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EFFECTS OF SOME ANTIDIABETIC MEDICINAL PLANTS ON PANCREAS AND LIVER OF DIABETIC ALBINO RATS

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Abstract

Background and aim of the study- Diabetes is a chronic metabolic disease which affects large number of population all over the world. More than 400 traditional medicinal plants have been recorded for helping in controlling such disease. This study investigated effects of some plants used in Saudi Arabia and some other Arab countries as antidiabetic agents.

Materials and Methods: One hundred fifty adult male Albino rats were divided into six experimental groups each consist of twenty five rats. The first group was considered as a control group. The rest of groups were affected by induction of experimental diabetes by subcutaneous injection of Alloxan. The second group consisted of diabetic rats without any treatment. The third group was treated by the aqueous extract of mixture contains Foenugreek, *Nigella* and Termis seeds. The fourth group was treated with the aqueous extract of *Nigella sativa* seeds, while the fifth group was treated with the aqueous extract of Foenugreek seeds.The sixth one was treated with the aqueous extract of Termis seeds with the administered dose of the plant extracts (100 mg/kg body weight).After four weeks of treatment, different biochemical parameters were performed including estimation of blood sugar level and serum insulin level. Pancreatic and liver samples were obtained and processed for microscopic and quantitative evaluation after staining the prepared sections with both heamatoxylin and eosin as well as a special stain for demonstration of the different pancreatic cells in the Islet of Langerhans.

Results: The usage of the mixture or each plant alone corrected the glucose level and insulin level. Microscopically there was definite decrease in the number and diameter of beta pancreatic cells in the diabetic group, while the other pancreatic cells were not affected (alpha and delta cells). The use of medicinal plants in the different groups of this study greatly improved such cellular changes and the level of blood sugar level was corrected. The present results showed that the activity of the mixture was the best when compared with *Nigell*a, Foenugreek and Termis seeds.

Conclusions: The water extract of the mixture is the most powerful in amelioration hyperglycemia and most of all damage effects of Alloxan on the liver and texture, hematological parameters, and lipid profile. So it is advised to use the plant mixture as an antidiabetic agent rather than the use of each plant separately. Repeating such study with the use of variable doses may be helpful in better evaluation for the required doses.

Key words: Alloxan - Diabetes - Antidiabetic plants - Pancreas

Introduction

Diabetes mellitus (DM) is possibly the world's fastest growing metabolic disease, so there is a great need for more appropriate therapies [(1).] Many plants have been investigated for their beneficial use in treating DM and reports are published in numerous scientific journals. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generation, increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver and antagonize the problem of insulin resistance [(2)]. Traditional remedies which are often free from side effects are still in use by some diabetic patients in developing countries, and may therefore; present new avenues in the search for alternative hypoglycemic drugs.

Literature survey revealed that *Nigella sativa* oil lowered blood glucose concentration in the diabetic rats and the hypoglycemic effect of *Nigella sativa* may be mediated by extra

pancreatic actions rather than by stimulated insulin release [3, 4]. Oral administration of ethanolic extract of *Nigella sativa* seeds to streptozotocin induced diabetic rats for 30 days reduced the elevated levels of blood glucose and improved altered levels of lipid peroxidation products and because of its antioxidant effects, its administration may be useful in controlling the diabetic complications [5, 6]. Foenugreek (*Trigonella foenum-gracaeum*) may increase plasma insulin level *in vivo* [7,8,9] .Similarly, various hypotheses about the mechanism of the hypoglycemic activity of Fenugreek have been postulated, including delayed gastric emptying and an agonist effect on insulin receptors[10]. The major free amino acid 4- hydroxyisoleucine constituent of Foenugreek stimulates insulin secretion from perfused pancreas *in vitro* [11].

Termis seeds (*Lupine*) are a medicinal plant with potential value in the management of diabetes with antihyperglycemic activity present in extracts of the whole seed. In white mice, extracts of seeds of the white lupine [*Lupinus albus* , Termis)] were associated with increased tolerance to an oral glucose [12]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Alternatives are clearly needed because of the inability of current therapies to control high cost and poor availability of current therapies for many rural populations, particularly in developing countries [13]. A scientific investigation of traditional herbal remedies for diabetes mellitus may be valuable and leads to development of an alternative drugs and therapeutic strategies.

This study was designed to examine the effects of a water mixture extract of *Nigella sativa*, Foenugreek and Termis and each of these plants alone on diabetic rats as well as the possible effects of these plants on the pancreatic cells types and numbers and on the liver.

Material and Methods

The experimental and feeding protocols of the animals used in this study was approved and performed according to the guidelines of Animal House and Ethical Standards of College of Medicine, Taif University, KSA.

Plant material

The dried seeds of *Nigella sativa*, Foenugreek and Termis purchased from a local market in Al-Taif, KSA in Jan., 2012.

Preparation of plant extract

The dried powdered seed of *Nigella sativa*, Foenugreek and Termis and a mixture of equal ratio of the powdered seeds were separately powdered and extracted with distilled water by decoction method followed by filtration. The obtained aqueous extracts of the four samples (*Nigella sativa*, Foenugreek, Termis and the mixture) were used.

Animal material

One hundred fifty adult male albino rats of local strain 10-12 weeks of age with body weight ranging between 180-200 gm were used in the current work. Animals that used in the experiments were obtained from the Laboratory Animal Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah. All experiments were taken place at the research laboratories, College of Medicine, Taif University. Animals were housed individually in clean rodent cages, in a room at relative humidity not less than 30% and not exceeding 70%, at room temperature 22 °C - 30 °C, with artificial lighting in a sequence 12 hours light and 12 hours dark. Animals were fed on conventional laboratory animal diet for rats with an unlimited supply of drinking water.Animals were randomly selected, marked to permit group identification. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal Welfare Act.

Induction of DM was done by giving subcutaneous injection freshly prepared Alloxan solution 120 mg/kg (powder from BDH chemical LTD, England), dissolved in acetate buffer (pH 5.5) prepared immediately before use. After an overnight fasting then 48 hours later, blood glucose level was determined by Glucometer for all animals. Rats with blood glucose level ranging from 180 to 250 were considered diabetic. Aqueous extracts of *Nigella sativa*, Foenugreek, Termis seeds and a mixture of equal ratio of the powdered seeds, each in 0.5% carboxymethylcellulose sodium (CMC) were separately given orally to male albino diabetic rats in a dose 100 mg/kg body weight.

The experiment was carried out on six groups; each group contains 25 rats as following:

The first group (Control group): normal rats were given subcutaneous (sc) saline solution (0.01 ml/100 gm body weight).

The remaining one hundred diabetic rats were classified as following:

The second group: was considered a diabetic group without receiving any treatment.

The third group: was treated with the aqueous extract of the mixture of equal ratio of the seeds under investigation.

The forth group: was treated with the aqueous extract of *Nigella sativa* seeds.

The fifth group: was treated with the aqueous extract of Fenugreek seeds.

The six group: was treated with the aqueous extract of Termis seeds.

After 4 weeks, all animals fasted overnight, and then weighted, blood samples were obtained and then rats sacrificed. Blood samples centrifuged at 4000 xg for 10 min at 4°C and supernatant kept at -70°C for further biochemical measurements.Pancreas, and liver removed, then homogenizes separately, in 2, 5, 20 ml ice cold PBS, respectively.

Biochemical assays:

Biochemical studies were done to assess the following biochemical parameters: serum glucose level, serum insulin level, serum aspartate transaminase (AST), serum total protein, serum total lipid concentration, serum triglyceride and serum cholesterol level. Blood indices RBCs, WBCs, haematocrite (Hct) and hemoglobin (Hb) were also assessed.

Histological studies:

The animals were killed by decapitation. The samples of pancreas and liver were obtained and fixed in 10% neutral buffered formal saline, dehydrated in ascending grades of alcohol and cleared in Benzol. Samples from each group were embedded in paraffin with a melting point between 55 °C and 56 °C for 4 hours and then paraffin blocks were prepared. Paraffin sections were made at 5 µm and stained with hematoxylin & eosin for demonstrating any histological changes. Modified aldehyde fuchsin stain was used for detecting different cells of islets of Langerhans [(14)] . Then sections were examined under the microscope. Image analysis system was used for determination alpha, beta and delta cells number and diameters in the islet of Langerhans.

Statistical analysis:

Data were analyzed using SPSS version 20. Normality of distribution was computed by Kolmogrov smirnov test and W Shapiro-Wilk's test. X^2 and Fisher exact tests were applied to observe association between qualitative variables. Quantitative variables were expressed in means ± SDs. The comparison of quantitative data was performed by independent t-test or Mann Whitney test according to normality of distribution for independent variables consisting of two groups and by ANOVA and Kruskal Wallis test according to normality of distribution for independent variables consisting of more than two groups. Post Hoc Turkey test was applied to observe which groups mean differs. Statistical significance was set at 0.05 levels [(15)]. A p-value of < 0.05 was considered as statistically significant

RESULTS

As shown in tables (1& 2), after induction of DM by Alloxan, the serum glucose level raised to about 266 mg/dl (p= 0.008) and serum insulin level was fallen to about 21 U/L (p= 0.008) with significant increase levels of total lipids, cholesterol, and LDL with non significant decrease in HDL level in all diabetes induced groups. After treatment, the results of glucose concentrations (mg/dl) for the groups of rats treated by mixture of the herbs, *Nigella*, Foenugreek and Termis seeds (after the 4 th week of treatment) with mean ± S.E. were 127 ± 1.58, 131.2 ± 4.43 , 131.2 ± 1.92 and 134.8 ± 0.83 respectively. The glucose lowering effect occur in all the treated groups with more marked decrease in the group of rats treated by mixture of herbs ($p<0.05$). Also table (2), showed that insulin was statistically increased in all the treated groups of rats as compared with non-treated group ($p = 0.001$). The rise was more marked in rats treated by the mixture of herbs ($p< 0.05$).

Table (2), revealed that the levels of total lipids, cholesterol, and LDL were significantly decreased in the treated groups than non-treated group ($p< 0.001$). The lowering effect was mo obvious in *Nigella* treated group for total lipids ($p<0.05$), and in Fenugreek for cholesterol and LDL ($p <0.05$). HDL showed non-significant increase among the treated groups.

Table (3) illustrated the effects of induction of DM by Alloxan on blood indices. RBCs number, Hct and Hb levels were significantly decreased in the diabetic compared to the control groups with no effect on WBCs number. Tables (4) revealed statistically increased RBCs, HCT, and Hb in all the treated groups as compared to non-treated group of rats ($p=0.001$). The effect was significantly more marked in mixture treated group ($p<0.05$) except for *Nigella* treated group,

where there was non-significant decrease in Hct and Hb. No significant differences were found among all the studied diabetic groups regarding the number of WBCs.

Table (5) showed a significant increase in AST and LDH with a significant decrease in serum protein in diabetic group. Table (6) showed a significant decrease of ALT and LDH in all the treated than non treated groups ($p= 0.005$ and < 0.001) respectively. This lowering effects of treatment on AST and LDH were significantly more marked in mixture treated group ($p<0.05$). Also, total protein levels were significantly raised in all the treated groups ($P<0.001$). No statistically significant differences were realized among the effects of different treatment herbs on total proteins.

Fig (4) revealed that diabetic liver showed periportal fibrosis, vacuolated cytoplasm and cellular infiltration. Fig (5) revealed a section in the liver of a diabetic rats (30 day treatment) revealed that the liver of diabetic- mixture ingested rat showed somewhat normal liver cells and nuclei with some pyknotic nuclei. Liver of the diabetic- *Nigella sativa* ingested rat showed infiltration with vacuolated cytoplasm Liver of the diabetic-Termis treated rat showed deeply basophilic vacuolated cytoplasm and pyknotic nuclei and liver of diabetic- Foenugreek treated rat showed normal distribution of hepatic cords with some vacuolated cytoplasm in hepatocytes.

Histological study of pancreas of rats in the treated and non-treated groups showed that induction of diabetes by alloxan in rats had no effect on alpha and delta cells (number, cell and nuclear diameters) (Tables 7, 8 and figures 1, 2, and 3). However, a significant decrease in beta cells number and increase in beta cells diameter as well as nuclear diameters was found in the diabetic group as compared to the control group ($P <0.001$). All the treated groups showed a significant increase in beta cells number and a decrease in beta cells diameter as well as nuclear diameters as compared to the non-treated groups ($P= 0.001$). However, the effect on beta cell number was more marked in rats treated by a mixture of plants ($p<0.05$). Other effects on beta cell diameter and nuclear diameter showed no significant differences among all the treated groups.

Discussion:

The diabetic patients need alternative therapies to control all the pathological aspects of diabetes and the high cost and poor availability of current therapies in developing countries [(4)].The traditional antidiabetic plants might provide this useful source of new oral hypoglycemic compounds. So, this study is a step to evaluate the effects of some water extracts of medicinal plants as antidiabetic agents individually and as a mixture. Severe hyperglycemia in diabetic rats recorded in the present work can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of Alloxan on the β-cells of the pancreas which has a direct effect on their membrane permeability by causing failure of ionic pumps and increased cells size [(16, 17, 18)].

Our results revealed that glucose lowering effect in all the treated groups after the 4 [th] week of treatment which was more marked in the group treated by mixture of herbs ($p<0.05$). Also, insulin was statistically increased in all the treated groups as compared with the non-treated group ($p= 0.001$). The rise was more marked in rats treated by mixture of herbs ($p< 0.05$).

Finally, our results revealed that the levels of total lipids, cholesterol, and LDL were significantly decreased in the treated groups than non-treated group ($p< 0.001$). The lowering effect was more obvious in *Nigella* for total lipids ($p<0.05$), and in Fenugreek for cholesterol and LDL ($p <0.05$). This may attributed to their stimulation to the most aspects of carbohydrate metabolism, including rapid uptake of glucose by the cells, enhanced gluconeogenesis, increased rate of absorption from the gastrointestinal tract and even increased insulin secretion with its resultant secondary effects on carbohydrate metabolism [(19, 20)]. **Marles *et al.*** [(4)] suggested that, the hypoglycemic effect of some medicinal plants could be attributed to factors other than stimulation of insulin release only, e.g. their effect on the number and /or affinity of insulin receptors on target cells and the post-receptors of these cells.

Abdel Moneim *et al*. [(16)] reported that the hypoglycemic effect of *Nigella sativa* may be attributed to an increase in the islet numbers and to its effect on the time-course of glucose resorption from the intestine. On the other hand, the treated group showed a significant increase in beta cell number and a decrease in their diameters as well a nuclear diameter was found in all the treated groups. These plants may have a stimulatory effect on the division of β-cells, block

the diabetogenic action of Alloxan and restore insulin production [21] .The significant hypoglycemic and insulinotropic effects induced in diabetic rats by treatment by such plants may result from their effect on the time course of glucose resorption from the intestine. The treatment with *Nigella sativa* induced islet cells regeneration with increased number of β-cells [16] .

Augusti and Sheela [22] mentioned that some plants exert their effect on beta cells through both protection of the already present beta cells due to their antioxidant effect and through stimulation of the beta cells to release insulin. The results of our study revealed significant decreased levels of total lipids, cholesterol, and LDL in all the treated groups compared to the non-treated group ($p< 0.001$). The lowering effect was more obvious in *Nigella* for total lipids ($p<0.05$), and in Fenugreek for cholesterol and LDL ($p <0.05$). **Zahida *et al.*** [23] stated that the *Nigella sativa* is effective to change the lipid profile significantly. **Akash *et al.*** [24] mentioned that some medicinal herbs may have antioxidant effects especially *Nigella sativa.*

Our results revealed a significant increase in AST and LDH with asignificant decrease in serum protein in the diabetic group. Also, diabetic liver showed periportal fibrosis, vacuolated cytoplasm and cellular infiltration. A significant decrease in ALT and LDH values was realized in all the treated than non treated groups ($p= 0.005$ and < 0.001) respectively. This lowering effects of treatment on AST and LDH were significantly more marked in the mixture treated group ($p<0.05$). Also total protein levels were significantly raised in all the treated groups ($P<0.001$). Histological study of the liver of the diabetic rats (30 day treatment) showed histological improvement of liver texture especially in the diabetic -mixture group, then liver of the diabetic- *Nigella sativa* group and liver of the diabetic -Foenugreek group with minimal effects in Termis group [25].

Our data illustrated the effects of induction of DM by Alloxan on blood indices where RBCs number, Hct and Hb levels were significantly decreased in the diabetic group compared to the control group. These parameters statistically increased RBCs, HCT, and Hb in all the treated groups as compared to the non-treated group of rats ($p=0.001$).The effect was significantly marked in the mixture treated group ($p<0.05$) except for *Nigella* treated group, where there was a non-significant decrease in Hct and Hb.This anemia could be attributed, to destruction of RBCs and reduced rate of red blood cells released from the bone marrow to blood. Several investigators attributed this anemia to the increase in lipid peroxidation of the erythrocyte cell membrane by the destructive effect of alloxan [26]. Finally, the water extract of the mixture is the most powerful in amelioration hyperglycemia and all damage effects of Alloxan on the liver and pancreas tissues, hematological parameters and lipid profile.

Conclusion:

The studied herbs in this work and water extract of the mixture of these plants appeared to be useful agents in reducing the hyperglycemia by increasing insulin level and regenerating beta cells of the pancreas. However, a mixture of these plants proved to be more effective than each of them without added side effects. The water extract of the mixture is the most powerful in amelioration most of all damage effects of Alloxan on the liver texture, hematological parameters, and lipid profile. So it is advised to use the plant mixture as an antidiabetic agent rather than the use of each plant separately. More studies on these plants are advised be done with different doses and for different periods before recommending their use on a wide scale.

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Table (1) Comparison between control and diabetic groups as regard metabolic profile

	Control	Diabetic	P
Blood glucose	136.8 ± 1.9	266.4 ± 0.89	0.008
Serum insulin	41.2 ± 0.83	20.2± 1.4	0.008
Total lipids	4.5 ± 0.33	13.7 ± 0.47	0.009
Cholesterol	143.4 ± 2.2	222.1± 1.1	0.009
LDL	69.3 ± 2.8	125.9 ± 2.05	0.009
HDL	47.2 ± 0.75	44.1 ± 1.4	0.009

Table (2) Comparison between diabetic groups as regard metabolic profile

	Diabetic untreated rats	Mixture treated group	Nigella treated group	*Foenugreek* treated group	Termis treated group	P
Blood glucose	266.4 ± 0.89	127 ± 1.58	131.2 ± 4.43	131.2 ± 1.92	134.8 ± 0.8	0.001
Serum insulin	20.2± 1.4	45.2 ± 3.3	32.4 ± 3.2	38.4 ± 1.5	34.6 ± 2.3	0.001
Total lipids	13.7 ± 0.47	6.7 ± 1.4	4.5 ± 0.09	5.7 ± 0.23	4.7 ± 0.37	<0.001
Cholesterol	222.1± 1.1	141.4 ± 0.79	191.1 ± 2.7	140.6 ± 1.3	190.5 ± 2.7	<0.001
LDL	125.9 ± 2.05	68 ± 0.92	114.8 ± 1.6	67.8 ± 2.05	72.2 ± 1.5	<0.001
HDL	44.1 ± 1.4	45.8 ± 0.93	45.3 ± 2.4	45.2 ± 3	45 ± 2.05	0.294

Table (3) Comparison between control and diabetic groups as regard CBC parameters

	Control Group	Diabetic Group	P
RBCs	7.5 ± 0.27	5.5 ± 0.38	0.009
WBCs	12.4 ± 1.2	12.3± 0.73	0.917
HCT	44.2 ± 1.5	40.1 ± 2.3	0.012
Hb	14.7 ± 0.52	12.6 ± 0.42	0.009

Table (4) Comparison between diabetic groups as regard CBC

	Diabetic untreated rats	Mixture treated group	Nigella treated group	Foenugreek treated group	Termis treated group	P
RBCs	5.5 ± 0.38	7.5 ± 0.30	5.8 ± 0.44	6.9 ± 0.14	6.7 ± 0.50	0.001
WBCs	12.3± 0.73	12.5 ± 1	12.3 ± 0.63	12.4 ± 0.55	12.5 ± 0.54	0.973
HCT	40.1 ± 2.3	43.8 ± 1.2	36.8 ± 2.8	44.2 ± 1.4	44.8 ± 1.1	0.001
Hb	12.6 ± 0.42	14.6 ± 0.42	11.6 ± 0.96	13.2 ± 0.43	12.4 ± 0.53	0.001

Table (5) Comparison between control and diabetic groups as regard liver function tests

	Control Group	Diabetic Group	P
AST	88.3 ± 3.9	101.8 ± 3.4	0.009
LDH	95.4 ± 0.61	192.4 ± 0.90	0.009
Total proteins	7.7 ± 0.33	5.6 ± 0.46	0.009

Table (6) Comparison between diabetic groups as regard liver function tests

	Diabetic untreated rats	**Mixture treated group**	**Nigella treated group**	**Foenugreek treated group**	**Termis treated group**	**P**
AST	101.8 ± 3.4	83.1 ± 4.3	87.9 ± 3.7	87.2 ± 2.7	87.8 ± 1.9	0.005
LDH	192.4 ± 0.90	84.5 ± 0.70	89.1 ± 0.82	92.1 ± 1.02	89.1 ± 0.63	<0.001
Total protein	5.6 ± 0.46	7.8 ± 0.31	7.1 ± 0.15	7.3 ± 0.33	6.8 ± 1.58	0.001

Table (7) Comparison between control and diabetic groups as regard pancreatic cells

		Control Group	**Diabetic Group**	**P**
Alfa cells	Number	2.83±0.64	2.43±1.04	0.01
	Nuclear Diameter	1.34±0.43	1.50±0.42	0.169
	Cell Diameter	3.03±0.60	3.48±0.78	0.016
Beta cells	Number	32.66±13.94	10.80±5.14	<0.001
	Nuclear Diameter	1.58±0.35	2.39±0.48	<0.001
	Cell Diameter	3.25±0.29	4.49±0.74	<0.001
Delta cells	Number	4.3±1.6	4.40±1.84	0.970
	Nuclear Diameter	2.01±0.47	2.01±0.56	0.929
	Cell Diameter	4.45±1.36	4.33±0.62	0.482

Table (8) Comparison between diabetic groups as regard pancreatic cells

		Diabetic untreated rats	**Mixture treated group**	**Nigella treated group**	**Foenugreek treated group**	**Termis treated group**	**P**
Alfa cells	Number	2.43±1.04	2.40±0.89	2.93±1.74	2.93±1.74	2.80±1.49	0.700
	Nuclear Diameter	1.50±0.42	1.27±0.31	1.25±0.28	1.25±0.28	1.36±0.39	0.077
	Cell Diameter	3.48±0.78	2.88±0.55	2.96±0.68	2.96±0.68	3.15±0.73	0.043
Beta cells	Number	10.80±5.14	34.12±16	25.90±13.70	25.90±13.70	26±10.94	<0.001
	Nuclear Diameter	2.39±0.48	1.32±0.17	1.31±0.27	1.31±0.27	1.33±0.21	<0.001
	Cell Diameter	4.49±0.74	3.02±0.46	2.70±0.38	2.70±0.38	3.06±0.50	<0.001
Delta cells	Number	4.40±1.84	3.40±1.10	3.46±0.89	3.43±1.04	3.40±1.16	0.091
	Nuclear Diameter	2.01±0.56	1.90±0.63	1.73±0.77	1.74±0.70	1.83±0.68	0.113
	Cell Diameter	4.33±0.62	3.66±1.06	3.34±0.64	3.33±0.67	3.03±0.46	0.091

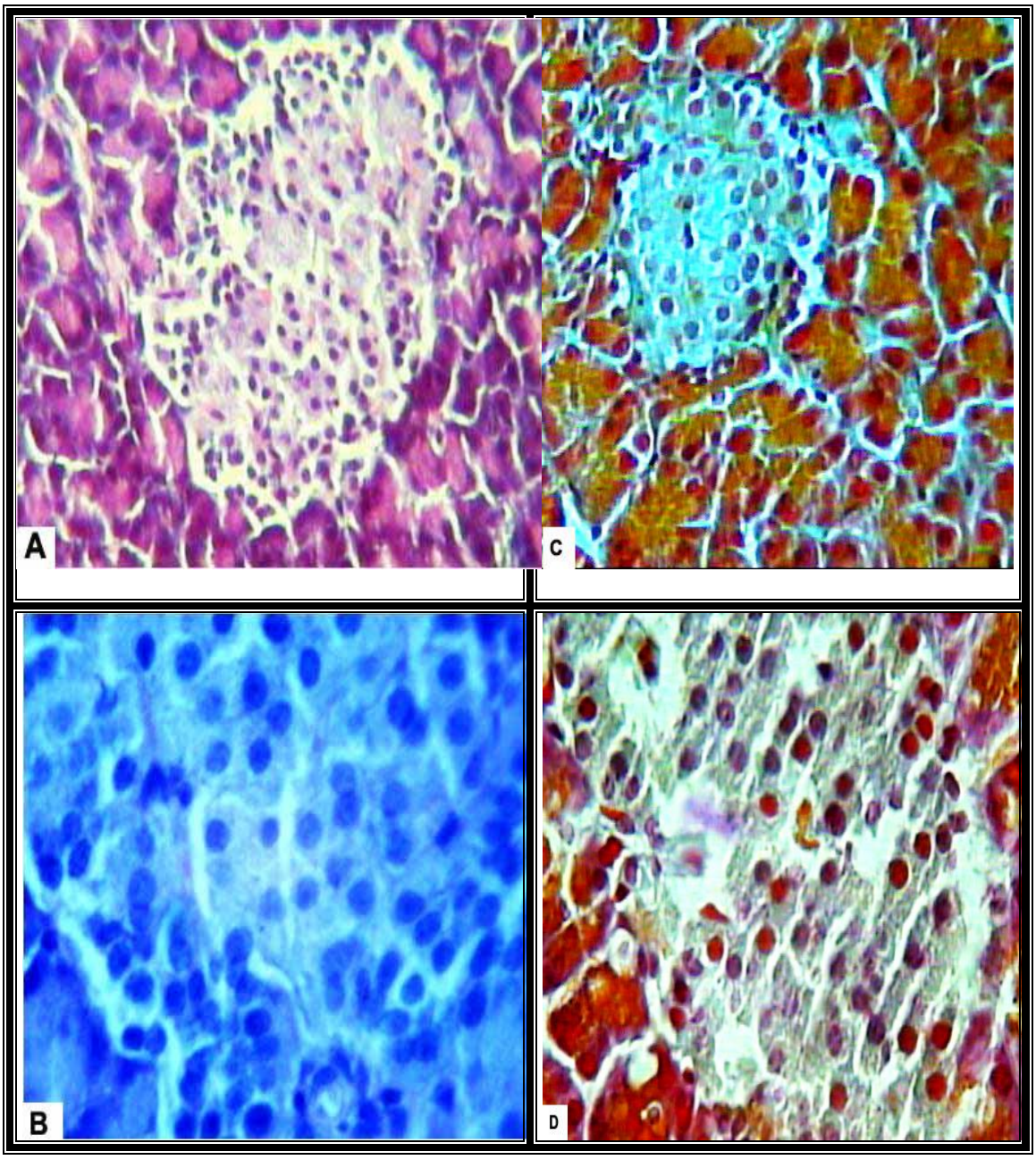

Fig (1): A photomicrograph of a section in the pancreas of control adult male rat showing:
A& B: rounded or oval and prominent nuclei within the B-cells, notice the deeply basophilic nuclei of A-cell.
(Hx & E (A) X400 (B) X 1200).
C&D: rounded or oval violet B-cell, oval green D cells and irregular yellow A-cell.
(Modified aldehyde fuchsin (C X 400 &D X 1200).

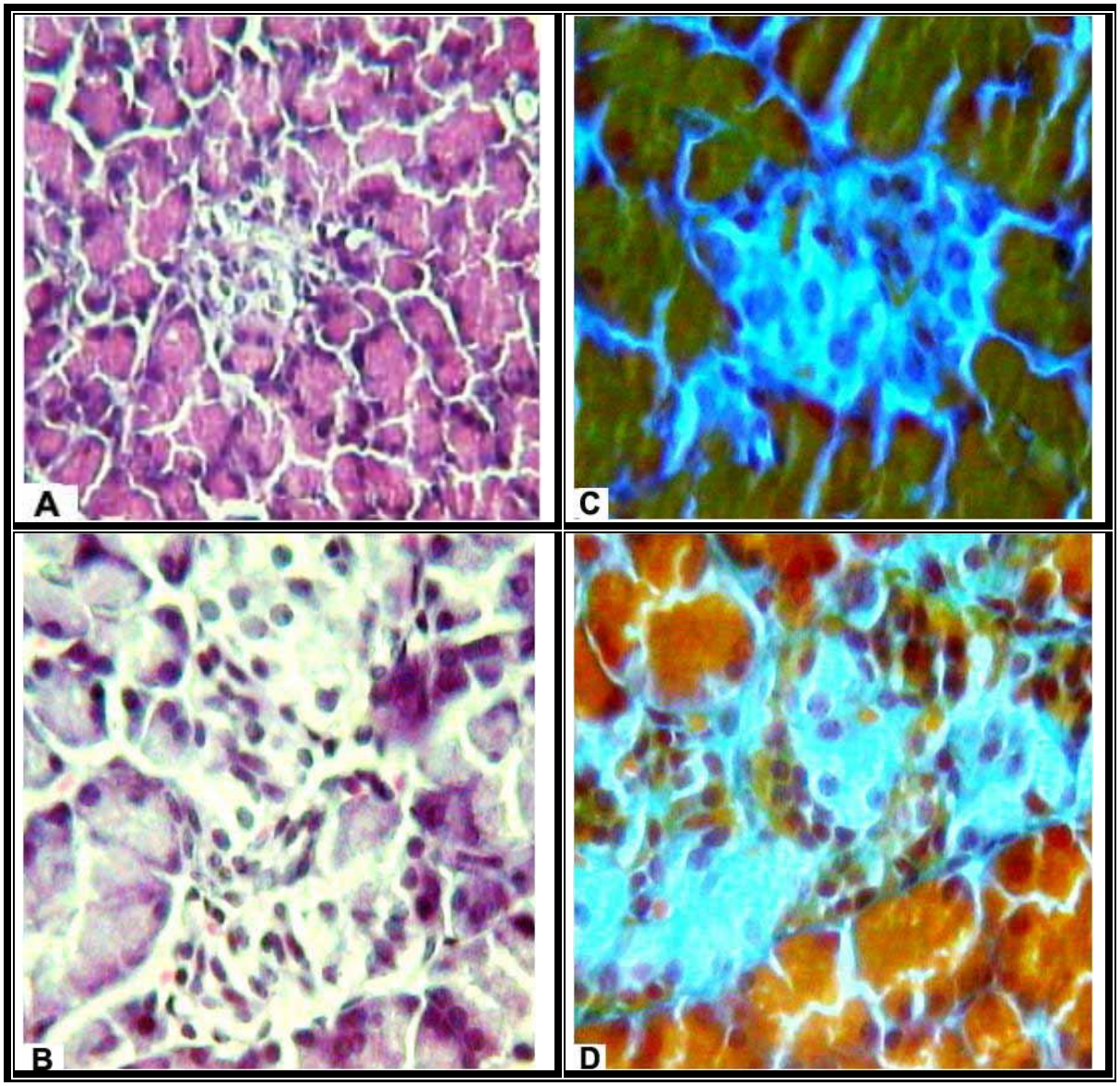

Fig (2): A photomicrograph of a section in the pancreas of a diabetic rat showing:
A&B: small sized islet with pale disintegrated nuclei and the intact dark cells at the periphery of the islet with normal structure of the exocrine pancreas. (Hx & E ,A X400 &B X 1200).
C&D: small sized islet with pale disintegrated nuclei, vacuolated B -cell, deeply green delta cell and faintly stained A-cell. (Modified aldehyde fuchsin ,C X 400 &D X 1200).

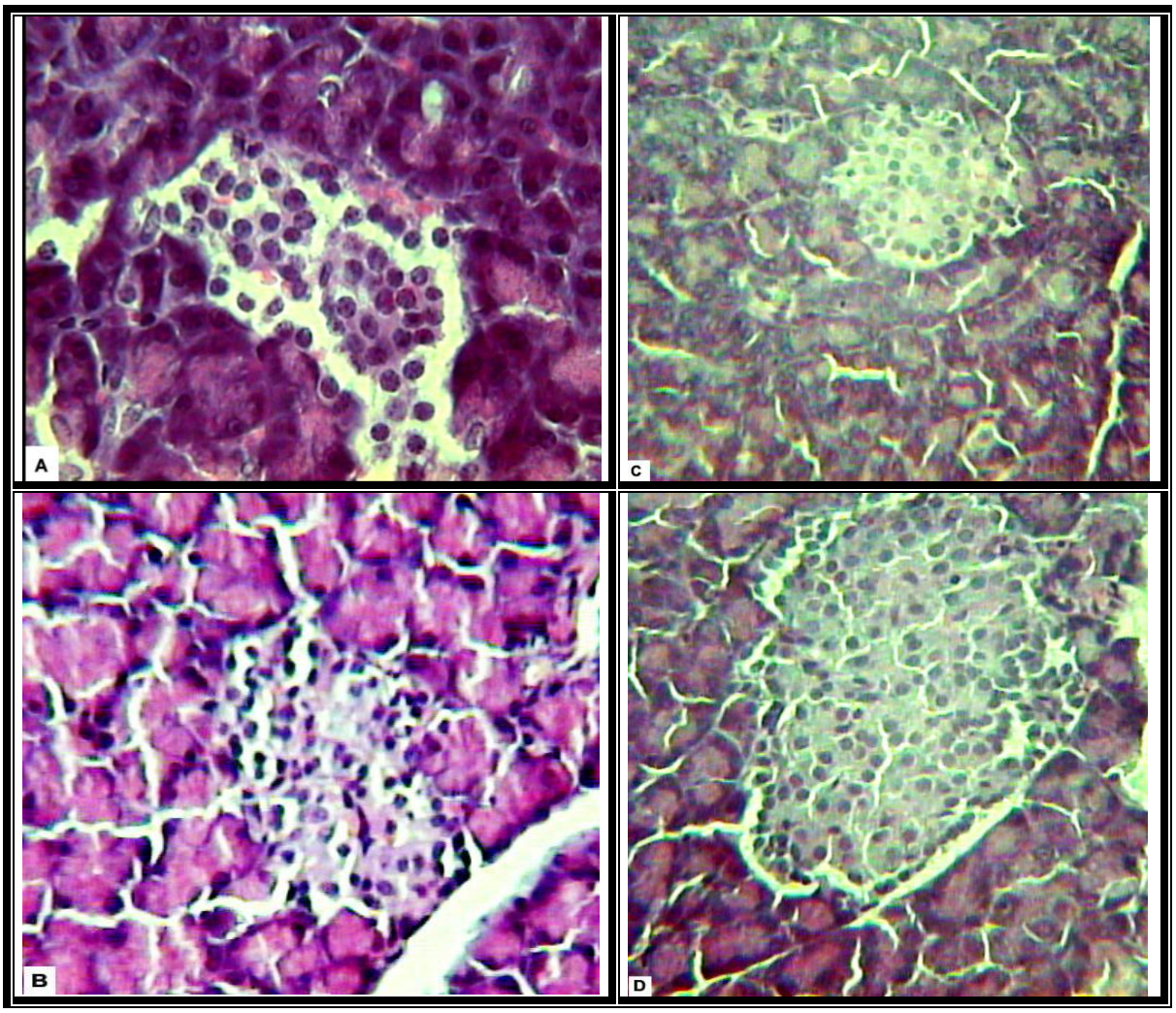

Fig (3): A photomicrograph of a section in the pancreas of a diabetic rat 30 day showing:

A - Section in the pancreas of a diabetic mixture ingested rat showing decreased signs of vacuolation in B-cell with normal islets.

B- Section in the pancreas of an adult male diabetic *Nigella Sativa* ingested rat illustrating vacuolated B-cellt, deeply stained nuclei with somewhat normal islets .

C- Section in the pancreas of an adult male diabetic Termis seeds ingested rat showing vacuolated and degenerated B-cell. Small islet having hypocellularity and poor vascularity.

D- Section in the pancreas of an adult male diabetic Foenugreek ingested rat showing less vacuolated B-cells, and within normal islets. (Hx & E X400).

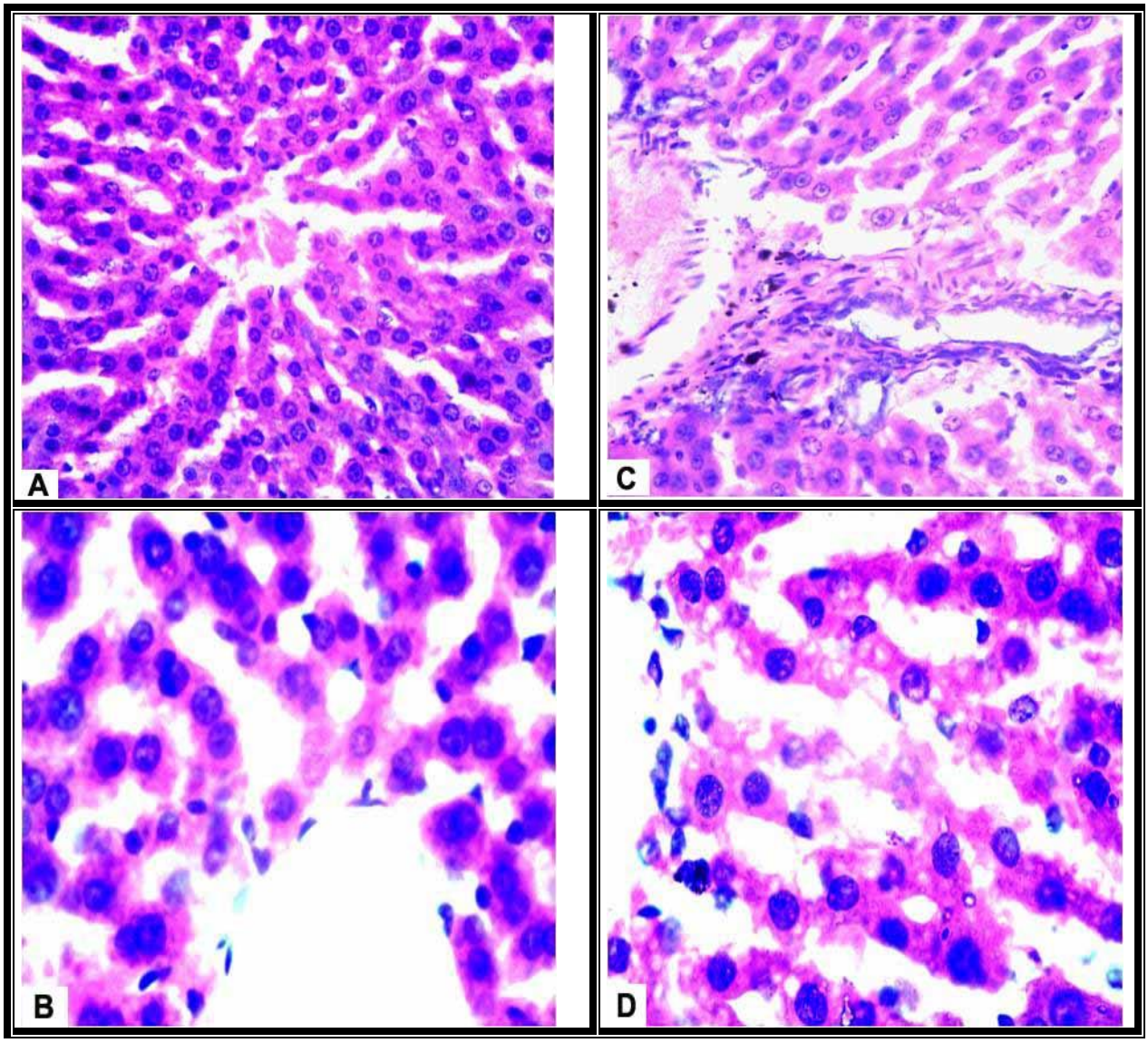

Fig (4): a photomicrograph of a section in the liver of the control and diabetic adult male rat.
A&B: sections of the control liver showing normal liver cells. (Hx & E ,A X400 &B X 1200).
C&D: sections of diabetic liver showing periportal fibrosis, vacuolated cytoplasm and cellular infiltration.
(Hx& E ,C X400 &D X 1200).

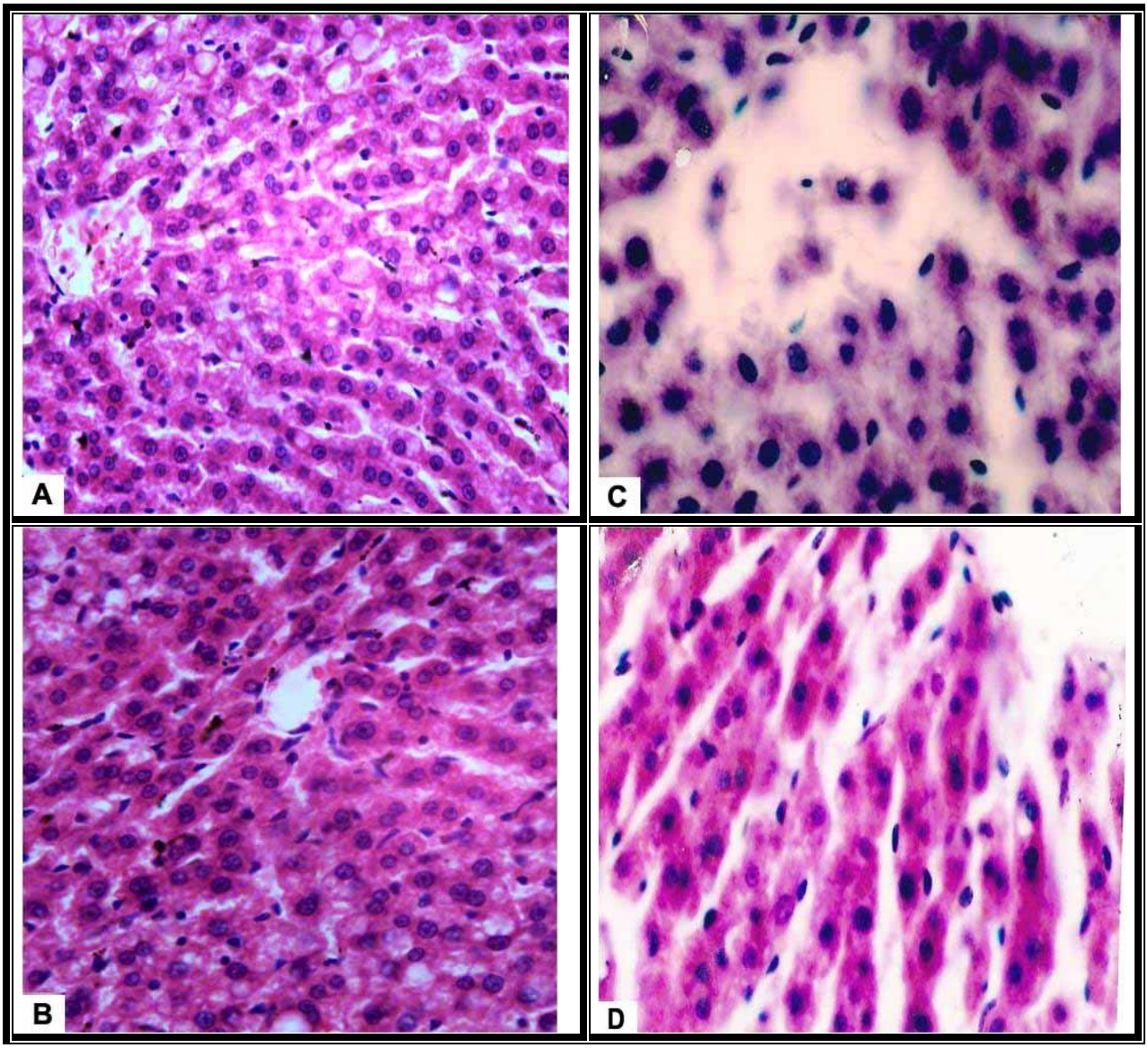

Fig (5): A photomicrograph of a section in the liver of a diabetic rat 30 day showing:

A - Section in the liver of a diabetic mixture ingested rat showing somewhat normal liver cells and nucei and some pyknotic nuclei.

B- Section in the liver of an adult male diabetic *Nigella sativa* ingested rat showing infiltration with vacuolated cytoplasm.

C- Section in the liver of an adult male diabetic Termis ingested rat showing deeply basophilic vacuolated cytoplasm and pyknotic nuclei

D- Section in the liver of an adult male diabetic Foenugreek ingested rat showing normal distribution of hepatic cords with some vacuolated cytoplasm. (Hx & E X400).