Biological and chemo-pathological comparative study on the effect of insulin and lettuce oil on experimentally-induced diabetic rats

Naglaa Z. H. Eleiwa *, Ibrahim S. Salem ** and Sherein S. Abd Elgayed ***

* Pharmacology Dept. Faculty of Veterinary Medicine, Zagazig University, Egypt.
** Nutrition and Food Science Dept. Faculty of Home Economics, Helwan University, Egypt.
*** Pathology Dept. Faculty of Veterinary Medicine, Cairo University, Egypt.

Abstract

Background: There is upward evidence that established the connection between dietary polyunsaturated fatty acids (PUFA) and insulin action and sensitivity.

Objective: The main objective is to compare the biological, biochemical and histopathological effects of insulin and lettuce oil (polyunsaturated fatty acids) on alloxan-induced diabetic rats.

Methods: 42 Albino rats were divided into 6 groups: the first group fed on basal diet (control negative). Rats in the other five groups were all injected subcutaneously with 150 mg/kg body weight of alloxan to induce hyperglycemia then those rats were subdivided into the followings: a group that remained as induced diabetic ‘control positive’ [2nd group], a group that fed on basal diet and subcutaneously injected with insulin 1 IU/kg body weight twice weekly [3rd group]; a group that fed on basal diet + injected with half of the insulin dose mentioned in the previous group + dietary supplemented with Lettuce oil 2% (LO2%) [4th group]; a group that fed on basal diet + dietary supplemented with Lettuce oil 4% (LO4%) [5th group], and the 6th group was fed on basal diet + dietary supplemented with Lettuce oil 6% (LO6%). At the end of the experimental period (8 weeks), different biological and serological parameters were estimated and specimens from the pancreas, liver and kidney were collected for histopathological examination.

Results: Diabetic rats either treated with insulin alone or combined with LO2% showed significant increase in the food efficiency ratio (FER) with marked decrease in food intake (FI). Rats in the all the intervention groups elicited variable degrees of decline in the serum glucose level. Insulin treated, insulin +LO2%, LO4% and LO6% groups showed significant decrease in the serum triglycerides, LDL and VLDL and elicited significant increase in serum HDL. Addition of LO2% to the diet of diabetic rats in combination with insulin injection displayed significant decrease in the urea nitrogen and creatinine and by increasing the amount of lettuce oil added to the diabetic rat diets, the mean values of uric acid & urea nitrogen and creatinine levels were directed toward the control negative values. Serum aspartate amino transferase (AST) level was significantly decreased in the groups treated either with insulin only or fed on LO4% & LO6%. Histopathological results showed that, the combination between insulin therapy and dietary supplementation with lettuce oil descend the curve representing tissue damage and histopathological lesions resulted from diabetes in the liver, kidney and pancreatic tissues.

Conclusion: The study recommended the beneficial dietary supplementation of lettuce oil in combination with insulin therapy in dealing with diabetes to decline the consequent diabetic complications.
Introduction

Although the discovery of insulin and its preparations in a form suitable for administration were of immeasurable benefit to many tens of thousands of diabetics, disadvantages were still evident and many lines of investigation had been followed in attempts to overcome the difficulties associated with insulin therapy [1]. Other attempts were to obtain a substitute to insulin. Mohan and Das [2] demonstrated that, ω-3 and ω-6 long-chain polyunsaturated fatty acids (LCPUFA) can attenuate chemically induced diabetes mellitus in rats by enhancing the antioxidant status and suppressing production of cytokines. Salmerón et al. [3] suggests that replacing 2% of energy from trans fatty acids isoenergetically with PUFAs would lead to a 40% lower risk of type 2 diabetes while prior treatment of Wistar rats with oils rich in eicosapentaenoic acid (EPA), arachidonic acid (AA), and γ-linolenic acid (GLA) prevents the development of alloxan-induced diabetes mellitus [4]. These findings suggest that LCPUFAs protect β cells from the cytotoxic actions of alloxan and also inhibit the production of TNF-α; which has an important role in the pathogenesis of diabetes; both in vitro and in vivo which may explain the beneficial effect of LCPUFAs in both type 1 and type 2 diabetes [4].

Other studies found a positive correlation between dietary polyunsaturated fatty acids and insulin action [5,6]. It was observed that increased intakes of (LCPUFAs) reduce insulin resistance and, thus, decrease the risk of type 2 diabetes as the number of insulin receptors increases when Ehrlich cells, which show all the binding characteristics of mammalian insulin receptor, were enriched in PUFAs [7,8]. Accordingly in male Wistar rats, fish oil (poly unsaturated fatty acids) intake resulted in a dose-dependent increase in glucose utilization and clearance in vivo, and an increase in insulin sensitivity Somova et al. [9].

Taken together, the current work was conducted to evaluate the biological, biochemical and histopathological effects of lettuce oil (polyunsaturated fatty acid) in comparison to insulin on alloxan-induced diabetic rats.

Materials and Methods

Materials:

-Insulin (Maxitard®); injectable solution produced by Novo Nordisk Co., Denmark; each ml contains 100 IU and each 1 unit equals 0.035 ml of anhydrous human insulin.

-Stem Lettuce (Lactuca sativa) seed oil was extracted [10] at Technology Research Institute, Cairo, Egypt and Gas Liquid Chromatography (GLC) technique was employed to identify the fatty acids composition of the obtained lettuce oil.

-Alloxan, casein, cellulose, vitamins and salts, absolute alcohol, Canada balsam, formalin, methyl alcohol, paraffin wax and xylene were purchased from EL-Gomhoria Company, Cairo, Egypt. Forty two male albino rats, each weighting 110 ± 5 gm were obtained from Helwan Farm.
Methods:

The experimental design:

Forty two male rats were kept in individual stainless steel cages under hygienic conditions and fed two weeks on basal diet for adaptation ad libitum in the animal house of Faculty of Home Economics, Helwan University. The basal diet in the experiment consists of casein (12.5%), corn oil (10%), choline chloride (0.2%), cellulose (5%), sucrose (22%), corn starch (45.3%), salt mixture (4%) and vitamin mixture (1%) [11].

After 2 weeks, the rats were divided into 6 groups; each of 7 rats; as follows: the first group fed on basal diet and kept as a control negative group. Rats in the other five groups were all injected subcutaneously with 150 mg/kg body weight of alloxan after fasting overnight to induce hyperglycemic diabetes [12], then those rats were subdivided as the followings: a group that remained as induced diabetic ‘control positive’ [2nd group], a group that fed on basal diet and subcutaneously injected with insulin 1 IU/kg body weight twice weekly [13] [3rd group]; a group that fed on basal diet + injected with half of the insulin dose mentioned in the previous group + dietary supplemented with Lettuce oil 2% (LO2%) [4th group]; a group that fed on basal diet + dietary supplemented with Lettuce oil 4% (LO4%) [5th group], and the 6th group was fed on basal diet + dietary supplemented with Lettuce oil 6% (LO6%).

At the end of the experimental period (8 weeks), blood samples were collected for serum separation to estimate serum cholesterol, triglycerides and HDL-c [14], LDL-c and VLDL-c [15], AST, ALT [16] and glucose [17]. Rats liver, kidney, heart, brain and spleen were isolated, weighted and % of organs to body weight was computed.

Statistical analysis:

The statistical analysis were carried out by using SPSS, PC statistical software (version 8.0 SPSS Inc., Chicago, USA). The results were expressed as mean ± SD. Data were analyzed by one way analysis variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at (P<0.05). [18].

Histopathological Technique:

Specimens of the liver, kidney and pancreas were fixed in 20% neutral formalin for histopathological examination using Lillie and Fulman technique [19].

Results and Discussion

- Chemical analysis of the obtained lettuce oil using GLC revealed the following fatty acids composition:
  Coprylic C8:0 (0%), Capric C10:0 (0%), Lauric C12:0 (0%), Myristic C14:0 (0.1%), Palmitic C16:0 (1.5%), Palmitoleic C16:1 (0.4%), Margaric C17:0 (0%), Hepta decenoic C17:1 (0%),
Stearic C18:0 (1.3%), Oleic C18:1 (30.9%), Linoleic C18:2 (33.2%), Linolenic C18:3 (26.8%), Arachidic C20:0 (0.9%), Ecosadienoic C20:2 (0.1%), Behenic C22:0 (0.5%), Erucic C22:1 (2.3%), 13,16Docosa dienoic C22:2 (1.5%), Lignoceric C24:0 (0.2%), Selacholeic C24:1 (0.3%), Total saturated Fatty Acids (SFA) (4.5%), Total monounsaturated Fatty Acids (MUSFA) (33.9%) and Total polyunsaturated Fatty Acids (PUSFA) (61.6%).

We notice from this analysis that, the polyunsaturated fatty acids (PUFA) forms the major proportion (61.6%) of lettuce oil fatty acid constituents so lettuce oil considers a good source of PUFA.

1. Biological Effects :-  
   1.A. Nutritional Evaluation:-  
   
   Effects of insulin and lettuce oil administration on food intake (FI), body weight gain (BWG) % and food efficiency ratio (FER) in alloxan-induced diabetic rats are shown in table 1. The mean values of FI (gm/day) of the control (+) group were markedly lower than that of the healthy normal rats control (-). Also, the control (+) group revealed a significant decrease in the mean values of FER and BWG % compared with those of the control (-) group.

   The weight loss observed in the induced diabetic rats was mostly attributed to the failure of the body to make use of the glucose so, the body directed toward muscles degradation and lipolysis to get energy therefore decrease in BWG % is familiar to be observed [20] and this weight loss take part in decreasing the blood glucose level in diabetes as reported by Al-Shamsi et al.[21].

   As exhibited also in table 1, diabetic rats treated with insulin only or in combination with dietary LO2% showed significant increase in the mean values of FER compared with the control (+) group while diabetic rats treated with insulin alone demonstrated significant increase in BWG% compared with the control (+) group.

   The formentioned findings highlightened the fact that, insulin is a key hormone in the regulation of food intake, nutrient storage and nutrient partitioning, and is linked to proper animal growth [22].

   This study documented that, diabetic rats fed LO4% and LO6% elicited improvement in FI and FER compared with the control (+) group.

   The previous findings stressed on the fact that, Polyunsaturated fatty acids (PUFAs) of the n–6 and n–3 families are necessary for proper growth and body function and it seems to be a beneficial effect on clinical outcomes by enrichment with dietary PUFAs. Several factors may account for these observations. First, serum LDL-cholesterol concentrations tend to decline when saturated fatty acids are replaced with PUFAs in the diet[23]. Second, PUFAs—may have antiatherothrombotic effects on growth factors, cytokines, and signal molecules [24,25]. Third, PUFA-rich food sources are often rich in antioxidants [23].
1.B. Organs weight as a percent of body weight:-

The mean values of (heart, liver, spleen, kidney and brain) weights as a percent of body weight in all the tested groups of rats are shown in table 1. The results revealed a significant decrease in the mean values of the heart weight as a percent of body weight in the control (+) group compared with the control (-) while there was a significant increase in the mean values of the kidney weight compared with the control (-). Non-significant changes in the liver, spleen and brain weights were observed in the control (+) rats compared with the control (-).

These results are fit with those obtained by Craven et al.[26] who reported a positive correlation between diabetes and increase kidney weight and they stated that untreated diabetic rats have shown two fold and seven fold increase in glomerular volume and albumin clearance, respectively.

Rats in the groups treated with (insulin +LO2%) and those dietary supplemented with LO4% showed significant increase in the mean value of liver weight while non-significant changes were recorded in the heart, spleen, kidney and brain weights in all treated groups compared with control (+). These result are match those obtained by Gaiva et al.[27] who reported that an enrichment of the diet with polynsaturated fatty acids produced significant changes in liver metabolism associated with increase in the liver weight and fat contents. Previous studies have shown that dietary PUFAs increased fat cell size or more fat cell numbers, and raised hepatic lipogenic enzyme activities which may account for the increased liver weight obtained. Types of PUFAs used, quantity of fat in the diets, and length of time studied are just a few of the factors that may affect the results [28].

2. Biochemical analysis of serum:-

2.A. Lipid fractions:-

Effects of insulin and lettuce oil administration on lipid fractions in alloxan-induced diabetic rats are presented in table 2. It could be noticed that the control (+) group showed a significant increase in the mean values of serum "triglycerides, LDL-C and VLDL-c " compared with those of control (-) group. Stamer et al.[29] supported the above mentioned results as they recorded that the most common lipid abnormalities found in diabetic individuals are hypertriglyceremia, elevated VLDL-c and decreased HDL-c.

Significant decrease in the mean values of serum triglycerides, LDL and VLDL were recorded in (insulin treated, insulin+LO2%, LO4% and LO6% groups) compared with the control (+) whole the group of rats that fed on a diet containing LO4% showed significant increase in total serum cholesterol level compared with the control (+). The mean value of serum HDL demonstrated significant increase in all the intervention groups compared with the control (+).

The previous findings is highly commendable by Lawson et al.[30] and Hayashi et al. [31] who stated that insulin therapy leads to significant rise in high density, lipoprotein cholesterol (HDL-C) level with a drop in the low density lipoprotein cholesterol (LDL-C) to HDL-C ratio and LDL-C level.

This effect of insulin may be supported by a concept that, insulin regulates both the secretion of VLDL-C, LDL-C from the liver into the plasma and its removal at the peripheral
tissue through its action on lipoprotein lipase "LPL" enzyme, a key enzyme in removing of triglycerides and lipoproteins from the blood stream so, inhibition of this enzyme by insulin treatment will results in a marked decrease in serum lipoprotein levels [32].

Diets rich in PUFA have been shown to facilitate the interaction of lipoprotein triglyceride with LPL by increasing the solubility of lipids in the circulating lipoproteins [33].

One possible alternative scenario is that, the decline in the serum lipid parameters( serum triglycerides, LDL and VLDL) which followed dietary supplementation of diabetic rats with polyunsaturated fatty acids may be due to suppression of the transcription of a wide array of hepatic lipogenic genes including fatty acid synthase (FAS) and acetyl-CoA carboxylase. Interestingly, the over-expression of sterol regulatory element binding protein-1 (SREBP-1) induced the expression of all of the enzymes suppressed by PUFA so it can be hypothesized that, PUFA coordinately inhibit lipogenic gene transcription by suppressing the expression of SREBP-1 XU et al.[34].

Harris and Bulchandani [35] stated that the triglyceride-lowering effect observed in rats fed on PUFA, has been attributed mostly to a decreased lipogenesis and partially to increased β-oxidation, consistent with increased mitochondrial compared with peroxisomal oxidation.

2.B. Kidney functions:-

Effects of insulin and lettuce oil on serum uric acid, urea nitrogen and creatinine in alloxan-induced diabetic rats are presented in table 3. Control (+) group showed a significant increase in the mean values of serum uric acid and urea nitrogen compared with those of the control (-) group. This is in agreement with both Asayama et al.[36] who found uric acid to be increased in the serum of diabetic rats and Imaeda et al.[37] who reported that blood urea nitrogen was increased as a result of injection with streptozotocin. This can be attributed to the increased rates of protein catabolism and gluconeogenesis for obtaining energy because of the body's inability to utilize blood glucose due to the lack of insulin production and/or action [38, 20, 39]. Renal dysfunction due to oxidative damage associated with diabetes is an important reason as well [40].

Diabetic rats treated with insulin only or in combination with LO2% elicited significant decrease in the mean values of serum urea nitrogen and creatinine compared with the control (+). Insulin treated rats showed significant increase in uric acid compared with control (-) group.

Yanan Zheng et al. [41] credited these findings to the fact that, insulin is known to bind to most of the nephron segments and to modify several functions of renal tubules. Little is known about roles of insulin receptor substrates (IRS) in the renal insulin actions but several intracellular proteins have been identified as phosphorylation substrates for the insulin receptor, when IRS-1 is activated by phosphorylation, it serves as a type of docking center for recruitment and activation of other enzymes that ultimately mediate insulin's effects [42].

On the other hand, hyperglycemia potentiates insulin antinatriuresis through an effect on the proximal tubule and Insulin antinatriuresis is accompanied by a reduction in the urinary excretion of uric acid [43] which may explain the increase in the serum uric acid level obtained in insulin treated rats.
In the current study, it has been shown that, dietary supplementation with LO4% and LO6% to diabetic rats showed significant decrease in the mean values of urea nitrogen compared with control (+) while there was a significant decrease in the uric acid & creatinine in the group fed on LO6% only compared with control (+) group.

It seems essential, from this prospective, to emphasize that polyunsaturated fatty acids (PUFA), either of n-3 or n-6 type are converted in the body into more complex PUFA called eicosanoids. These eicosanoids are hormone like molecules that have very pronounced effects on the regulation of numerous body functions and they exert renoprotective effects by reducing glomerular hypertension, intra renal inflammation, hyperlipidemia, lipid peroxidation, and intrarenal growth factor elaboration (a scaring type of growth). They stop and actually reverse the inflammation and prevent the formation of scar tissue that destroys normal renal function. [44].

2.C. Liver functions:-

Effects of insulin and lettuce oil administration on AST and ALT in induced diabetic rats are presented in table 4. Diabetic rats (control +) showed highly significant increase in both AST and ALT enzymes levels compared with the healthy rats (control -). Data showed that serum AST level was significantly decreased (P<0.05) in the groups treated with insulin only, fed on a diet containing LO4% and LO6% compared with the control (+). Diabetic rats in all the tested groups demonstrated significant increase in the mean values of serum ALT level compared with the control (-) group.

It is well known that high levels of AST and ALT in serum are indicators for liver dysfunction. The liver dysfunction associated with diabetic was reported by Vidro et al.[45] and can be attributed to elevated rates of lipid peroxidation and decreased level and/or activities of endogenous antioxidant enzymes in liver [46,47]. Imaeda et al.[37] supported the above mentioned results. They reported that injection with streptozotocin induced an increase in the serum levels of AST and ALT.

The improvement in liver function (AST level) observed in diabetic rats treated with insulin can be attributed, at least in part, to the concept that insulin attenuated hepatic damage by decreasing the hepatic enzymes and improving the hepatic integrity, hepatic glucose metabolism and hepatic function by increasing cell survival and attenuating the hepatic inflammatory responses by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade, thus restoring hepatic homeostasis, which has been shown to be critical for organ function and survival of critically ill patients [48,49].

On the other hand, PUFA has been shown to protect against various types of experimental liver damage in animal models and isolated hepatocytes [50] and administration of polyunsaturated fatty acids, regardless of whether they are of the n-6 or n-3 type, suppress the development of acute hepatitis and its associated elevation of liver enzymes levels [51].

It is now clear that PUFAs regulate fundamental adipose cell and liver functions through modulation of activity and abundance of key transcription factors that act as nutrient sensors,
including peroxisome proliferator-activated receptors (PPARalpha/delta/gamma), sterol regulatory element binding proteins (SREBP-1/2), and liver X receptors (LXR alpha/beta) [52].

2.D. Glucose:-

Effects of lettuce oil supplementation on serum glucose levels (mg/dl) in alloxan-induced diabetic rats compared with insulin are presented in table 5. Untreated diabetic rats revealed a highly significant increase in the mean value of serum glucose compared with the healthy normal rats. Frier et al, [53] and Beers and Berkow [54] attributed this effect to the lack of insulin level and/or action in diabetics. All the treated groups elicited significant decrease in the mean value of serum glucose level compared with the control (+) group and the highest significant decrease in the serum glucose level were recorded in diabetic rats treated either with insulin alone or in combination with LO2% compared with the control (+). Diabetic rats fed on diet contained LO6% showed more favorable significant decrease in the serum glucose level than the group fed on diet contained LO4% compared with the control (+).

Our findings could be cleared up by the view that dietary fatty acid composition seems to affect insulin secretion and insulin resistance [5,55,56] and there is a positive correlation between dietary polyunsaturated fatty acids and insulin action and this concept is supported by evidence indicating that increased intakes of (LCPUFAs) reduce insulin resistance as the number of insulin receptors increases when Ehrlich cells, which show all the binding characteristics of mammalian insulin receptor, were enriched in PUFAs [7,8]. The mechanisms linking dietary fat quality to insulin sensitivity are not completely understood; however, the effects of dietary fatty acids on this biological function are believed to be mediated, at least partially, through the fatty acid composition of cell membranes. A specific fatty acids profile in cell membranes could influence insulin action through several potential mechanisms, including altered insulin receptor binding or affinity, and by influencing ion permeability and cell signaling [56].

Insulin sensitivity may be improved as a result of the effects of polyunsaturated fatty acid intake on membrane fluidity [57, 58]. The improvement in glucose uptake after membrane enrichment with PUFA is apparently related to an increase in the residency time of glucose transporter 4 (GLUT4) in the plasma membrane, which leads to an expansion of the intracellular pool of glucose-6-phosphate [58] and to increased skeletal muscle glycogen synthesis [57].

On the other hand, ingestion of PUFA-rich diets has been shown to facilitate insulin action through a number of metabolic effects including suppression of hepatic lipogenesis, reduce the hepatic output of triglycerides, enhance ketogenesis, and induce fatty acid oxidation in both the liver and the skeletal muscle [59]. Taken together, these effects might explain an actual improvement in glucose uptake and insulin sensitivity after PUFA ingestion [59].

3- Histological Results:

-Liver-

The hepatic histopathological examinations denoted healthy intact hepatic tissue in the control negative group (fig.1). Severe lesions were detected in the control positive diabetic rats in the form of; large focal areas of coagulative necrosis with leucocytic infiltration(fig.2) and severely vacuolated and degenerated hepatocytes with binucleation. Both insulin treated and (insulin + dietary supplementation with LO2%) treated diabetic groups showed apparently intact healthy hepatic tissue (fig.3). Mild congestion in the central vein and blood sinusoids was the main pathological lesion that appeared in the diabetic rats fed on lettuce oil 4% (fig.4) while diet
supplemented with lettuce oil 6% revealed mild degree of hepatocytes degeneration with small focal necrosed area infiltrated with leucocytic cell (fig.5).

-Kidney:
Microscopical examination of the renal tissue revealed very mild degree of congestion in the control negative group (fig.6). Severely atrophied glomerular capillary tuft and severely degenerated renal tubules were recorded in the control positive group (fig.7). Insulin treated diabetic rats and (insulin +LO2%) treated rats denoted mildly degenerated renal tubules (fig.8). Vacuolar degeneration in the renal tubules was observed in the diabetic rats fed on lettuce oil 4% (fig.9) while diabetic rats fed on lettuce oil 6% showed focal scattered areas of coagulative necrosis among the renal tubules (fig.10).

-Pancreas:
Histological examination of the pancreatic tissue showed normal pancreatic lobules and normal pancreatic acini in the control negative group (fig.11). Severe multiple pathological lesions were seen in the control positive diabetic rats including; vacuolarly degenerated pancreatic acini, hyperplased pancreatic islets and severe hyperplasia in the pancreatic duct with newly formed pancreatic ductules (fig.12). Mild congestion and dilated pancreatic duct were observed in both insulin treated diabetic rats and insulin + LO2% treated diabetic rats (figs. 13 & 14). Diabetic rats fed on LO4% showed severe congestion in the pancreatic tissue (fig.15) while there were hyperplasia in both pancreatic ducts and pancreatic islets in the diabetic rats fed on LO6% (fig.16).

We really have no way of knowing how could insulin and lettuce oil improve these pathological lesions induced in diabetic rats. Acknowledging this limitation, shuffling through previous scholarly articles would enlighten us with quite unerring clue.

It is currently hypothesized that, dietary intake of PUFA ameliorate the histological tissue damage. The possible beneficial effect of PUFA is not only attributed to their inhibition of PGE2 and leukotriene B 4(LTB4) synthesis, but perhaps also to modulation of pro-inflammatory cytokines[60]. Similarly, Lee et al.[61] recorded that, diets enriched with PUFA may have antiinflammatory effects by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting the leukotriene B4-mediated functions of neutrophils.

On the same ground, Insulin decreased the hepatic inflammatory response signal cascade by decreasing hepatic pro-inflammatory cytokines mRNA and proteins interleukin-1 beta and decreasing hepatocyte apoptosis along with decreased caspases-3 and -9 concentration, thus improving liver morphology. Insulin also attenuates the hepatic damage and inflammatory response by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade and improve the hepatic integrity, thus restoring hepatic homeostasis [62].

Our data found common grounds with the results previously obtained by Ayan et al., [63] who reported that, insulin therapy can prevent or delay urodynamic and histopathological changes in diabetes mellitus.

It is trustworthy to mention that, exogenous insulin suppresses the expression of the glucose transporter 2(GLUT2) and insulin in beta-cells, and this may prevent the diabetogenic effect of streptozotocin which induces diabetes mellitus in experimental animals [64].
Table (1): Effects of insulin and lettuce oil administration on FI, BWG%, FER and Organs weight /Body weight % in experimentally - induced diabetic rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FI g/day</th>
<th>BWG % Mean±SD</th>
<th>FER Mean±SD</th>
<th>Organs weight / body weight % Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>Control (-)</td>
<td>10.35</td>
<td>8.694±6.25A</td>
<td>0.087±0.06A</td>
<td>0.522±0.18A</td>
</tr>
<tr>
<td>Control(+)</td>
<td>6.42</td>
<td>-2.894±4.68B</td>
<td>-0.117±0.16C</td>
<td>0.359±0.08B</td>
</tr>
<tr>
<td>Insulin</td>
<td>6.78</td>
<td>5.253±2.96A</td>
<td>0.046±0.02AB</td>
<td>0.270±0.03B</td>
</tr>
<tr>
<td>*Insulin+ LO2%</td>
<td>7.82</td>
<td>-4.145±5.92B</td>
<td>0.054±0.22AB</td>
<td>0.345±0.031B</td>
</tr>
<tr>
<td>LO4%</td>
<td>8.28</td>
<td>-7.691±9.04B</td>
<td>-0.068±0.08AB</td>
<td>0.405±0.14AB</td>
</tr>
<tr>
<td>LO6%</td>
<td>7.96</td>
<td>-9.249±5.69B</td>
<td>-0.075±0.04BC</td>
<td>0.346±0.05B</td>
</tr>
<tr>
<td>L.S.D</td>
<td>7.899</td>
<td>0.162</td>
<td>0.138</td>
<td>1.004</td>
</tr>
</tbody>
</table>

Control (-): Control negative ,Control(+) : Control positive. * insulin : half of the therapeutic dose of insulin. LO2%: Lettuce oil 2%, LO4%: Lettuce oil 4%, LO6%: Lettuce oil 6%.
FI: Food intake. BWG%: Body weight gain. FER: Food efficiency ratio.
L.S.D : Least significant differences.
Mean carrying different superscripts in each column are significantly different at (P<0.05).

Table (2): Effects of insulin and lettuce oil administration on lipid fractions in experimentally - induced diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lipid Fractions (Mg/dl)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>Control (+)</td>
<td>78.592±10.192C</td>
<td>42.760±3.965C</td>
</tr>
<tr>
<td>Control (+)</td>
<td>101.954±50.814RC</td>
<td>118.856±24.694A</td>
</tr>
<tr>
<td>Insulin</td>
<td>87.498±10.687RC</td>
<td>65.146±7.813B</td>
</tr>
<tr>
<td>LO4%</td>
<td>172.954±57.628A</td>
<td>69.658±8.810B</td>
</tr>
</tbody>
</table>

Control(-): Control negative, Control(+): Control positive,* insulin: half of the therapeutic dose of insulin ,LO2%: Lettuce oil 2%, LO4%: Lettuce oil 4%, LO6%: Lettuce oil 6%.
HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol,VLDL-c: Very low density lipoprotein cholesterol.
L.S.D : Least significant differences.
Mean carrying different superscripts in each column are significantly different at (P<0.05).
Table (3): Effects of insulin and lettuce oil administration on serum uric acid, urea nitrogen and creatinine levels (mg/dl) in experimentally-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Uric acid Mean ± SD</th>
<th>Urea nitrogen Mean ± SD</th>
<th>Creatinine Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>1.389±0.349C</td>
<td>16.020±1.053D</td>
<td>0.469±0.218AB</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>2.066±0.564A</td>
<td>28.219±2.157A</td>
<td>0.588±0.019A</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>2.118±0.481A</td>
<td>20.879±2.003B</td>
<td>0.380±0.029B</td>
<td></td>
</tr>
<tr>
<td>*Insulin+LO2%</td>
<td>1.846±0.098AB</td>
<td>20.173±2.199BC</td>
<td>0.384±0.097B</td>
<td></td>
</tr>
<tr>
<td>LO4%</td>
<td>1.638±0.424ABC</td>
<td>20.197±1.898BC</td>
<td>0.541±0.058AB</td>
<td></td>
</tr>
<tr>
<td>LO6%</td>
<td>1.166±0.175C</td>
<td>18.239±1.773CD</td>
<td>0.407±0.189B</td>
<td></td>
</tr>
<tr>
<td>L.S.D</td>
<td>0.503</td>
<td>2.463</td>
<td>0.166</td>
<td></td>
</tr>
</tbody>
</table>

Control(-): Control negative, Control(+): Control positive,
* insulin: half of the therapeutic dose of insulin,
LO2%: Lettuce oil 2%, LO4%: Lettuce oil 4%, LO6%: Lettuce oil 6%.
L.S.D : Least significant differences.
Mean carrying different superscripts in each column are significantly different at (P<0.05).

Table (4): Effects of insulin and lettuce oil administration on aspartate amino transferase (AST) and alanine amino transferase (ALT) "IU/L" in experimentally-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>AST Mean ± SD</th>
<th>ALT Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>14.593±3.946CD</td>
<td>7.514±3.625A</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>33.316±9.071A</td>
<td>14.953±4.603A</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>21.537±3.156BC</td>
<td>14.206±2.879A</td>
<td></td>
</tr>
<tr>
<td>*Insulin+LO2%</td>
<td>27.250±4.165AB</td>
<td>14.421±3.208A</td>
<td></td>
</tr>
<tr>
<td>LO4%</td>
<td>24.832±7.859B</td>
<td>16.090±1.846A</td>
<td></td>
</tr>
<tr>
<td>LO6%</td>
<td>12.930±3.466B</td>
<td>14.783±1.758A</td>
<td></td>
</tr>
<tr>
<td>L.S.D</td>
<td>7.517</td>
<td>4.107</td>
<td></td>
</tr>
</tbody>
</table>

Control(-): Control negative, Control(+): Control positive,
* insulin: half of the therapeutic dose of insulin,
LO2%: Lettuce oil 2%, LO4%: Lettuce oil 4%, LO6%: Lettuce oil 6%.
L.S.D : Least significant differences.
Mean carrying different superscripts in each column are significantly different at (P<0.05).

Table (5): Effect of lettuce oil administration on serum glucose levels (mg/dl) in experimentally-induced diabetic rats compared with insulin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Glucose level Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td></td>
<td>89.519±3.938B</td>
</tr>
<tr>
<td>Control (+)</td>
<td></td>
<td>165.914±1.463A</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>93.174±4.453B</td>
</tr>
<tr>
<td>*Insulin+LO2%</td>
<td></td>
<td>105.974±33.334B</td>
</tr>
<tr>
<td>LO4%</td>
<td></td>
<td>116.701±35.823B</td>
</tr>
<tr>
<td>LO6%</td>
<td></td>
<td>111.870±24.968B</td>
</tr>
<tr>
<td>L.S.D</td>
<td></td>
<td>29.456</td>
</tr>
</tbody>
</table>

Control(-): Control negative, Control(+): Control positive,
* insulin: half of the therapeutic dose of insulin,
LO2%: Lettuce oil 2%, LO4%: Lettuce oil 4%, LO6%: Lettuce oil 6%.
L.S.D : Least significant differences.
Mean carrying different superscripts in each column are significantly different at (P<0.05).
Fig. (1): Liver section from control negative rats showing apparently healthy intact hepatic tissue. (H&E X 100)

Fig. (2): Liver section from control positive diabetic rats showing large focal area of coagulative necrosis with leucocytic infiltration (H&E x 200).

Fig. (3): Liver section from diabetic rats treated with insulin + fed on LO2% demonstrating apparently intact healthy hepatic tissue (H&E x100).

Fig. (4): Liver section from diabetic rats dietary supplemented with LO4% showing mild congestion in the central vein and blood sinusoids in between the slightly normal hepatic tissue (H&E x 200).

Fig. (5): Liver section from diabetic rats dietary supplemented with LO6 % showing mild degree of hepatocytes degeneration with small focal necrosed area in between infiltrated with leucocytic cells (H&E x 200).

Fig.(6) : Kidney section from control negative rats showing mild congested renal tubules. (H&E X 100)

Fig. (7): Kidney section from control positive diabetic rats showing severely atrophied glomerular capillary tuft with severely degenerated renal tubules (H&E x 400).

Fig. (8): Kidney section from diabetic rats treated with insulin + fed on LO2% illustrating mildly degenerated tubules (H&E x 200).
Fig. (9): Kidney section from diabetic rats dietary supplemented with LO4% demonstrating vacuolar degeneration in the renal tubules (H&E x 200).

Fig. (10): Kidney section from diabetic rats dietary supplemented with LO6% showing congested glomerular capillary tuft with focal area of coagulative necrosed tubules (H&E x 200).

Fig. (11): Pancreatic section from control negative rats showing normal pancreatic lobules with normally included pancreatic acini (H&E X 100)

Fig. (12): Pancreatic section from control positive diabetic rats showing sever hyperplasia in the pancreatic duct (d) with newly formed pancreatic ductules. (arrow) (H&E x100).

Fig. (13): Pancreatic section from diabetic rats treated with insulin only illustrating mild congestion and dilated pancreatic duct (H&E x200).

Fig. (14): Pancreatic section from diabetic rats treated with insulin + fed on LO2% demonstrating moderately dilated pancreatic duct (H&E x100).

Fig. (15): Pancreatic section from diabetic rats fed on LO4% displaying severe congestion (H&E x200).

Fig. (16): Pancreatic section from diabetic rats fed on LO6% showing moderate hyperplasia in the pancreatic duct and pancreatic islets (H&E x200).
REFERENCES


دراسة بيولوجية وكيمياء–بايثولوجية مقارنة على تأثير الإنسولين وزيت الخس على الجرذان المصابة

تجريبياً بالسكر

نجلاء زكريا حلمى عليوه – إبراهيم سعيد سلام **– شهير سعاد عبد الجيد **

قسم الفارماكولوجى–كلية الطب البيطرى: جامعة الزقازيق *
قسم التنسيق وعلوم الأطعمة–كلية الاقتصاد الواقعية جامعة حلوان **
قسم الباثولوجى–كلية الطب البيطرى–جامعة القاهرة

الملخص العربي

لقد اجريت هذه الدراسة تأثير زيت الخس (الذي يحتوي على نسبة عالية من الامراض الدهنية المتعددة الغير مشبع) على الجرذان المصابة تجريبيا بمرض السكر مقارنة بالانسلين وذلك من الناحية البيولوجية والكيميائية والبايثولوجية.

تم استخدام 42 جرذ من النوع الأفريقي في هذه الدراسة حيث تم تقسيمهم إلى 6 مجموعات متساوية.

المجموعة الأولى "الض chatte السليبة–ال.randn السكر و غير المعالجة"، المجموعة الثانية "التصابة بمرض السكر والانسلين حقا تحت الجدل" (وهي دفعة نجلي من وزن الجسم مرتين آسيا)، المجموعة الثالثة "التصابة بمرض السكر وعالية النسبة تحت الجدل (نصف درجة المستخدم في المجموعة السابقة)« زيت الخس بنسبة 2% مضافة إلى غذائها»، المجموعة الخامسة "التصابة بمرض السكر + زيت خس بنسبة 6% مضافة إلى غذائها».

وقد أوضحت النتائج أن الجرذان المصابة بالسكر في المجموعة الأولى والثانية أظهرت زيادة ملونية في معدل التحويل الغذائي مع نقص ملموس في مستوى ذاتيا الغذائي. كما اوضحت النتائج أن الجرذان المصابة بالسكر في كل المجموعات المعالجة أظهرت درجات متقارنة من الانخفاض في مستوى الجلوكوز بالدم.

كذلك فإن الجرذان في المجموعات الثالثة والرابعة والسادسة أظهروا نقصا ملموسا في مستوى الجلوكوز والبروتئينات الدهنية المنخفضة الكثافة وانخفاض القناع جذا في السيرم ورغم ذلك حدوث زيادة معنوية في نسب المضادات الدهنية عالية الكثافة في السيرم.

كما أوضحت النتائج أيضا أن إضافة زيت الخس بنسبة 2% إلى غذاء الجرذان المصابة بالسكر متناول مع الحق بالانسلين أظهرت نقصاً ملموساً في مستوى السكر والكربوهيدرات. وقد وجد أنه زيادة نسبة زيت الخس المضاف إلى غذاء الجرذان المصابة بالسكر فإن معدلات السكر نباتية زيتية وحماض البوليك و الكربوهيدرات تتجاه نحو مثيلاتها في المجموعة المعالجة بالسكر.

كذلك وجد أن مستوى انزيم "الأسبارتيت أمينو ترانز فريز" قد أظهر نقصاً ملموساً في المجموعات الثلاث الرابعة والسادسة. وقد أظهرت الدراسات البيولوجية أن التزام بين السكر والانسلين وضمت زيت الخس إلى غذاء الجرذان المصابة بالسكر قد أحدث انخفاضاً ملموساً في معدل نسب التغيرات البيولوجية الناجمة عن الإصابة بمرض السكر في أنفس كل من الكبد والكلي والبنكرياس.

وقد حصلت الدراسة إلى أهمية اضافة زيت الخس إلى غذاء مرضى السكر بالالتزام مع حقن الإنسولين و وذلك للحد من المضاعفات الناجمة عن الأصابة بمرض السكر.