Biochemical and histopathological studies on the influence of aqueous extract of fenugreek seed (Trigonella foenum graecum) on alloxan diabetic male rats.

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Abstract

Diabetes and the related complications continue to be a major medical problem in Egypt. In spite of the warning of adaptation for regime diet, practicing exercise and administering hypoglycaemic drugs.

The toxic side effects, contraindication and sometimes diminutions in response after prolonged use of antidiabetic drugs encouraged to search for therapeutic herbal remedies for safety, efficacy and economy. Diabetes is manifested by multiple disturbances in the metabolic processes of the body, which are attributed to an insufficient supply of insulin. Many plants were known for their activity as antidiabetic agents.

This investigation aims to clarify the role of fenugreek seed aqueous extract in its therapeutic dose on beta cells number, blood glucose and plasma insulin levels in alloxan diabetic rats. Also to illustrate the functional biochemical changes and the associated histopathological alternations in pancreas, liver, kidney, aorta and testes as influenced by fenugreek and alloxan induced diabetes for 4-weeks of treatment.

24 adult male albino rats were divided into four groups, control, diabetic, diabetic rats treated with 0.1mg/kg B.W. of fenugreek seed aqueous extract and 0.1mg/kgB.W. fenugreek treated group for 4-weeks. The results indicated that, in the diabetic state significant increase in plasma glucose accompanied by significant reduction in plasma insulin and beta cells number. Significant increase in serum AST, ALT and ALP associated with dilatation and severe congestion of central vein and sinusoids, vacuolization and necrotic areas. Inflammatory area around portal tract. Significant increase in serum urea and creatinine accompanied by atrophy of most glomerular tuft, others displaying swelling and hyperemia. Diffuse extravagations of red blood cell between the degenerated renal tubules. Dilatation and severe congestion of blood vessels. Dilated convoluted tubules, contain hyaline casts in their lumens were observed. Significant increase in serum total cholesterol and triglycerides accompanied by degenerative changes in aorta and formation of medial calcinosis in some cases, significant decrease in serum testosterone levels associated by germ cells depletion as well as sloughing and degeneration of sperms.

In conclusion, fenugreek seeds exhibited antioxidant property could ameliorate the alternations induced in diabetes.

This investigation recommended that higher concentrations of debitterized fenugreek seeds may double the regeneration of beta –cells in pancreas, further studies would be done in mammals.

Fenugreek seed aqueous extract exhibited antioxidant property which ameliorated the biochemical and histopathological alternation induced by alloxan.

In conclusion, higher concentrations of debitterized fenugreek seeds can regenerate beta cells in pancreas; further studies would be done in mammals.

Introduction

Fenugreek-El Helba- (Trigonella Foenum Graecum) L.[Fam.Fabaceae]. It used for thousands of years to reduce blood sugar, increase lactation, stomach ulcers, appetite loss, Fever, catarrh of the respiratory tract, bronchitis, pellagra and eczema (Blumenthal et al,2000).

Many studies have shown that the fenugreek seeds exhibit anti diabetic action (Sharma et al,1996), hypolipidaemic effect
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Feungreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30 proteins high in lysine and tryptophan, 5-10% fixed oils (lipids), pyridine-type alkaloids mostly trigonelline (0.2-0.36%), choline (0.5%), gentianine, and carpaine, flavonoids, free amino acids, calcium and iron, saponins, glycosides, cholesterol and sitosterol, vitamins A1, B2, B3, B,C and D, nicotinic acid and volatile oils (Blumenthal et al, 2000, and Shang et al, 1998).

Diabetes is a common chronic disease of man race is manifested by multiple disturbances in the metabolic processes of the body, which are directly attributed to an insufficient supply of insulin.

This investigation aims to clarify the role of fenugreek seed aqueous extract in its human therapeutic dose on beta cells number, blood glucose and plasma insulin levels in alloxan diabetic rats. As well as to illustrate the functional biochemical changes and the associated histopathological alternations in pancreas, liver, kidney, aorta and testes as influenced by fenugreek and alloxan for 4-weeks of treatment.

Materials and Methods

Plant material

Fenugreek seeds were obtained from local market, cleaned of extraneous matter and ground into a fine powder. 200ml boiling distilled water were added to 2.5gm powder fenugreek seed, left it for 10minutes and filtered. The filtrate was dried at 35-45C in incubator.

Animals

Male albino rats weighing 120 ±10 gm bodyweights were purchased from Helwan Farm.

The animals were kept at the room temperature of 25 ±5C with a natural lighting cycle (12) hours. They were kept under observation for about two weeks before the initiation of the experiment.

Diabetes were induced in overnight fasted animals by a single subcutaneous injection of alloxan monohydrate (Sigma USA). In a dose of 120mg /kg Bwt.

Dissolved in acetate buffer immediately prepared before usage. Seven days after injection of alloxan blood glucose levels of all surviving rats were determined. Only rats with glucose levels above 200mg/ml were considered diabetic and employed for the assay.

Experimental design

Twenty –four adult male albino rats weighing (110-130gm). They divided into four groups 6 each. Group I, represented control, group II diabetic rats, group III diabetic rats treated with 0.1mg of fenugreek seed aqueous extract and group IV treated with 0.1mg of fenugreek extract, that dose equivalent to human therapeutic dose (Paget and Barnes, 1964) daily for 4 weeks.

Blood Sampling

Blood samples were collected from retro-orbital vein in three separate tubes, one tube with EDTA for determination of haematological parameter. Red blood cells (RBCs) and (WBCs) were counted in Neubarger Hemocytomter. The second tube containing potassium oxalate and sodium fluoride for estimation of glucose (Trinder,1969) and insulin. The third containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for determination serum aspartate amino transferase (AST), alanine amino transeferase (ALT) (Reitman and Frankle,1957), alkaline phosphatase (ALP) ( German Society for clinical chemistry,1972 ) ,urea (Tabacco et al,1979) ,creatinine (Bartls et al,1971) ,total cholesterol (Allain et al., 1974), Triglycerides (Bucolo and David,1973) , total protein (Doumas,1975), albumin (Doumas et al.,1971), and globulins were calculated as the difference between total protein and albumin. Insulin and testosterone were estimated by Kits obtained from IMX Abbott Labs, IL/USA.
**Histological examination**

Fresh liver, kidney, aorta and pancreas samples were collected in formal saline. Testes were collected in Bouin's fluid. All samples were stained with H&E. Some sections of pancreas were fixed with modified aldehyde–Fuchsin (M.A.F.) (Kiernan, 1999).

**Statistical analysis**

All data obtained were analyzed using student 't'-test according to (Sendecor and Coebram, 1969).

**Results and Discussion**

Diabetes is manifested by multiple disturbance in the metabolic processes of the body, which are directly attributed to an insufficient supply of insulin. This investigation aims to clarify the role of fenugreek seed aqueous extract in its human therapeutic dose on beta cell numbers, blood glucose and plasma insulin levels in alloxan diabetic rats. As well as to illustrate the functional biochemical changes and the associated histopathological alternations in pancreas, liver, kidney, aorta and testes as influenced by fenugreek and alloxan induced diabetes for 4-weeks of treatment.

A decrease in rat body weight was noted in alloxan–induced diabetic rats for 4-weeks in comparison with control (table 1). A significant decline in rat body weights treated with alloxan (Joy and Kuttan, 1999), and streptozotocin (Bwititi et al, 2000). Alloxan a cytotoxic agent affect organs and metabolism which lead to significant decline in the growth rate. But when the diabetic rats treated with fenugreek seed aqueous extract, the decrease in body weight was nearly suppressed after 4-weeks. Fenugreek extract treated rats showed non-significant increase in body weight over the experimental period.

Fenugreek aqueous extract exhibited antioxidant property (Thirunavukkarasu et al., 2003), which protects the functional organs and increase body weight

Table (1) displayed lower blood parameters of the diabetic rats against the control values. The red blood cells were significantly decreased after 30 days in diabetic rats due to the increase in lipid peroxidation of the erythrocyte cell membrane and destruction of red blood cells (anemia) (Kang-Xin et al., 1990). Reduction in haemoglobin concentration is considered as useful indicator for lower number of red blood cells.

Non-significant change of white blood cell at 4-weeks by alloxan treatment was detected. Rats treated by fenugreek extract displayed non-significant variation from the corresponding control after month in RBCs, Hb% and in WBCs. Fenugreek treated diabetic displayed non-significant alternation in RBCs, Hb% and in WBCs comparable to control (table 1).

The anti–oxidant property of fenugreek (Thirunavukkarasu et al., 2003), inhibit lipid peroxidation of the erythrocytes. Fenugreek contains iron (Blumenthal et al., 2000), it can improve anemia conditions.

Plasma glucose levels were significantly elevated in alloxan diabetic rats and this was associated with low plasma insulin concentration (table1). This result was due to the cytotoxic agent alloxan which cause a massive reduction of the β-cells of the islets of langerhans in pancreas of diabetic rats comparable to control (figs.1 & 1a). Also severe β-cells necrosis and intracellular vacuolation were observed (figs. 2 & 2a), which induced hyperglycemia. Diabetic animals treated with fenugreek extract had lower blood glucose and this was accompanied by an increase in plasma insulin concentration (table1). The number of β-cells relatively increased, the islets appeared more organized and less vacuolated (figs. 3 & 3a). Fenugreek extract treated rats(figs.4 & 4a)displayed lower blood glucose and increase insulin level. The hypoglycemic effect of fenugreek extract may be due to the major existence of 4- isohydroxy leucine which stimulates insulin secretion from pancreas (Al-Habori and Raman 1998) & (Haefele et al, 1997). Sharma et al (1996) recorded that 100gm of fenugreek debitterized powdered seeds for 10 days significantly reduced blood sugar for type I diabetes. It may be due to the regeneration β-cells number. Puri et al, (2002) reported that hypoglycemic effect
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of fenugreek may be mediated through stimulating insulin synthesis and/or increasing secretion β pancreatic cells of langerhans.

In the diabetic non–treated rats (table 1) serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase increased significantly compared to their corresponding control. The augmentation in these enzymes may be due to the hepatotoxic effect of alloxan or may be a result following hepatic necrosis (Tanak et al, 1988 and Cornelius,1989). Also liver obtained various pathological changes in comparison with control(fig.4) were dilatation and severe congestion of central veins and sinusoids necrotic areas of hepatocytes (figs.4a & 4b ). Inflammatory area around portal tract was observed (fig.4c). Diabetic rats treated with fenugreek displayed significant decrease in Serum AST, ALT and ALP (table 1). Liver showed amelioration in histological pattern (fig.5). Fenugreek extract treated rats showed non significant increase in serum AST, ALT and ALP (table1). Liver displayed nearly normal architecture (fig.6). Bin-Hafeez et al, (2003) observed that , no elevation in liver function enzymes by fenugreek treatment. As well as (Duke, 1985) & (Kapoor,1990) recorded that fenugreek has no inflammatory disorder in liver

Significant increase in urea and creatinine was observed in diabetic rats (table 2) which attributed to increased protein catabolism (Finco, 1984) and to glomerular or tubular destructive changes (Ravel, 1984). The pathology of kidney appeared as dilatation and severe congestion of blood vessels (fig.10). Majority of convoluted tubules cloudy swelling contained hyaline cast in their lumen and vacuolar degenerative changes were observed in some renal tubules (fig.10c). Diffuse extravagations of red blood cells between the degenerated renal tubules (fig.10). The glomerular tuft were congested, atrophied (fig.10a) and some of them were swollen(fig10b) . In fenugreek treated diabetes significant decrease was noticed in urea and creatinine levels .An improvement in kidney structure occurred (fig.11). The involvement of free radicals in alloxan nephrotoxicity may be normalize by fenugreek (Ravikumar and Anuradha 1999). fenugreek treated rats, showing normal appearance in kidney (fig.12)

The analysis of data showed marked increase in serum total cholesterol and triglycerides (table,2) which accompanied by degenerative changes of aorta (fig.14) and in some cases formation of medial calcinosis (Fiq.14a ) in diabetic rats comparable to control ones (fig.13).

Atherosclerosis is a diabetes macro-vascular complication, which account for most of mortality in diabetic population (king, et al.,1990). Fenugreek treated diabetic rats significantly reduced serum total cholesterol and triglycerides (table,2) and prevented degenerative changes in aorta and atherosclerosis (Fiq.15). In rats treated with fenugreek aorta revealed normal pattern (fig.16).

Fenugreek contains lecithin which dissolve cholesterol and contains lipotropic (fat dissolving ) substances ,which dissolves deposits of fat ,prevents fatty accumulates and water retention (Blumenthal et al., 1998) . Al-Habori and Raman (1998) reported that fenugreek hypocholesterolemic effect has been attributed to increased conversion of hepatic cholesterol to bile salts due to loss,of complexes of these substances ,in the feces, with fenugreek fiber and saponins.

Serum total protein and albumin was significantly reduced in diabetic rats (table 2) which attributed to increased protein catabolism as a result of insulin deficiency (Jefferson et al., 1983). The decrease of serum albumin and the increase in globulin level (table 2) was another confirmation of liver damage (Kanko, 1989). In diabetic rats treated with Fenugreek, serum total protein and albumin levels restored to normal values (table 2). This result was due to fenugreek antioxidant property which improves organ functions (Devasena and Menon, 2002 & Thirunavukkarasu et al, 2003). Rats treated with Fenugreek recorded non-significant increase in serum total protein, albumin and globulin compared with the control ones.
Alloxan induced diabetes in male rats significantly lowered serum testosterone level. Also the testis of diabetic animals showed inactive seminiferous tubules, characterized by focal disorganization of germ cells (fig.18) in comparison with control (fig.17). The testis has been described to be a sensitive target for diabetic complications (Tarleton et al., 1990 & Gondos and Bevier, 1995). Fenugreek treated diabetic rats serum testosterone level revealed non significant decrease from control group. Testis showed partial recovery (fig.19). Rats treated with fenugreek recorded significant increase from the control ones. Testis displayed normal pattern (fig.20). Fenugreek has estrogenic effect (Duke et al., 2002), low levels of dietary phytoestrogen have a biological effect in the testis (Robertson et al, 2002).

Fenugreek seeds are rich in flavonoids (Gupta and Nair, 1999), thus the phenolic structure of fenugreek makes it a radical scavenger for the alternations induced by cytotoxic alloxan.

In conclusion, fenugreek seeds exhibited antioxidant property could ameliorate the alternations induced in diabetes.

This investigation recommended that higher concentrations of debitterized fenugreek seeds may double the regeneration of beta –cells in pancreas, further studies would be done in mammals.

Table (1): Showing the effect of treatment 0.1mg/kgBW fenugreek seeds aqueous extract for 4 weeks in body weights, some hematological parameters, blood glucose, insulin and some liver function tests in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic treated with fenugreek</th>
<th>Normal treated with fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight Gm</td>
<td>145.83 ±4.92</td>
<td>123.33*↓ ±6.83</td>
<td>140 ±4.2</td>
<td>155.83 ±5</td>
</tr>
<tr>
<td>R BCs x10^6</td>
<td>5.35 ±0.66</td>
<td>2.25*↓ ±2</td>
<td>4.5 ±0.5</td>
<td>6.2 ±0.35</td>
</tr>
<tr>
<td>W BCs x10^7</td>
<td>4.66 ±0.6</td>
<td>6.76 ±0.8</td>
<td>6 ±1</td>
<td>4.6 ±0.54</td>
</tr>
<tr>
<td>Hb Gm%</td>
<td>16.33 ±1</td>
<td>12.5*↓ ±0.8</td>
<td>15.16 ±0.75</td>
<td>17.33 ±1.63</td>
</tr>
<tr>
<td>Glucose mg/dl</td>
<td>85 ±2</td>
<td>280*↑ ±5</td>
<td>150*↑ ±10</td>
<td>79*↓ ±2.1</td>
</tr>
<tr>
<td>Insulin μU/dl</td>
<td>12.5 ±0.5</td>
<td>7.5*↓ ±0.8</td>
<td>10 ±1</td>
<td>14 ±0.5</td>
</tr>
<tr>
<td>AST U/L</td>
<td>39 ±4.5</td>
<td>52*↑ ±2.3</td>
<td>42 ±4</td>
<td>40 ±3.5</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>24 ±2.5</td>
<td>44*↑ ±3.1</td>
<td>35 ±4</td>
<td>27 ±3</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>56 ±6</td>
<td>97*↑ ±3.4</td>
<td>70 ±3.8</td>
<td>58 ±4.8</td>
</tr>
</tbody>
</table>

Number of rats in each group=6  P<0.05 in comparison to control group
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Table (2): Showing the effect of treatment 0.1mg/kg BW fenugreek seeds aqueous extract for 4 weeks in some kidney function tests, cholesterol, triglycerides, total proteins, albumin, globulins and testosterone in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic treated with fenugreek</th>
<th>Normal treated with fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td><strong>Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea mg%</td>
<td>22 ± 2</td>
<td>34*↑</td>
<td>28 ± 1</td>
<td>24 ± 2.5</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.7 ± 0.035</td>
<td>1.5*↑</td>
<td>1.1 ± 0.05</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td>128 ± 4.2</td>
<td>200*↑</td>
<td>140 ± 3.8</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td>50 ± 7</td>
<td>105*↑</td>
<td>70 ± 6</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>Total proteins g/dl</td>
<td>6.5 ± 0.5</td>
<td>5*↓</td>
<td>6 ± 0.5</td>
<td>7 ± 0.3</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.5 ± 0.2</td>
<td>3</td>
<td>3.5 ± 0.2</td>
<td>4 ± 0.1</td>
</tr>
<tr>
<td>Globulins g/dl</td>
<td>3 ± 0.3</td>
<td>2</td>
<td>2.5 ± 0.3</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>Testosterone ng/ml</td>
<td>1.6 ± 0.02</td>
<td>0.71*↑</td>
<td>1.5 ± 0.06</td>
<td>2.5↑</td>
</tr>
</tbody>
</table>

Number of rats in each group=6
*P<0.05 in comparison to control group

Legend of figures

**Fig.1:** Control rat pancreas showing islet of langerhans containing α, β and δ cells. β cells are the most abundant cells M.A.F. x 200.

**Fig.1a:** Control rat pancreas M.A.F. x1000

**Fig.2:** Diabetic rat pancreas showing, reduction in the pancreatic β cell numbers, β cell vacuolization and necrosis in some surviving β cells M.A.F. x 400.

**Fig.2a:** Diabetic rat pancreas M.A.F. x1000

**Fig.3:** Fenugreek extract treatment, showing relatively increasing β cells number M.A.F. x 400

**Fig.3a:** Fenugreek extract treated rats M.A.F. x1000

**Fig.4:** Fenugreek extract treated diabetic rats showing slight improvement in β cell numbers M.A.F. x400

**Fig.4a:** Fenugreek extract treated diabetic rats M.A.F. x1000

**Fig.5:** Control rat liver showing the normal histological structure of hepatic, with hepatic cords, hepatic sinusoids and central vein H & E x400.
**Fig. 6:** Diabetic rat liver, showing dilatation and severe congestion of central vein, vacuolization and necrotic area of hepatocytes. Inflammatory cells in the hepatocytes. 
H & E x400.

**Fig. 6a:** Diabetic rat liver, showing dilatation and severe congestion of sinusoids and inflammatory cells in the hepatocytes. 
H & E x400.

**Fig. 6b:** Diabetic rat liver, showing dilatation and severe congestion of portal tract and inflammatory area around it. 
H & E x400.

**Fig. 7:** Liver of diabetic rat treated with fenugreek extract, showing ameliorating in the histological structure. 
H & E x100.

**Fig. 8:** Normal rats treated with fenugreek extract showing nearly normal appearance of hepatic architecture. 
H & E x100.

**Fig. 9:** Kidney of rats in control group, showing normal histological structure of glomeruli and renal tubules. 
H & E x400.

**Fig. 10:** Kidney of diabetic rats, showing dilatation and severe congestion of blood vessels, diffuse extravasation of red blood cells between renal tubules. 
H & E x200.

**Fig. 10a:** Kidney of diabetic rats showing glomerular atrophy. 
H & E x200.

**Fig. 10b:** Kidney of diabetic rats showing glomerular swelling and tubular degenerative changes. 
H&E x400.

**Fig. 10c:** Kidney of diabetic rats showing glomerular atrophy, dilated convoluted tubules contain hyaline cast in their lumen. 
H & E x400.

**Fig. 11:** Kidney of diabetic rats treated with fenugreek, showing ameliorated histological structure. 
H & E x400.

**Fig. 12:** Kidney of normal rats treated with fenugreek extract, showing normal appearance. 
H & E x400.

**Fig. 13:** Aorta of rat in control group. 
H & E x200.

**Fig. 14:** Aorta of diabetic rat showing degenerative changes. 
H & E x400.

**Fig. 14a:** Aorta of diabetic rats, showing formation of medial calcinosis. 
H & E x200.

**Fig. 15:** Aorta of diabetic rats treated with fenugreek extract, showing normal histological appearance. 
H & E x200.

**Fig. 16:** Fenugreek treated rats, showing normal pattern. 
H & E x400.

**Fig. 17:** Testis of rat in control group, showing the normal histological structure of the seminiferous tubules with different series of spermatogenic layers and spermatozoa. 
H & E x400.

**Fig. 18:** Testis from diabetic rats showing abnormal seminiferous tubules characterized by germ cells depletion as well as sloughing, degeneration of sperm and debris of spermatogenesis cells could be observed. 
H & E x400.

**Fig. 19:** Testis of diabetic rat treated with fenugreek extract, showing partially recovery in seminiferous tubules. 
H & E x400.

**Fig. 20:** Testis of normal rats treated with fenugreek for 4 weeks showing well developed seminiferous tubules and well developed spermatogenetic activity and Leydig cells. 
H & E x400.
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References


دراسات كيميائية حيوية وهistonopathological على تأثير المستخلص المائي لبذور الحلبة في ذكور الجرذان المصابين بمرض السكر المستحدث بالألوكون

أيناس على مهدي خليل

 الهيئة القومية للرقابة والبحوث الدوائية

لازال مرض السكر ومراضاته من أكبر المشاكل الطبية في مصر رغم التحذيرات لاتباع رجيم غذائي ومارسات الرياضة وتناول الأدوية المضادة لمريض السكر وتظهر الأدوية المضادة لمريض السكر أعراض جانبية ضارة وعكسية وأحيانا عدم استجابته الجسم للدواء بعد الاستعمال لفترة طويلة.

ويرفع مرض السكر إلى نقص هرمون الأنسولين ولهذا يؤدي إلى اضطرابات الحيوية في الجسم وعرف بعض النباتات لفعاليتها كعلاج لمرض السكر ولذلك سمى البحث لتوضيح دور المستخلص المائي لبذور الحلبة بجرعته العلاجية للانسان على خلايا بيتا وعلى مستوى السكر والأنسولين في الدم وأيضاً توضيح التغييرات الكيميائية الحيوية والهستوپاثولوجية في الخلايا للأدوية والأورتي والخصبة لمدة أربعة أسابيع.

وقد تم تقسيم عدد 24 من ذكور الجرذان البالغة إلى أربع مجموعات مجموعه ضابطه، ومجموعة مصابه بمرض السكر و مجموعة تعامل فقط بالمستخلص المائي لبذور الحلبة، ومجموعة مصابه بمرض السكر و معامله بالمستخلص المائي لبذور الحلبة.

وتنتج عن معاملة الفئران بمرض السكر ارتفاع في مستوي سكر الدم ونقص الأنسولين مصحوباً بنقص عدد خلايا بيتا، أيضاً ارتفاع في مستوي الأنزيمات الناقل للتمزقات، وخفض خلايا الصفية من الحيوانات المستور بالرغم من توزع خلايا الكبد وجود خلال الفترات التي قبل الفرضية، وقد لوحظ أيضاً ارتفاع ذات دلالات إحصائية في نسبة مصل البيوريا والكرياتين مع ضخما وأضعاف وأحذق في معظم كرات مليجي انساع وأحذق وانعكاس الدمائي وترتر الربع الخلايا المبطنة لانائية الكلي ونحو أرتفاع ذو دلالات إحصائية لكل من الكوليستيرول الكلي والدهون الثلاثية مصحوب بتغيرات من مرض في الأورطي وظهور في بعض الحالات تكون جدار الأورطي ووجود أيضاً أطفال في مستوي هرمون التنسستيرون مصحوب بنقص عدد الخلايا الجرثومية وكذلك وتزكر كثير من الحيوانات المدونه.

ويكتب المستخلص المائي لبذور الحلبة ظاهره المضادات للأكسدة حيث تم تحسن في التغييرات الكيميائية الحيوية والهستوپاثولوجية المستحدثه بالألوكون في ذكور الجرذان وبحث هذا البحث على إجراء تجارب على الحلبة المنزوعة المزارة ذات التركيز الأعلى لتقدير مدى نية عدد خلايا بيتا في الثدييات.