	3.8±0.37[ab]	4.80.37[ab]
Total structural aberration	6±0	32.4±0.4[a]	8±0.32[ab]	12.4±0.6[ab]	14.8±0.37[ab]
Polyploidy	3.2±0.37	6.6±0.4[a]	2.6±0.24[b]	3.4±0.24[b]	2.4±0.51[b]
Total numerical aberration	3.2±0.37	6.6±0.4[a]	2.6±0.24[b]	3.4±0.24[b]	2.4±0.51[b]

[a]: Significant change at p<0.05 with respect to control group.[b]: Significant change at p<0.05 with respect to diabetic group. L: *Lupinus termis*, H: *Hyphaene thebaica*, G: gliclazide, STZ: streptozotocin.

Table (2): Mitotic activity percentage recorded in rat bone marrow cells after four weeks of oral administration of different treatments.

Treatments / No. of animals	Number of dividing cells / 1000 cell / animal						
	I	II	III	IV	V	M ± SE	MI%
Normal	60	59	63	60	61	60.6±0.68	6.06%
STZ	14	18	21	17	18	17.6±1.12[a]	1.76%
STZ+T	51	55	59	55	56	55.2±1.28[ab]	5.52%
STZ+H	43	51	49	47	50	48±1.41[ab]	4.8%
STZ+G	34	37	31	34	33	33.8±0.97[ab]	3.38%

Total number of examined metaphases 500 (5 animals/group). Data were expressed as Mean±Standard Error (M±SE). [a]: Significant change at p<0.05 with respect to control group.
[b]: Significant change at p<0.05 with respect to diabetic group. L: *Lupinus termis*, H: *Hyphaene* thebaica, G: gliclazide.

Discussion

From our results in histopathology for pancreas and liver, it was found that pancreas of STZ-diabetic group showed necrosis of β cells of islets of Langerhans and vacuolations in both β cells and epithelium lining of pancreatic acini. Examined sections of diabetic rat treated with termis showed normal histological structure of pancreatic tissue, reinforcing its hypoglycemic action. Also treatment with doum suspension showed marked improvement. The hypoglycemic effect on blood glucose level could be through increased serum insulin levels provided by repair / regeneration of the endocrine pancreas **(14)**. The histopathological result of liver of diabetic group showed kupffer cells activation, cytoplasmic vacuolization of hepatocytes and

loss of hepatic archeticture. It is known that Liver enzymes (ALAT, ASAT, γGT and ALP) activities were used as important biomarkers for detection of hepatotoxicity. The membrane bound enzymes like ALP and γGT are released unequally into bloodstream depending on the pathological phenomenon **(15)**. However, treatment with *Lupinus termis* and *Hyphaene thebaica* suspension showed improvement in liver histopathology.

In the present study, STZ induced genetic changes in rat bone marrow cells. It reduced the number of dividing cells causing inhibition in the mitotic index than normal. Our results showed that aquous suspension of *Lupinus termis* have the ability to reduce the frequency of chromosomal aberrations induced by DM in rat bone marrow cells. Lupin is a legume with a rich source of plant protein and amino acid **(16).** Similar to other legumes, lupine contains phenolic compounds and carbohydrates that may affect human health or results in a reduced risk of disease **(16,17)**. Flavonoids are the major group of phenolic compounds, thus biologically active and may be potent antioxidants **(18)**.

Also, oral treatment of aquous suspension of doum pulp inhibited the frequency of chromosomal aberrations significantly (**19).** The present results are also in agreement with those of **Abdou *et al*. 2011** on female rat bone marrow cells **(20)**. It contains flavonoids which have been shown to reduce oxidative stress in experimental animals **(21,22).**

In conclusion, it can be suggested to use the *Lupinus albus* and *Hyphaene thebaica* as dietary adjuvant to upgrade and alleviate diabetes damage.

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