Efficacy and Biosafety of *Lepidium sativum* Seeds on Hyperglycemia and *Villin* Gene of Renal Brush Border in Alloxan-Induced Diabetic Mice

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Abstract: *Diabetes mellitus* is a metabolic disorder constituting a major health concern today. The study used *Lepidium sativum* (LS), Garden cress, as an edible plant and is native to Egypt. Furthermore, aqueous LS seeds are known for their various ethnic pharmacological properties as mentioned in some medical researches. The present study aimed to examine the hypoglycaemic efficacy of aqueous LS seeds on alloxan-induced diabetic male mice by applying biochemical analysis of BUN and creatinine, and glucometer in sera, besides PAS-glycogenic inclusions in liver and kidney cells. In more precise manner, the present work was constructed to detect the point of mutation of alloxan by the protocol of renal *villin* gene, which expressed in the brush-border membranes of proximal tubules in mice kidney. The alteration of that gene as a result of alloxan-injected diabetic mice was reflected in its impacts on PAS-carbohydrate contents in hepatocytes and renal brush border. Such parameters were used to evaluate the ameliorative potential of LS on these processes. The study used forty mice, were divided into four groups of 10 animals each as follows: Control, alloxan-induced diabetic group, besides the third group of injected alloxan+LS aqueous extract, and the fourth group fed only LS. The biochemical results recorded the ability of LS as a hypoglycaemic and has ameliorative efficacy on renal functions and manifested a distinct mitigation on PAS-glycogenic content in alloxan-diabetic mice at significance decrease (p<0.05). PCR product of mouse *villin* gene patterns by specific primers for both treated groups (alloxan in a lone manner or injected before LS-fed group) were exhibited a progressive variation between these specimens comparable to control once, except LS group, which is corresponded to controls. The study suggested the biosafety of LS on carbohydrate inclusions in hepatocytes and renal tubules, beside its relative alleviation on damaged-mouse *villin* gene at proximal brush border and improver to alloxan-kidney dysfunctions.

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1. Introduction

Lepidium sativum (LS), Garden cress, is an edible plant botanically related to mustard and is native to Egypt and South west Asia (Doke and Guha, 2014). LS is an annual herb belonging to the family Brassicaceae (Cruciferae), which is continued to be the most widely to the present used as vegetables or sources of industrial and cooking oils, forage, and condiments (Al-Shehbaz et al., 2006). LS (*L. sativum*) seeds are well known for their various ethnic pharmacological properties (Vohora and Khan, 1977; Al-Sheddi et al., 2016), and also as a natural food supplement and in traditional medicine (Datta et al., 2011; Al-Sheddi et al., 2016), such as anti-inflammatory, antipyretic, antihypertensive, anti-ashthamatic, anticancer, and anti-oxidant. However, a preliminary work on chemical composition of seeds was carried out and the possibility of using it as nutraceutical food ingredient in dietary fiber formulation was explored by Gokavi et al. (2004). Seeds of LS are rich source of proteins, dietary fibers, omega-3 fatty acids, iron, other essential nutrients and phytochemicals (Doke and Guha, 2014). Therefore, Gokavi et al. (2004); Zia-Ul-Haq et al. (2012) recorded that the whole meal, endosperm and bran of LS (*L. sativum*) seeds had 22.5, 27.7 and 12.6% protein, 27.5, 33.1 and 6% fat, 30, 13.6 and 75% dietary fibers, and 1193.00, 945.15 and 1934.57 mg% potassium respectively. In addition, the same authors (Gokavi et al., 2004) added that the most abundant amino acid was glutamic acid (19.3%) and the essential amino acids, leucine was the highest (8.21%) and methionine was the lowest (0.97%), beside the major fatty acid was linolenic acid (30.2%) and low amount of erucic acid (3.9%) was also present. But the study by Zia-Ul-Haq et al. (2012) indicated that oleic acid (19.50%) and linoleic acid (52.0%), besides alpha linolenic acid (32-34.0%), polyunsaturated fatty acids (46.8%) and monounsaturated fatty acids (37.6%) (Diwakar et al., 2010) were the major fatty acid profile.

Sakran et al (2014) detected a new isoflavonoid, 5,6-dimethoxy-2',3'-methylenedioxy-7-C-β-d-gluco-pyranosyl isoflavone, which was isolated by NMR spectrometry from the seeds of LS (*L. sativum* l.) along with two known isoflavonoids, 7-hydroxy-

4',5,6-trimethoxyisoflavone and 7-hydroxy-5,6-dimethoxy-2',3'-methylenedioxyisoflavone. They also found such compounds have ability to reduce the hepatotoxicity induced by paracetamol in male rats with a significant improvement of total antioxidant capacity, normalizing the levels of liver enzymes GSH, SOD, GPX, CAT and GST compared to control group. LS seeds have been applied as a poultice to pains and hurts, and are also used in the treatment of bacterial and fungal infections (Mukhopadhyay et al., 2010). The toxicity tests by Al-Yahya et al. (1994) on an ethanolic extract of LS seeds showed that the administration of extract in single doses of 0.5 to 3.0g/kg did not produce any adverse effects or mortality in mice, beside the animals treated with extract (100 mg/kg/day) for a period of 3 months in drinking water showed no symptoms of toxicity with a statistically insignificant higher mortality rate.

Eddouks et al. (2005) reported that after an acute (single dose) or chronic (15 daily repeated administration) oral treatments of the aqueous LS extract (20 mg/kg) produced a significant decrease on blood glucose levels in both normal and streptozotocin-induced diabetic rats without affecting insulin secretion. The study by Amawi and Aljamal (2012) was indicated that the administration of LS (*Lepidium sativum*) seed extract (20 mg/kg) for 4 weeks showed a better lipid profile as well as decreases in the blood glucose level (30%) in hypercholesterolemic (4% cholesterol for 2 weeks) of alloxan-diabetic rats. The lipid profile of this investigation showed significant lower values of cholesterol 22%, triglycerides 25%, LDL 23% and increase in HDL 32%. Shukla et al. (2012) reported that the potential antidiabetic efficacy of (50, 150 and 250 mg kg-1, i.p.) of the major components of LS seed total alkaloid fractions (i.e.; lepidine and semilepidine), on alloxan induced diabetic rats upon 21 days continuous treatment, was significantly (p<0.001) suppressed blood glucose, cholesterol, triglyceride and urea levels. Daily oral administration of the aqueous LS extract (20 mg/kg for 3 weeks) exhibited a significant decrease in blood pressure (p < 0.01) in spontaneously hypertensive rats while in normotensive animals no significant change was noted during the period of treatment, and both concomitant with a significant increase of urinary excretion (Maghrani et al., 2005). Althnaian (2014) detected that LS reduced total cholesterol, ALT and serum triacylglycerol, and repaired the histopathological alterations in livers of rats fed with high cholesterol (1%) diet. Bigoniya and Shukla (2014) stated that *Lepidium sativum* Linn. (Cruciferae), commonly known as garden cress is widely used for treatment of asthma, diabetes and rheumatic pain in Indian medicine. The plant is used as salad and seeds are used for its nutritious value. The seed contains glucosinolates and imidazole alkaloids. Furthermore, Al-Sheddi et al. (2016) investigated the protective effects of chloroform extract of LS seeds against oxidative stress and cytotoxicity induced by hydrogen peroxide (H_2O_2) in human liver cells (HepG2).

Diabetes mellitus is a metabolic disorder constituting a major health concern today whose prevalence has continuously increased worldwide over the past few decades (Kruger et al., 2012). Moreover, it has been considered as an incurable metabolic disorder affecting about 2.8% of the global population (Rohilla and Ali, 2012). Mouse animal model has been developed for the past few decades for studying *Diabetes mellitus* and testing anti-diabetic agents that include chemical and genetic manipulations (Chatzigeorgiou et al., 2009; Bu et al., 2012) for the understanding toxicity of such disease. One of the most potent methods to induce experimental *diabetes mellitus* is chemical induction by alloxan (Etuk, 2010; Rohilla and Ali, 2012). It is a well- known diabetogenic agent that is used to induce type-I diabetes mellitus in different experimental animals (Viana et al., 2004; Hu et al., 2013). Alloxan is a urea derivative which causes selective necrosis of the β-cells of pancreatic islets (Dunn et al., 1943), involving oxidation of essential sulphydryl; -SH groups (Szkudelski, 2001), generation of free radicals, disturbance in intracellular calcium homeostasis and inhibition of glucokinase enzyme (Dhanesha et al., 2012). The reduction of this enzyme (glucokinase) decreased both glucose-stimulated insulin release and glycogen synthesis by alloxan (Dhanesha et al., 2012). The underlying mechanism of alloxan involves the selective uptake of that compound as due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells (Lenzen, 2008 and Viswanathaswamy et al., 2011). Thus, it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas; hence it is used to induce diabetes in laboratory animals, especially mice when exposed to supplemented drugs (Takemoto et al., 2015; Wang et al., 2016). Alloxan arise hyperglycemia-induced oxidative stress has been concerned in the development of diabetic nephropathy, which may cause kidney damage associated with inflammation and fibrosis (Kim and Lim, 2013).

The present study aimed to examine the hypoglycaemic efficacy of aqueous *Lepidium sativum* (Garden cress) herb seeds on alloxan-induced diabetic male mice by two techniques of glucometer and PAS-glycogenic inclusions in liver cells. In more precise manner, the present work was constructed to detect the point of mutation of alloxan by the analysis of

renal *villin* gene and its function at brush border having carbohydrate contents and the ameliorative role of LS on this process.

2. Materials and Methods

The Experimental Animals

Thirty adult male Swiss albino mice (*Mus musculus*), morphologically healthy with weights ranged between 25 and 28 g at the beginning of experiment, and were obtained from Theodor Bilharz Research Institute, Giza, Egypt. These animals (30 mice) were randomly allocated to three groups of 10 animals each and kept in polypropylene cages and housed under standard conditions of temperature, humidity and dark light cycle. Mice were housed in suitable cages for two weeks for adaptation to laboratory conditions. All animals were fed on standard diet (hay, wheat and milk). Food pellets and water were available *ad libitum*.

Material agents used

1- Extraction procedure of *Lepidium sativum*

The used dry seeds of *Lepidium sativum* (Garden cress) were purchased from a local market in Cairo, Egypt, and authenticated by an expert taxonomist at Agriculture College, Cairo University.

According to Patel et al. (2009) with minor modifications, the aqueous extract was prepared by boiling 1 g of dried powdered seeds of *L. sativum* in 100 ml of distilled water for 10 min and left for 15 min to infuse. Thereafter, the extract was cooled and filtered to remove particulate matter. The filtrate was lyophilized by first freezing at -22°C in a deep freezer for 8 h. The frozen material was then dried and sterilized in autoclave and powdered using a laboratory grinder. The required doses were taken and reconstituted in 10ml of distilled water just before oral administration.

2-Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine 5,6-dioxyuracil) is an oxidation product of uric acid, having the molecular formula: C4H2N2O4.H2O, and its vial has Batch No. A-00446, Oxford Laboratory Reagent, Mumbai – 400 002, India, which is an oxygenated pyrimidine derivative and is present as alloxan hydrate in aqueous solution .Alloxan monohydrate was purchased from sigma Fine chemicals (USA). Alloxan-induced diabetic mice by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg b.wt.) that was dissolved in normal sterile saline, as designed by Yimam et al. (2014) to induce hyperglycaemia.

According to Slavin et al. (2010), prior to sacrifice, mice were fasted for 15 hours before collection of blood through the retroorbital sinus within 90 seconds of initiating ether anaesthesia at 3 hours into the light phase of the diurnal cycle. Blood samples were collected in iced EDTA and plasma kept at −70°C until analyzed in duplicate for glucose. Subsequent to alloxan administration, the mice had free access to food and water and were provided with 50% glucose solution to drink overnight to induce hyperglycaemic shock. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of one touch ultra-glucometer (G-423 test meter, Bioland, Germany) and compatible blood glucose strips, as recommended by Trinder (1969). Mice showing a fasting blood glucose (FBG) level greater than 140 mg/dl were considered diabetic and they were selected for treatment with *Lepidium sativum* (50 mg/kg b.wt.) was orally administered, once in a day for 30 days.

Experimental protocol

Mice were kept under suitable laboratory conditions during the whole period of experimentation. As recommended by Ferdowsian and Beck (2011), at the end of experimental protocols all the animals were killed humanely taking into account the ethical and scientific considerations regarding animal testing and research.

Mice were allotted to four groups:

Group 1. Served as Control.

Group 2. Mice injected only once with alloxan dosage of 150 mg/kg body weight.

Group 3. Mice injected with both alloxan 150 mg/kg body weight + LS aqueous extract of 50 mg/kg body weight/ daily for successive 30 days.

Group 4. Mice fed only with aqueous extract of LS 50 mg/kg body weight/ daily for successive 30 days.

Cytochemical and Histological Preparations

The carbohydrate inclusions as polysaccharide (glycogen) were cytochemically detected by periodic acid Schiff (PAS) technique of Drury and Wallington (1976). After fixation with alcoholic Bouin's fluid, the paraffin sections of liver and kidney were deparaffinized, oxidized with 0.5% periodic acid and reacted with Schiff's reagent. Then they dehydrated in alcohol, cleared in xylol and mounted in DPX. A positive reaction was indicated by the presence of magenta (pink) colouration; the intensity of which was highly dependent on the available amount of PAS–positive inclusions. Some renal specimens were processed in the usual manner for staining with Mallory's triple stain for those fixed in Zenker's fluid.

Biochemical Analysis

Blood urea nitrogen (BUN) was estimated in serum content according to urease-colorimetric

method described by Patton and Crouch (1977). Whilst the level of serum creatinine was determined using the method of Bowers and Wong (1980).

Molecular Protocol

Genomic DNA extraction

According to Maniatis et al. (1982) and Ausubel et al. (1989) with modifications by Kumar et al. (2016), the extraction of genomic DNA from kidney of mice for the four groups involving: 1-control, 2-alloxan-induced diabetic mice, 3-alloxan-induced diabetic + LS-gavage mice and 4-LS-gavage mice in a lone manner) was applied. The DNA pellet was carefully dried in laminar airflow. The dried DNA was dissolved in minimum amount of TE buffer (pH 8.0). The quantity of total DNA isolated was checked by adding 2 µl 6× orange loading dye (Fermentas, USA) to 2 µl of isolated DNA. Four micro liters of this isolate was loaded in a well of 0.8% w/v agarose gel containing 0.05% of ethidium bromide.

DNA amplification for mouse *villin* gene amplification

For mouse *villin* gene amplification, the study used 5′-CAACTTCCTAAGATCTCC-3′ coding strand primer and 5′-GCAACAGTCGCTGGACATCACAGG-3′ noncoding strand primer were used, generating a ~ 470 bp product, as recommended by Pinto et al. (1999). The purified oligonucleotides were supplied by PRIMM (Milan, Italy). Amplification was performed in 25 µl reaction volumes containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP and 12.5 µl of Taq DNA Polymerase (Roche). The protocol consisted of an initial denaturing step of 2 min at 95 °C, followed by 40 cycles at 95 °C for 1 min (denaturation), 52 °C for 1 min (annealing of primers), and 72 °C for 2 min (extension). Cycling was concluded with a final extension at 72 °C for 4 min, and then held indefinitely at 4°C. Optimization of amplification conditions was carried out by ranging the template DNA from 100 ng, the primers from 10 pmol/µl, $MgCl_2$ from 0.5 to 5.0 mM. A negative control, containing all reaction ingredients except for template DNA, was included for each amplification. The thermal cycler used was a Mastercycler personal (Eppendorf, Hamburg, Germany). All amplifications were done in triplicate and on different days. Ten microliters of amplification products were separated electrophoretically in a 1 % agarose gel using Tris–borate–EDTA (TBE 1X), Sigma-Aldrich buffer system (Lonza) for about 1 h at 130 V. Appropriate DNA marker (XIII, Boehringer Mannheim GmbH, W. Germany) was loaded in an adjacent well. Fractionated bands were detected by ethidium bromide fluorescence under UV light and photographed with a Digital camera.

Statistical Analysis

Statistical analysis of the data was carried out by *t*-test, SPSS statistics 17.0. Whereas, *p*-value ($p<0.05$) was considered as a statistically significant*, whilst *p*-value ($p<0.0001$) was represented as a highly statistically significant**.

3. Results

General carbohydrates in mice liver

Carbohydrate inclusions are markedly abundant in the cytoplasm of the hepatic cells of **control** adult male mice, as reflected by the strong PAS reaction illustrated in figures (1). Nonetheless, these polysaccharides had been ascertained to be mainly "glycogen" as verified by diastase digestion. These inclusions existed usually in the form of intense reddish–magenta coloured particles aggregated together forming dense clumps condensed mainly at one pole of the cells, beside a relatively few particles scattered in the rest of the cytoplasm. Such polarization is usually referred to as "glycogen flight"; a phenomenon known to take place under the influence of the penetrating fixatives which sweep carbohydrate materials in front of them until they settle at the opposite pole of the cells. Investigation of liver tissues after treatment with an aqueous extract of **LS** 50 mg/kg body weight/ daily in a lone manner for successive 30 days (Fig.2). The PAS preparations photographed from these specimens revealed that almost all hepatocytes corresponds to their control configuration in the reactivity of carbohydrate components with the PAS reagent to a considerable extent for the mode of both localization and intensity of glycogen reactivity as marked in figure (2). Examination of liver tissues of **alloxan-diabetic mice** revealed a severe diminution of carbohydrates or glycogen components of the constituent hepatocytes (Fig. 3&4). Moreover, the remaining parts of the cytoplasm of those devastated cells did not appear to contain any apparent traces of glycogen. Inspection of liver tissues after administration with a single **alloxan-induced diabetic** concomitant with **LS**-orally fed (for 30 days) showed a distinct amelioration for the restoration of their polysaccharide contents as manifested by their moderate PAS-reactivity and some areas appear devoid from glycogenic materials, as detected in figures (5&6).

Histology and general carbohydrates of mice kidney

The mice kidney specimens stained with Mallory's stain to show the normal appearance of histological structure of both control group (Fig. 7)

and LS-fed mice for 30 days (Fig. 8), and in alloxan-induced diabetic mice exhibiting marked alterations (Fig. 9), beside the prominent ameliorative of LS on kidney structure after alloxan treatment (Fig.10). Furthermore, the study of affinities of PAS reactivity in brush border of the proximal tubules and glomeruli in the kidney sections of the four examined groups were also detected in control group (Fig. 11), LS-fed mice (Fig. 12), alloxan-injected mice (Fig. 13) and the group of alloxan-treated mice followed by feeding with LS for 30 days (Fig. 14). This point of work was based on the brush border of the proximal tubules has affinity by the reagents used of PAS-stainability as degrees of pink colouration.

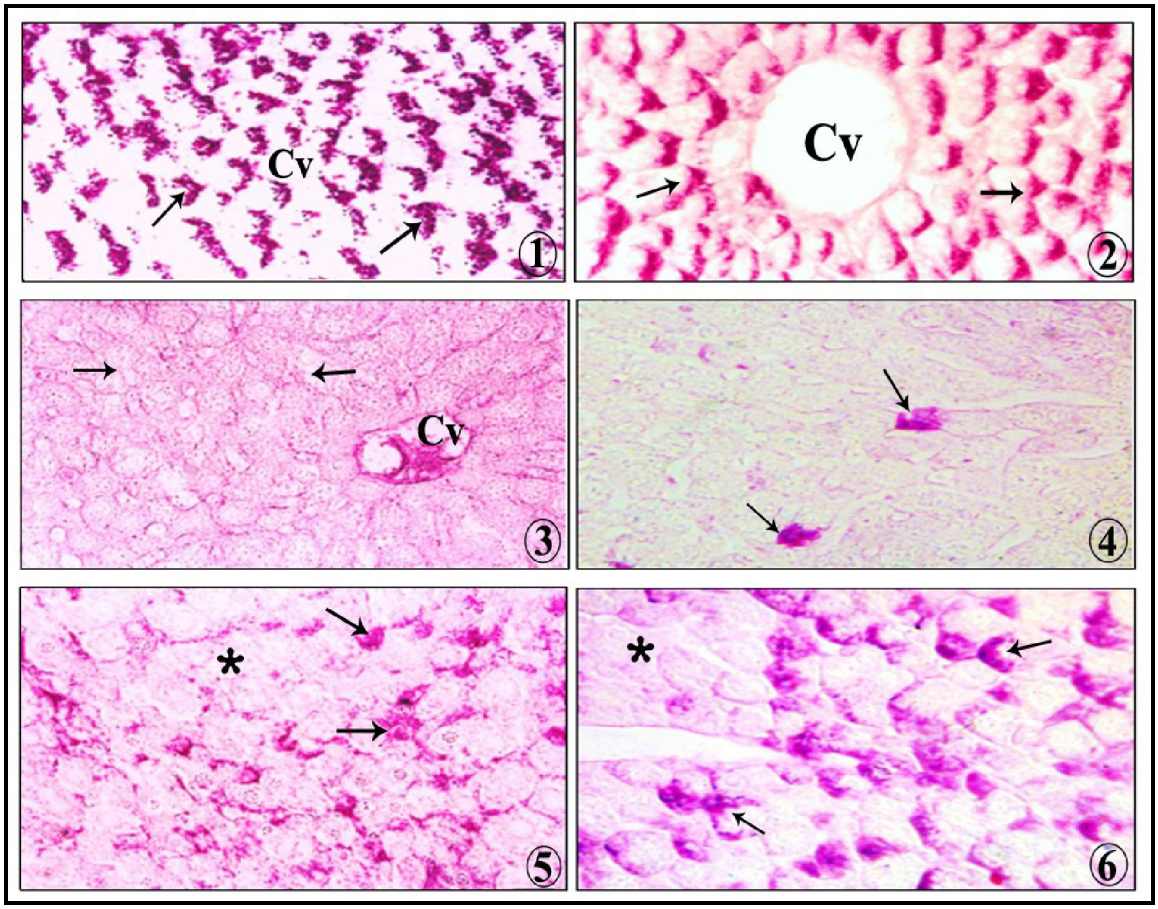
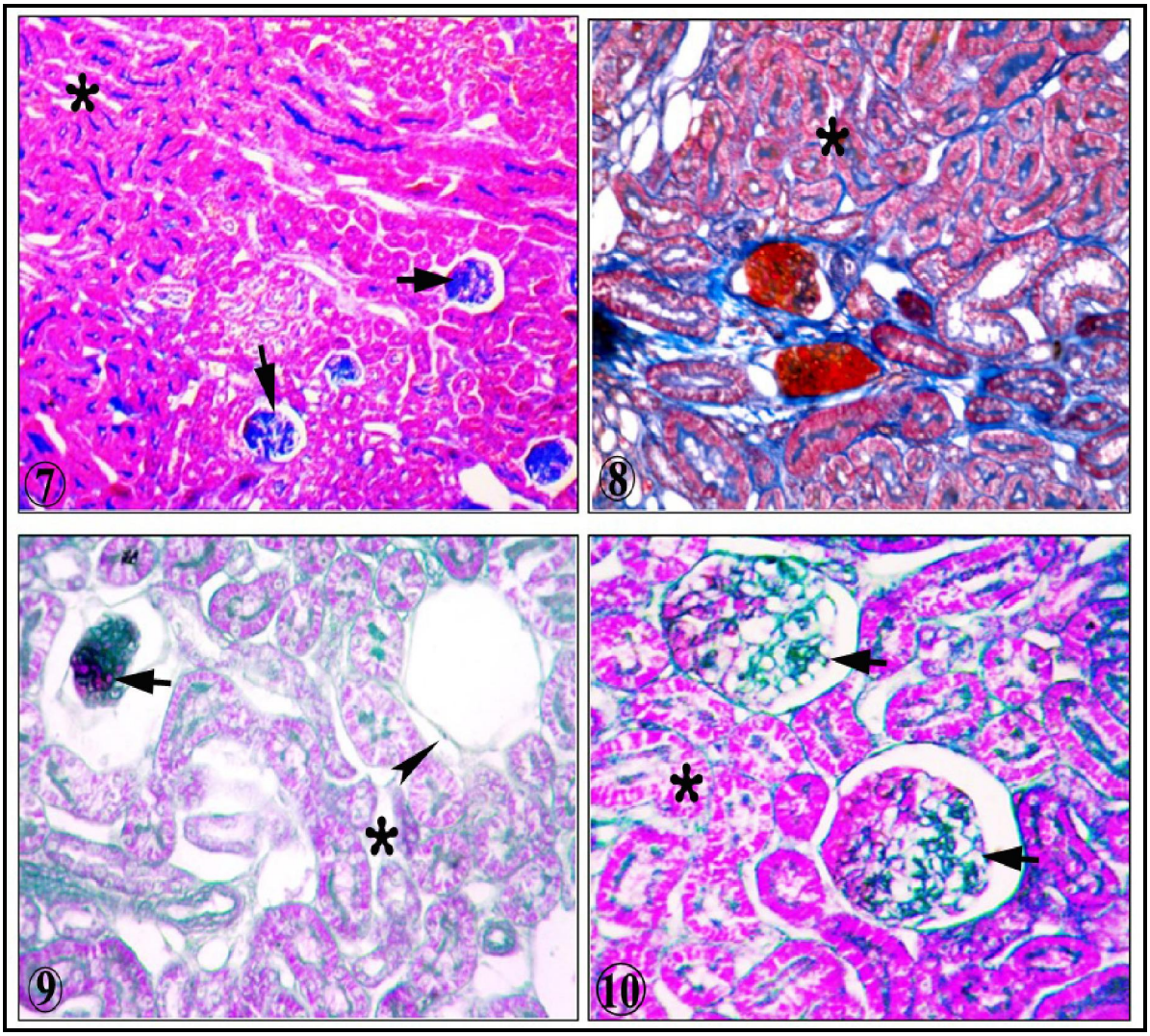


Fig. 1: A liver section of **control** mouse, showing a positively–PAS reaction as a darkly pinkish granules in the cytoplasm of pericentral hepatocytes at one pole of the cell reflecting the phenomenon of "glycogen flight", as exhibited by arrows. The central vein (Cv) as well as the hepatic sinusoids (arrows) are completely devoid of carbohydrate materials (X400).

Fig. 2: Illustrating a normal configuration of liver section from mouse given **LS**, reflecting a classical phenomenon of strongly polarized PAS stainability of glycogen inclusions (arrows) around the central vein (Cv) (X400).

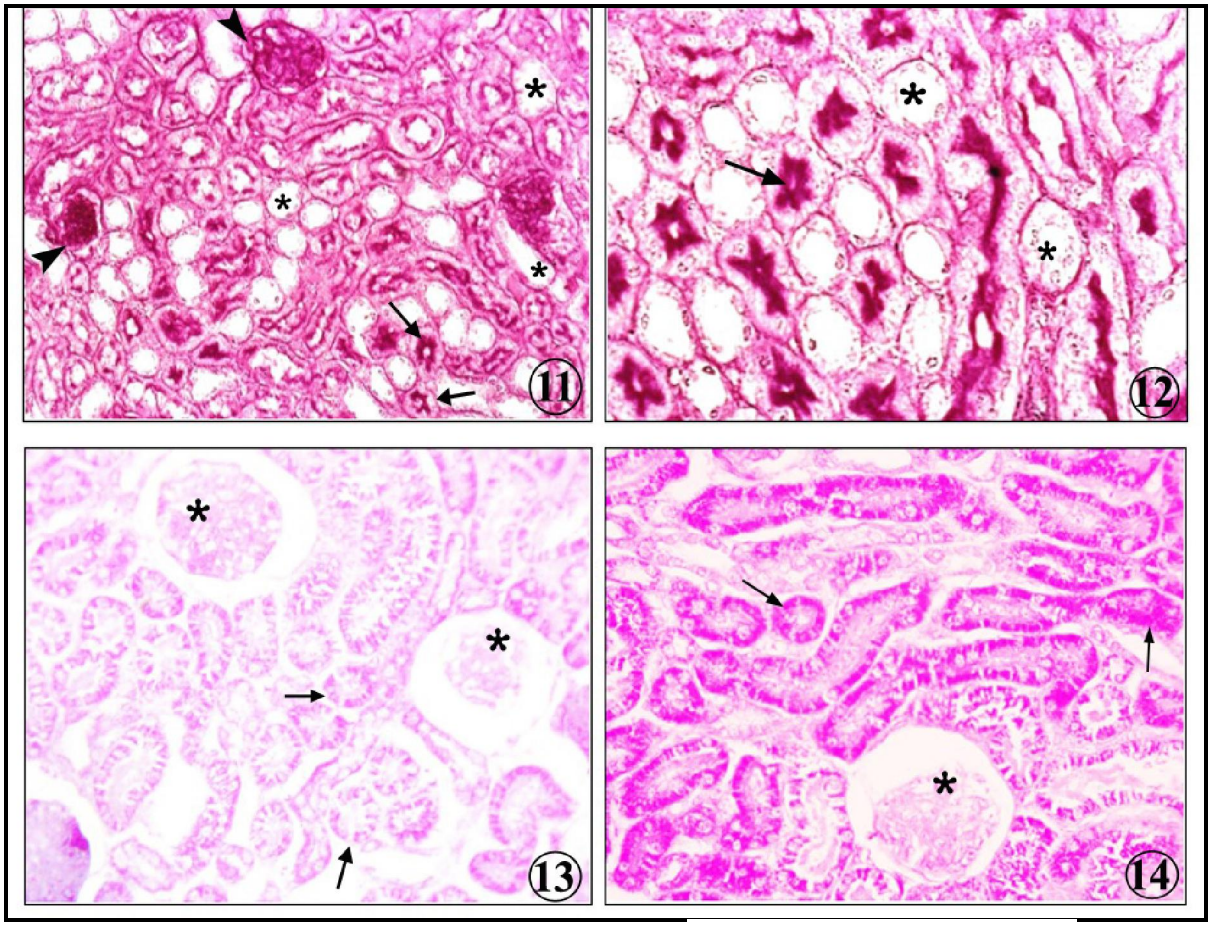
Fig. 3 & 4: Pericentral hepatocytes of **alloxan-induced diabetic mouse** illustrating a progress loss of PAS stainability for glycogen materials (**Fig.3**; arrows). Although, the central vein (Cv) revealing a presence of remaining glycogenic substances inside it with a moderately magenta colouration (**Fig.3)** and some cells still having traces of glycogen (**Fig.4**; arrows) (X400).

Figs. 5 & 6: A liver sections of mice treated with both **alloxan-induced diabetic + LS**-orally fed indicting a marked restoration of its carbohydrate contents with a moderate PAS-reactivity (arrows). Besides, some hepatocytes still appear devoid of glycogenic materials (*) (X 400).



Figs. 7-10: Photomicrographs of mice kidney sections stained with Mallory's stain to show the normal appearance of histological structure of both control group **(Fig. 7)** and LS-fed mice for 30 days **(Fig. 8)** illustrating well developed glomeruli (arrows) and brush borders of proximal convoluted tubules taking a dark bluish stainability (*). **Fig. (9)** exhibits marked alterations in the group of alloxan-induced diabetic mice representing with atrophied glomeruli (arrow) or totally degenerated from their Bowman's capsules (arrowhead) with losing the characterized renal brush border (*).

Fig. (10) shows a magnified part of kidney specimen of alloxan-treatment plus LS-fed mice indicating the prominent ameliorative of LS on configuration of kidney components. The figure illustrates a normal well-formed glomeruli (arrows) and abundant of brush border lining the intact renal lumens with a bluish green colouration (*). (Fig 7; X100 & Figs.8-10; X400)



Figs. 11-14: Photomicrographs of affinity of **PAS stainability** in brush border of the proximal tubules and glomeruli in the kidney sections of the four examined groups: Control group (Fig. 11), LS-fed mice (Fig. 12), alloxan-injected mice (Fig. 13) and the group of alloxan-treated mice followed by feeding with LS for 30 days (Fig. 14).

Brush border of the proximal tubules has affinity by the reagents used of the periodic acid of Schiff' pink coloration (arrows).

Fig. 11: A kidney section of **control** mouse, showing a positively–PAS reaction as a darkly pinkish layer of brush border enclosing the lumens of renal proximal convoluted tubules (arrows) and strongly pinkish masses of glomeruli (arrow heads), contrasts with the clearer and less abundant PAS-positively of the distal tubules (*). (X100)

Fig. 12: A magnified section of mouse kidney from **LS-treated group** exhibiting proximal tubules are characterized by an abundant carbohydrate content at brush borders (arrow), with a little affinity of PAS-philic cytoplasm. However, the figure showing PAS-negatively in the distal tubules (*). (X400)

Fig.13: A magnified section of mouse kidney was taken from **alloxan-injected group** revealing severe alterations in the response of PAS reactivity in almost renal brush borders of proximal convoluted tubules (arrows), and the glomeruli appear shrinkage in degenerated appearance with totally loss to their affinity to PAS stainability (*). (X400)

Fig. 14: A magnified section of mouse kidney of **alloxan-injected + LS-fed mice** exhibiting a moderate improvement by LS on PAS reactivity of the carbohydrate content after exposure to alloxan, whilst the glomeruli a relatively still suffering from the effect of alloxan (*). Whereas, the most renal tubules (arrows) restored their PAS positively of magenta colouration. (X400)

Biochemical Results

The data represented in table (1) display the influence of both alloxan and LS in a separate or concomitant fashion on the serum BUN and creatinine levels to examine the kidney functions in male mice. The two biochemical parameters (BUN and creatinine) score a highly significant increase (p<0.0001) after exposure to alloxan injection showing the progressive effect of alloxan on the renal physiology in alloxan-diabetic mice compared with

control group. Such impacts were also markedly recorded the serum glucose level in alloxan-hyperglycaemic mice as due to the known effect of alloxan on generating diabetic mice revealing a highly significant increase (p<0.0001) comparable to control ones (Table 1). Although, the examined glucose parameter of LS-groups (3&4) recorded a distinct ameliorative efficacy of LS on hyperglycaemic mice for those tested groups (alloxan-diabetic+LS-Group, and LS-group) manifesting significant decreases of the serum glucose level (p<0.0001 & p< 0.05) compared with group (2) and control group (1), respectively, as illustrated in table (1). Besides, the other two measured criteria (BUN and creatinine) of kidney functions proved the mitigated role of LS on their records in comparable with LS-negative groups and the control samples scoring significant decreases, as evidenced in table (1).

Table 1: Levels (Mean ±SD)$^{n=8}$ of BUN, creatinine and glucose in sera in four experimental cases involving: control, alloxan-induced diabetic mice, alloxan-induced diabetic + LS-gavage mice and LS-gavage mice in a lone manner, and their statistical significances (p-values).

Experimental Mice Groups	**Biochemical Parameters (Mean ±SD)**		
	Serum BUN (Blood Urea Nitrogen) (mmol/L)	**Serum Creatinine** (µmol/L)	**Serum Glucose Level** (mg/dl)
1. Control-Group	**6.96 ± 0.80**	**22.98 ± 0.674**	**115.5 ± 7.54**
2.Alloxan-diabetic Group	**↑ 17.63 ± 4.46 ****	**↑ 28.33 ± 0.981 ****	**↑ 392 ± 7.34 ****
3. Alloxan-diabetic + LS-Group	**8.18 ± 0.809 (↑*)** comparable to controls, and **(↓**)** when compared with alloxan-diabetic group	**24.28 ± 0.727 (↑*)** comparable to controls, and (↓**) when compared with alloxan-diabetic group	**227.8 ± 9.04** (↑**) comparable to controls, and (↓**) when compared with alloxan-diabetic group
4. LS-Group	**↓ 5.93 ± 0.220 ***	**23.15 ± 0.633**	**↓ 97 ± 2.82***

Level of significance:
***p*> 0.05 is a statistically non-significant.**
*** *p*< 0.05 is a statistically significant.**
*****p*< 0.0001 is a statistically highly significant.**
↑ represents a significant increase.
↓ represents a significant decrease.

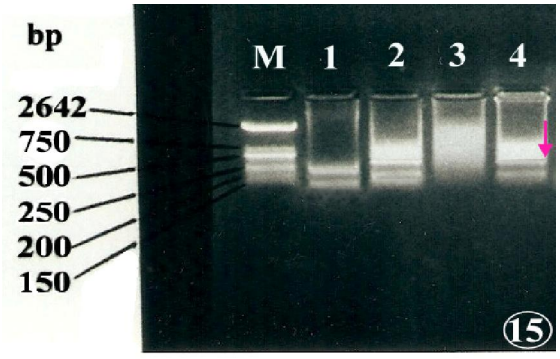


Figure 15: PCR pattern of *Villin* gene amplified with specific primers (5′-CAACTTCCTAAGATCTCC-3′ coding strand primer and 5′-GCAACAGTCGCTGGACATCACAGG-3′ noncoding strand primer) at the band ~ 470 bp (arrow) for alloxan-induced diabetic+LS-gavage mice (lane 1), control (lane 2), alloxan-treated samples (lane 3, hasn't the characteristic band but reveals DNA-fragmented smear) and LS-gavage mice (lane 4). Lane M: represents the DNA molecular size marker.

Molecular protocol

PCR product of mouse *villin* gene patterns of both treated and control groups were represented in figure (15). The figure shows the characteristic fragment ~470 bp of amplified mouse *villin* gene segment were detected in LS-treated group as well as the control one. However, such band is not observed in the two alloxan-treated groups (involving alloxan-treated diabetic mice beside the group of alloxan-diabetic + LS-fed mice), reflecting the progressive variation between alloxan-diabetic samples and the ameliorative LS on the mouse *villin* gene. Such observations may be regarded to the relative ability of LS on improvement of alloxan-induced DNA alterations at the level of mouse *villin* loci of the renal brush border under the examined conditions.

4. Discussion

Marles and Farnsworth (1995) stated that *Diabetes mellitus* is a debilitating and often life-threatening disease with increasing incidence in rural populations throughout the world, predicting some

plants with hypoglycemic potential activity and promising directions for future research on antidiabetic plants. If LS seeds evaluated from traditional medicine, thus many diabetic people turn to complementary therapies to control the chronic nature and threat to quality of life eventually reducing the complications (Israili et al., 2007). For a long time plants based herbal medicines have been the major source of drugs for treatment of *Diabetes mellitus* in Ayurveda and other ancient systems of medicine (Akhtar and Ali, 1984).

Concerning the determination of dietary feeding of LS dosage, Datta et al. (2011) reported that by conducting the acute doses (0.5 –5.0 g/kg b. wt.) for 72 hrs. or subchronic toxicity (1.0 – 10.0%) for 14 weeks of LS seed powder did not induce any symptoms of toxicity or mortality of male and female rats to assess its safety to humans, particularly for its use in pharmaceutical preparations. Moreover, the previous study was also found no significant changes in food intake, gain in body weight, and relative weight of organs, hematological parameters, macroscopic and microscopic changes in vital organs. As recommended by Yimam et al. (2014), insulin dependent diabetes was induced by administering a single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg to CD-1 mice.

Antioxidant Activity of LS seeds as interpreted by Kasabe et al. (2012); Doke and Guha (2014) was illustrated in the following: Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant has ability to trap these free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids in LS seeds (as assayed by Kasabe et al., 2012; Zia-Ul-Haq et al., 2012; George et al., 2015) scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Bhasin et al. (2011) investigated the antioxidant activity of LS seed extracts in different solvents (ethanol, chloroform, methanol, benzene, hexane, propanol, glacial acetic Acid, petroleum ether, acetone, and ethyl acetate). Ethanolic extract of LS seeds was reported to possess the strongest antioxidant activity (Bhasin et al., 2011; Yadav et al., 2011). In turn, the antioxidant activity of an ethanolic extract of LS seeds symbolizes its reducing potential and its tendency to neutralize reactive oxygen species available in the environment (Mukhopadhyay et al., 2010).

The present investigation recorded a distinct ameliorative efficacy of LS on hyperglycaemic mice for those tested groups (alloxan-diabetic+LS-Group, and LS-group) manifesting significant decreases of the serum glucose level ($p<0.0001$ & $p< 0.05$) compared with group Alloxan-diabetic Group and control ones, respectively. Such results interpreted by Eddouks et al. (2005), who concluded that the aqueous extract of LS exhibits a potent hypoglycaemic activity in rats without affecting basal plasma insulin concentrations. Shukla et al. (2012) interpreted the potential antidiabetic effect of *L. sativum* alkaloid against alloxan-induced diabetes may be through reducing oxidative damage and modulating antioxidant enzymes. The possible mechanism by which LS alkaloid brings about its anti-hyperglycaemic action may be by potentiation of pancreatic secretion of insulin from the remaining islet β-cells. In more depth, Mahassni and Al-Reemi (2013) reported that glucosinolates were the major secondary metabolites of LS and its extracts had chemopreventive effects in inhibiting carcinogenesis. The mechanism explaining the observed hypoglycaemic activity of this plant in normal and diabetic mice was suggested by Goumenos et al. (2002); Eddouks and Maghrani (2008), who stated that the aqueous LS extract (20 mg/kg for 15 days) caused a potent inhibition ($p<0.001$) of renal glucose reabsorption with a potent enhanced glycosuria by elevating urinary excretion with urinary transforming growth factor-beta1, which was mainly localized within the cytoplasm of renal tubular epithelial cells, so in turn reduced blood sugar in both normal and diabetic rats. Whereas in newly diagnosed diabetic patients, hyperglycaemia seems to represent the principal factor leading to urinary transforming growth factor-beta1 overproduction and it is also an important mediator of the immune and anti-inflammatory responses (De-Muro et al., 2004).

In this concern, administration of *Lepidium sativum* (LS) seed total alkaloid extract effectively prevented the increase in blood glucose level without causing a hypoglycemic state (Amawi and Aljamal, 2012). These authors suggested some hypotheses to interpret the mechanism of lower value of serum glucose by LS, as following: It may be due to restoration of the delayed insulin response and slow absorption of glucose. The possible mechanism by which the extract of garden cress (LS) seeds brought its anti-hyperglycemia action might be by potentiation of pancreatic secretion of insulin from the remaining islets of β-cells. Other probable mechanism by which the alkaloid of LS lowered blood glucose levels in diabetic rats might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver or reducing the absorption of glucose from the intestine or these might have improved insulin resistance.

The present study used PAS-glycogenic reactivity in both hepatocytes and brush border of renal tubules, beside the biochemical analysis as

evidences on the effect of alloxan on *Villin* gene, responsible for structure-function of renal brush border, and the hypoglycaemic and improver role of LS on this process. In this concern, glomerular filtration rate showed a significant increase after oral administration of LS in normal rats ($p< 0.001$) while in spontaneously hypertensive rats, no significant change was noted during the period of treatment (Maghrani et al., 2005). The findings by Kassie et al. (2003) showed that water and garden cress juices (0.1–1.25 µl/ml) are highly detoxification and chemoprotective against benzo(a)pyrene-induced DNA damage in a pronounced dose-dependent manner in human derived cells using the single cell gel electrophoresis assay and that their effects cannot be explained by their isothiocyanate contents. LS is a natural antioxidant and effective herbal medicine improved the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose (Kooti et al., 2016).

Ongeri et al. (2011) interpreted the mice kidney alterations with meprins, metalloproteinases abundantly expressed in the brush-border membranes of rodent proximal kidney tubules, have been implicated in the pathology of renal injury induced by ischemia-reperfusion.

The present work was constructed to detect the point of mutation of alloxan by the analysis of renal *villin* gene and its function at brush border containing carbohydrate contents and the ameliorative role of LS on this process. The *villin* gene is located on chromosome 2q35-36 in humans and on chromosome 1 in mice. Villin belongs to a family of calcium-regulated actin-binding proteins that share structural and functional homologies (Pringault et al., 1991). The villin gene is expressed mainly in cells that develop a brush border, such as mucosal cells of the small and large intestine and epithelial cells of the kidney proximal tubules. *villin*, a calcium-regulated actin-binding protein, modulates the structure and assembly of actin filaments *in vitro* (Friederich et al., 1990). *villin* is mainly produced in epithelial cells that develop a brush border and which are responsible for nutrient uptake. Beauregard et al. (1995) used an antisense RNA strategy to investigate the role of the actin-associated protein, *villin*, in the brush-border morphogenesis of human intestinal cells. In the core of each microvillus, actin filaments are bundled by two proteins, *villin* and fimbrin, which are present in the intact kidney proximal tubules and in others alimentary passages (Pinson et al., 1998).

Expression of the *villin* structural gene is precisely regulated during mouse embryogenesis and is restricted in adults, to certain epithelia of the gastrointestinal and urogenital tracts, suggesting that *villin* plays a key role in the morphogenesis of microvilli (Friederich et al., 1990). The findings by Hoboth et al. (2015); Mziaut et al. (2016) showed that *villin* controls the size of the F-actin cages restricting newly synthesized insulin-containing secretory granules (SGs), in pancreatic islet b cells, and, thus, regulates their dynamics and availability for exocytosis. Evidence that *villin* acts downstream of *Ica512* also indicates that SGs directly influence the remodeling properties of the cortical actin cytoskeleton for tight control of insulin secretion.

The presence of others bands beside the main band detected in specimens of may be due to polymorphic loci of that designed primer to match the *villin* gene, which were characterized by means of inter-simple sequence repeats (ISSRs) to discern the extent of genetic variation and to develop a fingerprinting key between the mice individuals, as observed by Adhikari et al. (2014), who found that the number of primers producing polymorphism is 12 from the Total number of 15 primers screened, recording the percentage of total loci which are polymorphic 87.01%, to interpret the genetic diversity (polymorphism) between the selected individuals (Parveen et al., 2013).

In conclusion

The study suggested the biosafety of LS on carbohydrate inclusions in hepatocytes and renal tubules, beside its relative alleviation on damaged-mouse *villin* gene at proximal brush border and improver to alloxan-kidney dysfunctions. The study also detected the hypoglycaemic efficacy of aqueous *Lepidium sativum* on alloxan-diabetic mice.

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