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Dynamics of a Vaccinium Corymbosum Lam. Leaves Extract Action on Some Biochemical, Structural and Ultrastructural Parameters in Rats with Experimentally Induced Diabetes

C. CRĂCIUN[1], ALINA ILIE[1], J. MADAR[2], VERONICA CRĂCIUN[1], M. TĂMAŞ[3], V. MICLĂUŞ[4], I. ILYES[2]

[1]"Babes-Bolyai" University, Cluj-Napoca, [2]Biological Research Institute, Cluj-Napoca,
[3]University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca,
[4]Faculty of Veterinary Medicine, Cluj-Napoca

ABSTRACT.

The aim of the present study is to investigate the effect of a hydroalcoholic extract of *Vaccinium corymbosum Lam.* (highbush blueberry) leaves on plasma and cholesterol levels, on the structure of pancreas and on the ultrastructure of liver, in streptozotocin-diabetic rats. Plasma glucose levels were found to drop by about 30% after 10 and 20 days of treatment with the leaf extract, while plasma cholesterol levels slightly decreased only after 20 days of treatment. There was no evident improvement in the streptozotocin-altered structure of the pancreas after 3 and 6 days of treatment with the extract. The extract was effective in improving the shape and structure of the nucleus of hepatocytes altered by streptozotocin.

KEY WORDS: diabetes, streptozotocin, leafs extract, highbush blueberry, glycemia, cholesterolemia, structure, ultrastructure, pancreas, liver.

INTRODUCTION

In the last 10-20 years, the research works concerning the action of plant extracts on in vivo models have gradually developed. In this context, a special attention was granted to the plants with antidiabetic action. Thus, the benefic role of blueberry (*Vaccinium myrtillus*) leaves extract in lowering the level of glycemia in diabetic patients is well known. Furthermore, it was already introduced in the composition of some hypoglycemic drugs, like Hipogalegin (UMF-Cluj-Napoca) and Normogluc (Plantavorel). Because leafs of *Vaccinium myrtillus* are difficult to obtain (because of its spreading in mountain areas, its low efficiency due to the reduced size of leafs) Plafar thought a solution might be the use of *Vaccinium corymbosum* leafs instead (Tamas et al., 1997).

Vaccinium corymbosum has been cultivated in Romania for 10-15 years, especially for its large, edible fruits. The hybrid cultivated in Romania is called "Blueray" and is a result of crossbreeding between *V. corymbosum* and hybrids originated from North-American species (Eck and Childers, 1992). In order to introduce leafs of "Blueray" in phytotherapy, pharmacological and phytochemical investigations are compulsory.

Several classes of active substances were identified in leafs of *V. corymbosum* ("Blueray") (Tamas et al., 1997): flavones, proanthocyanins and tanoid polyphenols. The amount of flavones in the *V. corymbosum* ("Blueray") leafs is more than twofold higher than in *V. myrtillus* leafs (3-3.68% / 1.5%) and depends on the time of leaf harvesting. Thus; leafs harvested after fruits (August) contain more flavones than leafs harvested in June, before fruit harvesting. Because of the very high content of proanthocyanins (14.2-16.2‰, 4-fold more than in *V. myrtillus* leafs), leafs of *V. corymbosum* ("Blueray") have an important P-vitamin-like effect. The percentage of tanoid polyphenols is also higher in *V. corymbosum* ("Blueray") leafs (17.32-18.6%) than in *V. myrtillus* leafs (10.43%).

There are only few studies concerning the effects of *V. corymbosum* leaf extract on animal organisms (Benigni, 1962, Pirvu, 1998). The aim of the present study is to investigate the effects of a *V. corymbosum* ("Blueray") hydroalcoholic leaf extract on some biochemical (glycemia, cholesterolemia), structural and ultrastructural parameters in rats with experimentally induced diabetes mellitus.

MATERIALS AND METHODS

The experiment was performed on white Wistar rats of 150-175 g b.w. After a fasting period of 24 hours, diabetes was induced by i.v. injection of a single dose (3 mg/100 g b.w.) of streptozotocin (Serva) solved in sodium citrate 10 mM. After 30 minutes, 1 ml glucose 33% / animal was injected intraperitoneally, in order to prevent a hypoglycemic shock.

The animals were divided into 4 groups: control group (none injected rats), diabetic group (rats with experimentally induced diabetes), non-diabetic group treated with the *V. corymbosum* ("Blueray") leaf extract and diabetic group treated with the extract.

The extract had been administered for 20 days, by intragastric tubing, in a daily dose of 0.6 g/kg b.w. Glycemia was enzymatically

established, by using a GOD-Perid Glucose Kit ("Boehringer-Mannheim" GmbH, Mannheim-Germany), while cholesterolemia was measured by the method of Zlatkis, after 10 and 20 days of treatment with the leaf extract.

Fragments of pancreas and liver were collected after 3 and 6 days of treatment. For the histological study, tissue fragments were fixed in 10% formol, embedded in paraffin, sectioned at 5 micrometers and stained by the three-cromic method of Goldner. For the ultrastructural study, tissue fragments were prefixed in 2.7% glutaraldehyde solution, postfixed in a 2% osmic acid solution, dehydrated in acetone and embedded in Vestopal 310 (Fluka). Ultrathin sections were obtained by using a LKB III ultramicrotome. The sections were contrasted with uranyl acetate and lead citrate and examined at a TESLA BS-500 electron microscope.

RESULTS

The administered streptozotocin induced a moderate hyperglycemia (180% after 10 days and 189.5% after 20 days), as compared to the control group (81% after 10 days and 84% after 20 days). There was a decrease of glycemia by ~30% in the diabetic group treated with the leaf extract, comparative to the diabetic untreated group; this effect did not depend on the period of treatment with the leaf extract (10 or 20 days). In the non-diabetic group, the treatment with the extract induced a slight hypoglycemia (72% after 10 days, 73% after 20 days) as compared to the control group. (Tables 1, 2; graphs 1 and 2).

Streptozotocin induced also a slight hypercholesterolemia (152% after 10 days, 180% after 20 days) as compared to the control group (145% after 10 days, 158% after 20 days). Only after 20 days of treatment with the leaf extract, one can observe the tendency of normalization of cholesterolemia in the diabetic treated group.

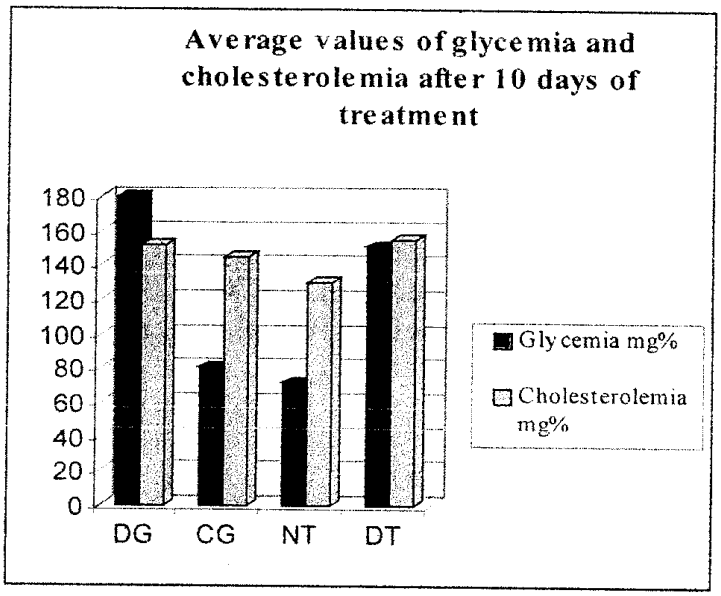
Table 1. Average values of glycemia and cholesterolemia in diabetic rats treated with highbush blueberry (*Vaccinium corymbosum*) leafs extract for 10 days.

Groups of rats	**Glycemia mg%**	**Cholesterolemia mg%**
Control group	81	145
Diabetic group	180	152
Non-diabetic group treated with the extract	72	130
Diabetic group treated with the extract	151	155

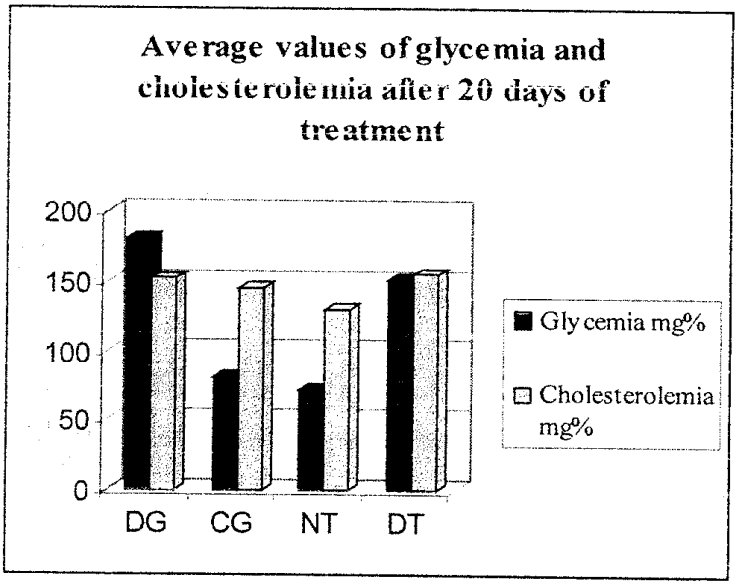
Table 2. Average values of glycemia and cholesterolemia in diabetic rats treated with highbush blueberry (*Vaccinium corymbosum*) leaves extract for 20 days.

Groups of rats	**Glycemia mg%**	**Cholesterolemia mg%**
Control group	84	158
Diabetic group	189.5	180
Non-diabetic group treated with the extract	73	150
Diabetic group treated with the extract	159.5	164

Graph. 1



Graph. 2



The histological investigation of pancreas in the ***diabetic group***, 3 days after streptozotocin administration, showed a slight atrophy of pancreatic islets, with almost all cells affected. Many cells present cytoplasmic vacuolisations of different degrees, ranging from small vacuoles to the complete cytoplasm vacuolisation. These cells have a larger size than the surrounding ones, suggesting a tumefaction process of the cytoplasm. Some cells display picnotic nuclei. The most damaged is the central zone of the islets of Langerhans, where a reticular material is visible, probably resulted from the disintegration of some cells (Fig. 1). The increase of glycemia and its maintenance at high levels after the applied treatment prove the damaging of pancreatic islet cells in different grades.

The aspect of the pancreas in the ***group treated for 3 days with the leaf extract*** is comparable to that of the diabetic group, but the number of cells with partial or total vacuolisations is greatly reduced. Cells with picnotic nuclei are still present (Fig. 2).

Six days after streptozotocin administration, the atrophic aspect of pancreatic islets is evident. The disposition of cells is somehow disordered, and most cells have picnotic nuclei. In some areas only cell debris are persisting (Fig. 3). In the ***group treated for 6 days with the leaf extract***, the intensity of the vacuolisation process is greatly reduced, but cells with picnotic nuclei are still present (Fig. 4).

The ultrastructural investigation of liver was realized comparatively in the control group and in the treated groups.

In the ***control group***, hepatocytes are normally organized in cell cords. Their nuclei are large, spherical, central, and contains scattered clumps of chromatin and 1-3 prominent nucleoli. Mitochondria are abundant and have spherical or elongated shapes. The rough endoplasmic reticulum has a typical arrangement in parallel profiles. Lysosomes are present especially in the form of peribiliary bodies (Figs. 5, 6).

In the ***diabetic group***, streptozotocin influenced the size and shapes of hepatocyte nuclei, which are smaller, have irregular shapes and no visible nucleoli (Figs. 7, 8). Mitochondria are swollen and have a rarefied matrix (Figs. 7, 8). Mitochondria of some hepatocytes have a polymorph and vacuolated appearance (Fig. 9). The rough endoplasmic reticulum is nearly absent and the number of peribiliary bodies is increased (Fig. 7). These data

suggest a strong decrease of protein synthesis in hepatocytes. The bile canaliculi are almost closed by their dilated microvilli. Due to the stasis of red cells, blood capillaries are congested (Fig. 10) and in some cases the endothelium and the sinusoidal surface of hepatocytes are broken, resulting in migration of cell content into sinusoids (Fig. 11). The lipid amount of hepatocytes is extremely reduced. Furthermore, there is a slight collagen proliferation in the space of Disse. Kupffer cells have a relative intense activity (Fig. 12).

In the ***diabetic group treated for 3 days with the V. corymbosum leaf extract*** the nuclei of most hepatocytes are normally shaped and sized and have evident nucleoli (Figs. 13, 14). Mitochondria are still swollen and have rarefied cristae and matrix (Fig. 13). Some hepatocytes display a slightly increase of smooth endoplasmic reticulum vesicles and an accumulation of lipid droplets (Fig. 15). Ito cells don't contain lipids, suggesting an impairment of lipid transit. There is a stasis of red cells in blood capillaries in some areas, but the capillary walls are unimpaired (Fig. 14).

In the ***diabetic group treated for 6 days with the V. corymbosum leaf extract***, most hepatocytes display a normal aspect of their nuclei and mitochondria (Figs. 16, 17, 18). Kupffer cells have an intense activity, probably in order to eliminate the streptozotocin-impaired structures (Fig. 17). The stasis of red cells in blood capillaries is still present, suggesting that this streptozotocin-induced effect have not been removed yet (Fig. 18).

DISCUSSION

Diabetes is a syndrome that comprises a heterogeneous group of disorders, but all have in common hyperglycemia associated with lipid and protein metabolism alterations. Hyperglycemia and other secondary modifications of the other metabolisms are results of an absolute or relative insulin insufficiency.

Diabetes mellitus can be induced experimentally by different chemicals. One of these is streptozotocin, a nitrous derivative of nitrozamine, which destroys selectively the B-cells of pancreatic islets of Langerhans, already one hour after its i.v. injection. As a result, glucose is not able to pass into tissues and hyperglycemia arises. Also lipids are moved into blood because of lack of inhibition exerted normally by insulin upon

liver lipase. The increased level of blood cholesterol promotes atherosclerosis (Dorofteiu, 1992).

Hyperglycemia represents the essential pathogenic factor that induces specific injuries in different organs. Its negative effects are due to secondary metabolic modifications: protein glycosylation, accumulation of polyol intermediaries, and overproduction of oxygen free radicals (Gherasim, 1996).

In hyperglycaemia, proteins undergo a process of non-enzymatic glycosylation, that continues as the level of glycemia increases. The catabolism of glycosylated proteins results in formation of final products of advanced glycosylation that form abnormal peptide bonds between collagen molecules. Furthermore, synthesis of collagen is increased and its degradation is decreased (Gherasim, 1996). This could explain the collagen proliferation observed in the space of Disse in the liver of the diabetic group.

Another result of hyperglycemia is the intensified autooxidation of glucose and the consecutive formation of very reactive free radicals that lead to lipid peroxidation and induces vascular lesions, observed in the thymus and liver of diabetic rats. Lipid peroxidation also leads to important structural modifications of the Golgi apparatus in the liver (Kordowiak et al., 1997; Dabros et al., 1998).

Plant extracts are very important in improving diabetic lesions. The efficiency of blueberry (*Vaccinium myrtillus*) leafs in the treatment of diabetes is due to a glycoside of galic acid called neomyrtilin (Ciulei et al., 1993). Cignarella et al. (1996) observed a decrease by ~26% of plasma glucose level in two different stages of diabetes, in rats with streptozotocin-induced diabetes and treated for 4 days with a hydroalcoholic blueberry leaf extract. They also noticed a decrease of plasma triglycerides by 39% following treatment. Other researches (Laplaud et al., 1997) proved the protective, antioxydating effect of a blueberry extract (rich in flavonoids, which are phenol compounds with antioxydating activity) on low-density lipoproteins (LDL), in a copper-mediated oxidation in vitro. It seems that this extract is more potent than either ascorbic acid or butylated hydroxytoluene in the protection of LDL particles from oxidative stress.

The hypoglycemic activity of a *Vaccinium corymbosum* leaf extract was first proved by L'Edgars (cited by Benigni, 1962), who extracted a

hypoglycemic glycoside from leafs of *V. corymbosum* named neomyrthilin. Our study shows a decrease by ~30% of glycemia in the diabetic group treated for 10 and 20 days with the *V. corymbosum* leaf extract. This effect does not depend on the period of treatment. The slight hypercholesterolemia induced by streptozotocin tends to normalise only after 20 days of treatment with the leaf extract.

The structure of endocrine pancreas was strongly impaired by streptozotocin and the administration of the leaf extract for 3 and 6 days was not enough to improve it. In turn, the ultrastructure of liver (especially shape and ultrastructure of hepatocytes nuclei) was greatly improved after the treatment, suggesting the implication of the extract at this level.

CONCLUSIONS

The treatment with a *V. corymbosum* leaf extract has a moderate hypoglycemic activity, relatively independent of the period of administration. The leaf extract has also a slight antihypercholesterolemic action.

The period of treatment with the extract was too short to improve the streptozotocin-altered structure of the endocrine pancreas.

The administered leaf extract had a favourable effect on the liver, especially in improving the structure of hepatocyte nuclei and mitochondria affected by streptozotocin.

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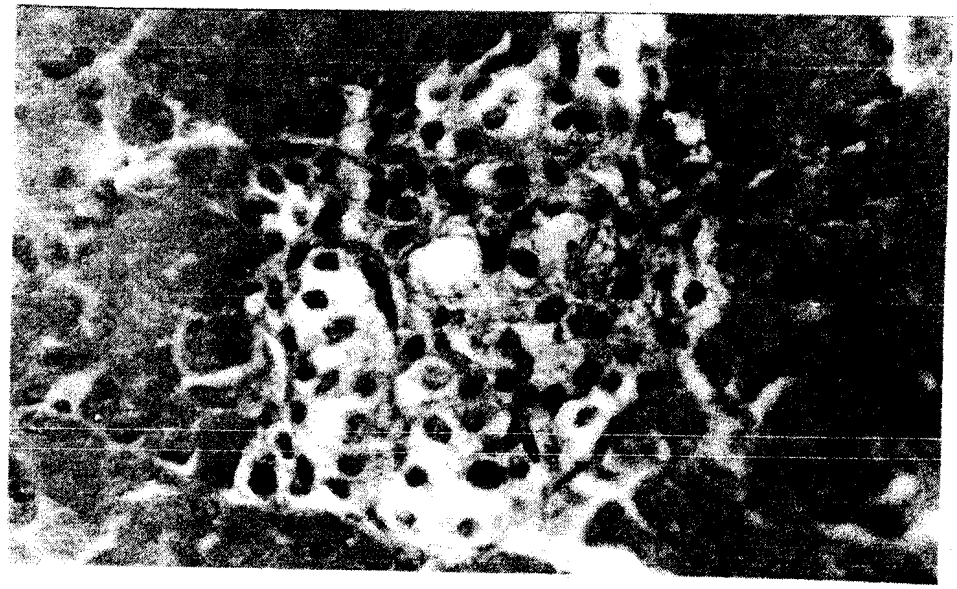


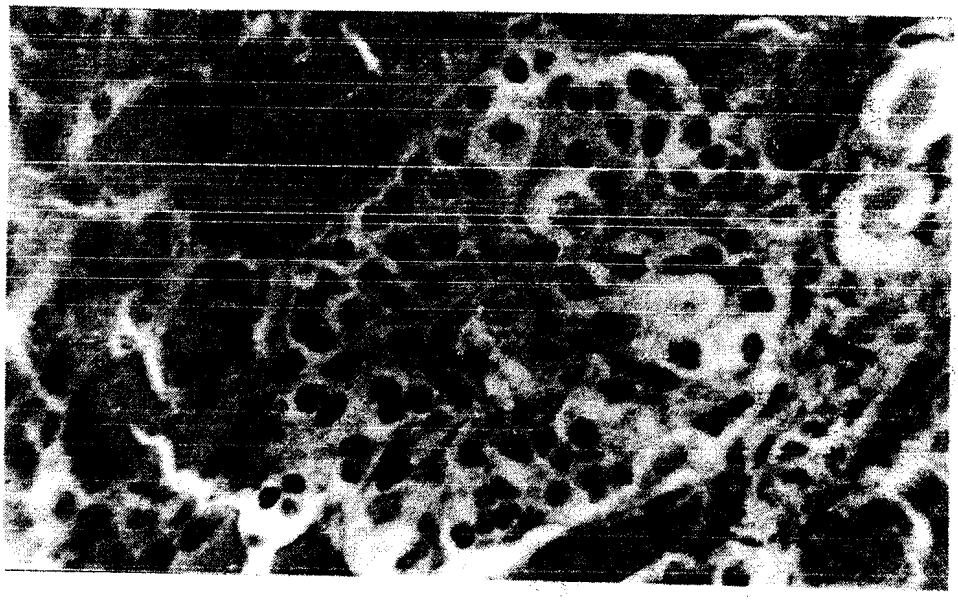


Fig. 1. Pancreas – 3 days after streptozotocin-induced diabetes.
Fig. 2. Pancreas – diabetic group treated for 3 days with the leaf extract, ob. 40.

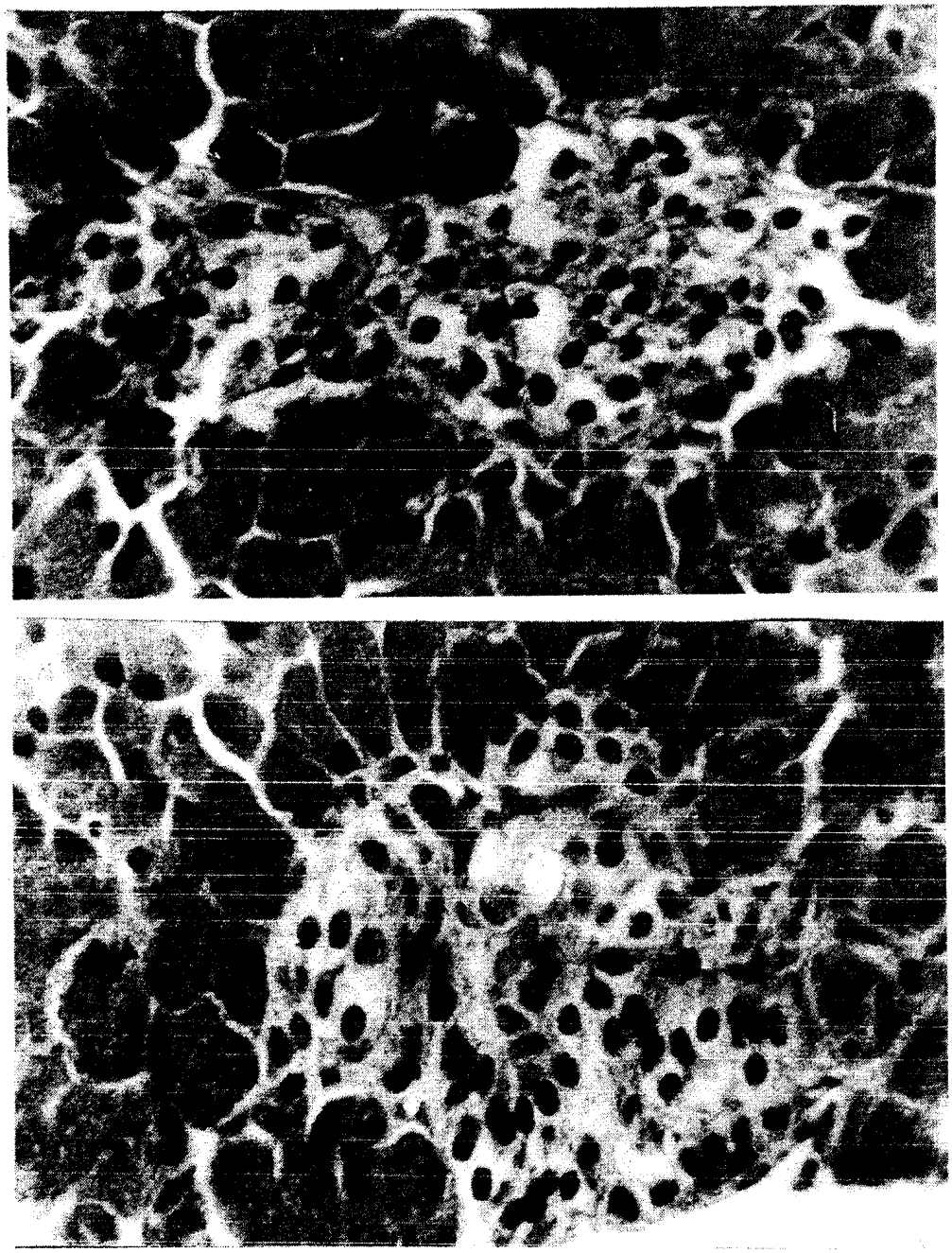
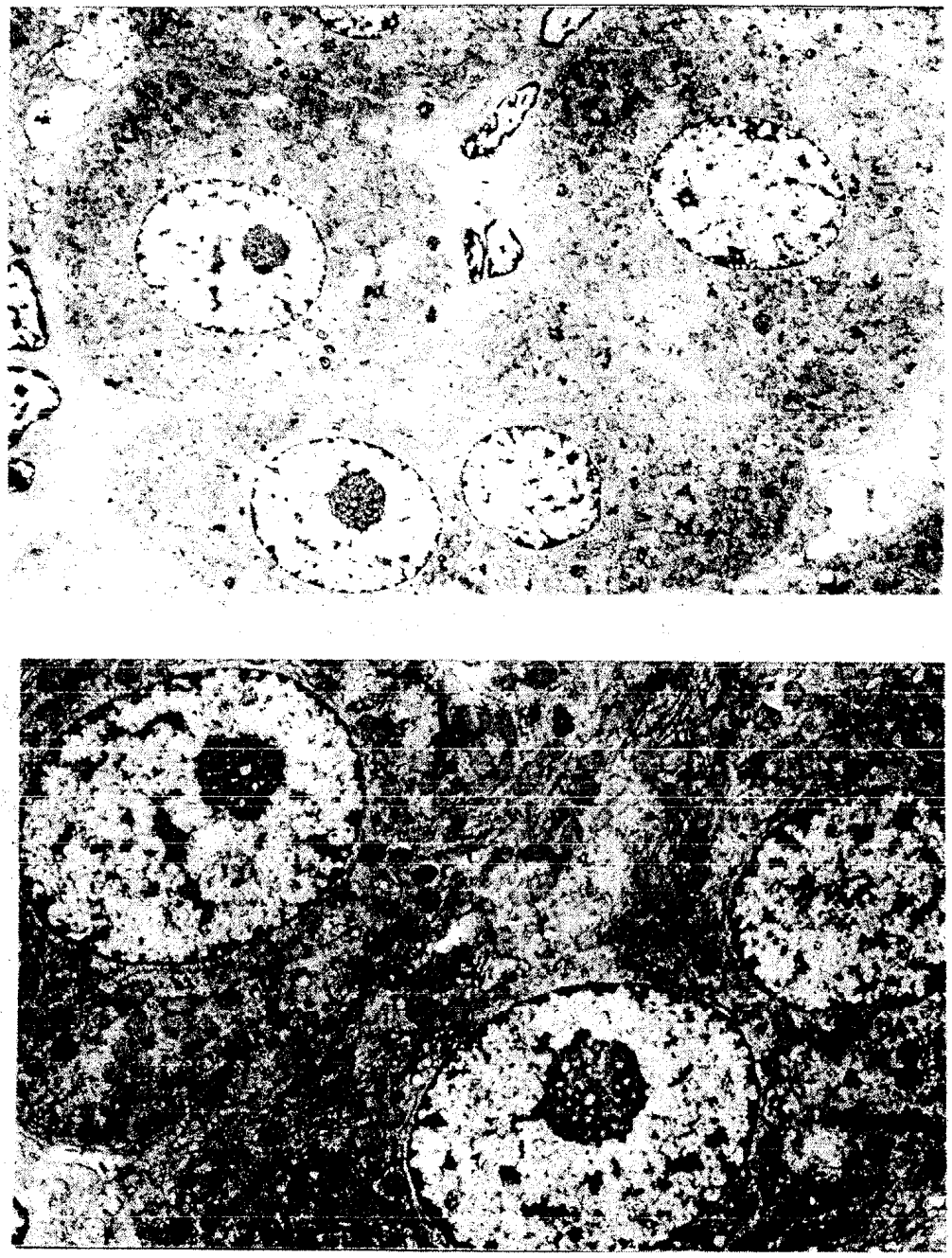
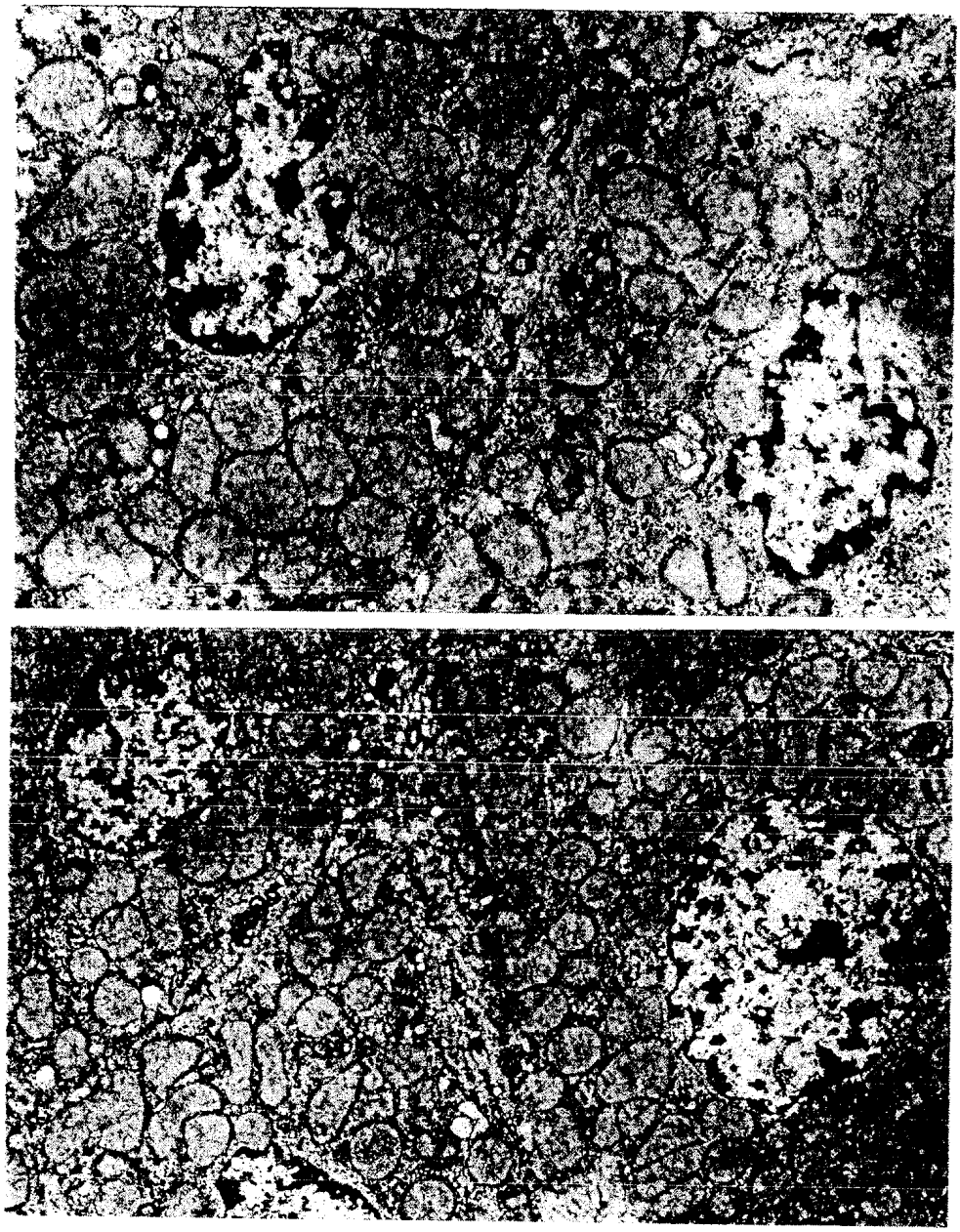


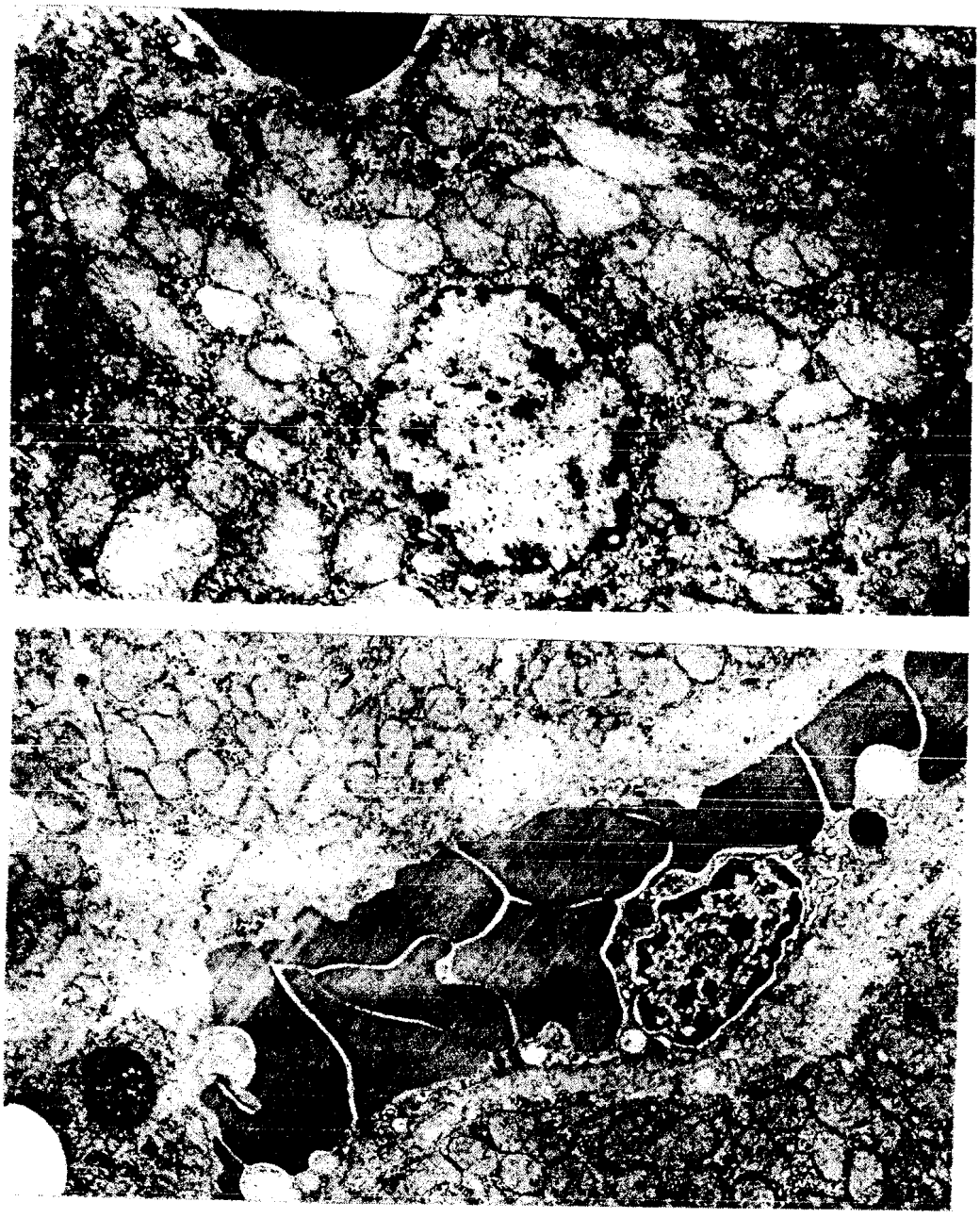
Fig. 3. Pancreas – 6 days after streptozotocin-induced diabetes. Fig. 4. Pancreas – diabetic group treated for 6 days with the leaf extract, ob. 40.



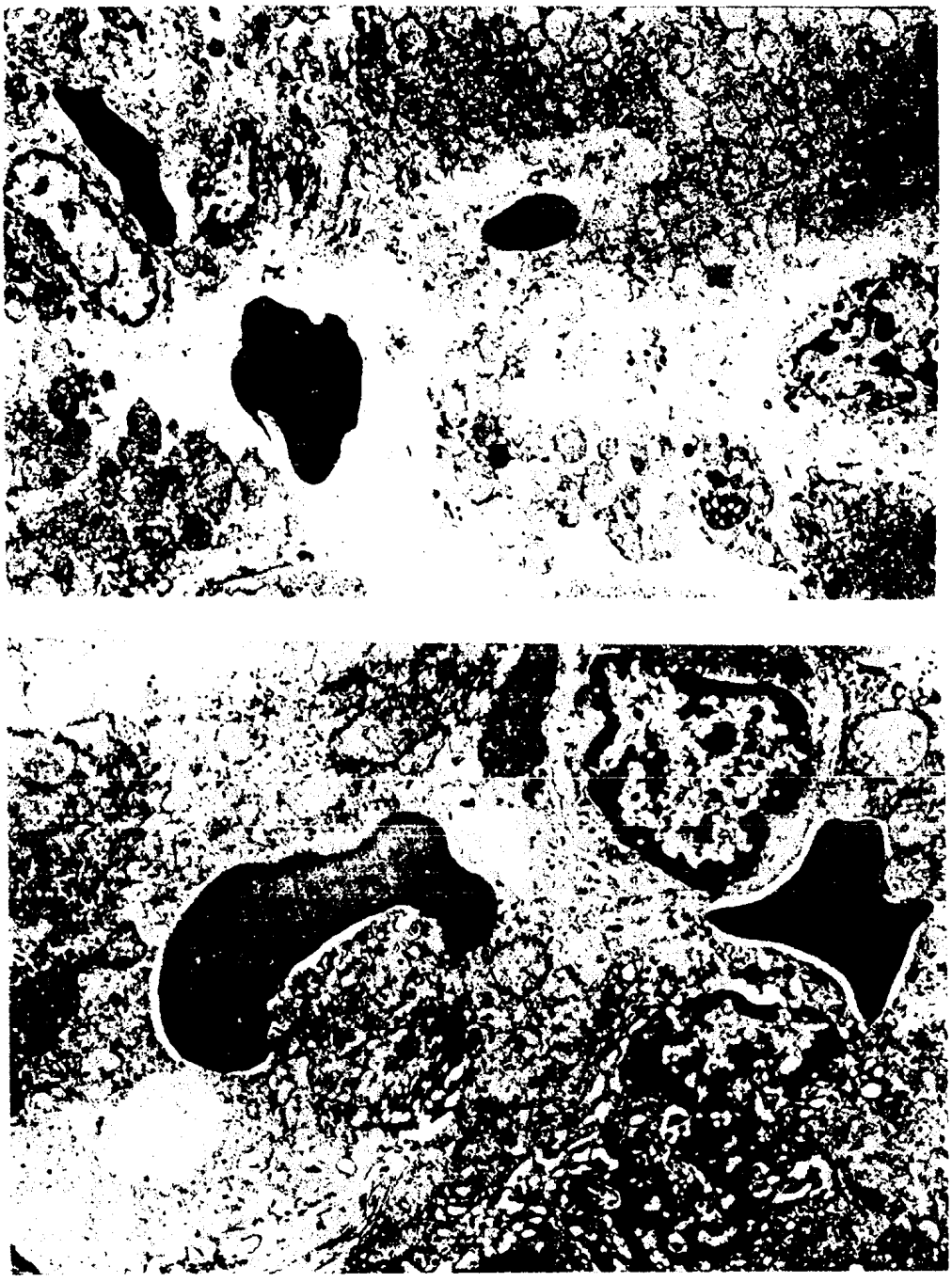
Figs. 5, 6. Liver – control group. Fig. 5, x 3990. Fig. 6, x 5890.



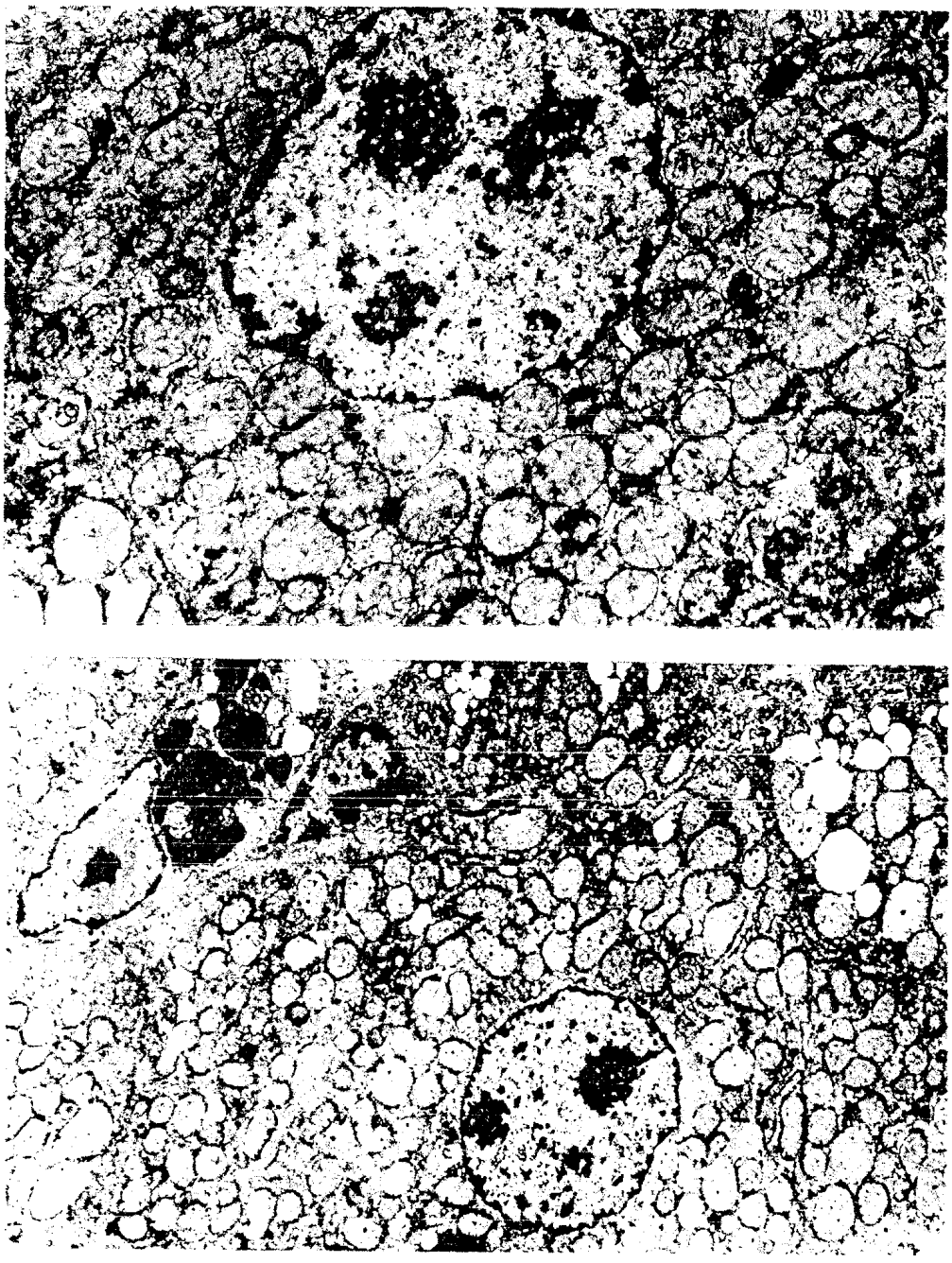
Figs. 7, 8. Liver–diabetic group. Fig. 7, x 7600. Fig. 8, x 5700.



Figs. 9, 10. Liver–diabetic group. Fig. 9, x 7600. Fig. 10, x 5700.



Figs. 11, 12. Liver- diabetic group. Fig. 11, x 4560. Fig. 12, x 7600.



Figs. 13, 14. Liver–diabetic group treated for 3 days with the leaf extract.
Fig. 13, x 7600. Fig. 14, x 4370.

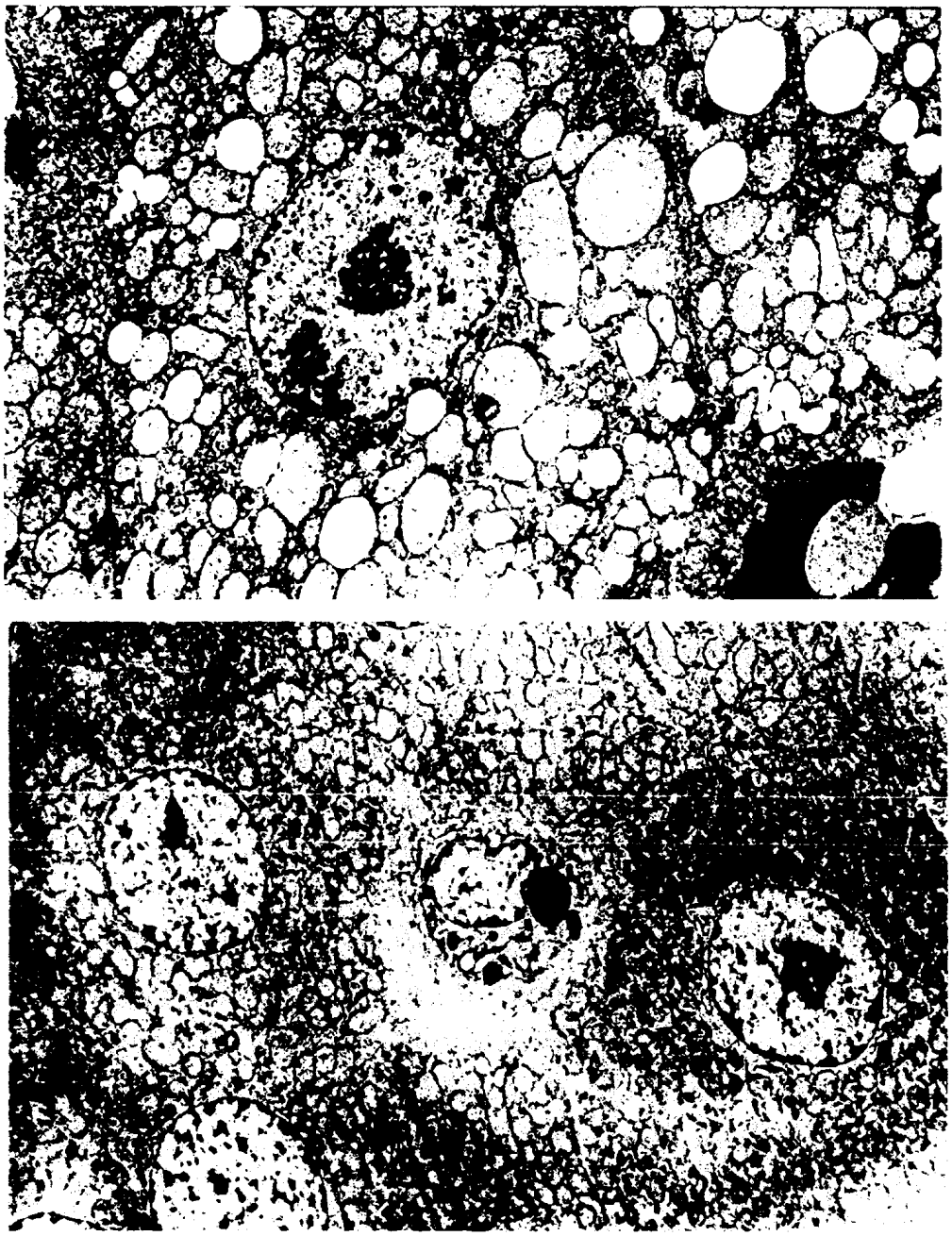
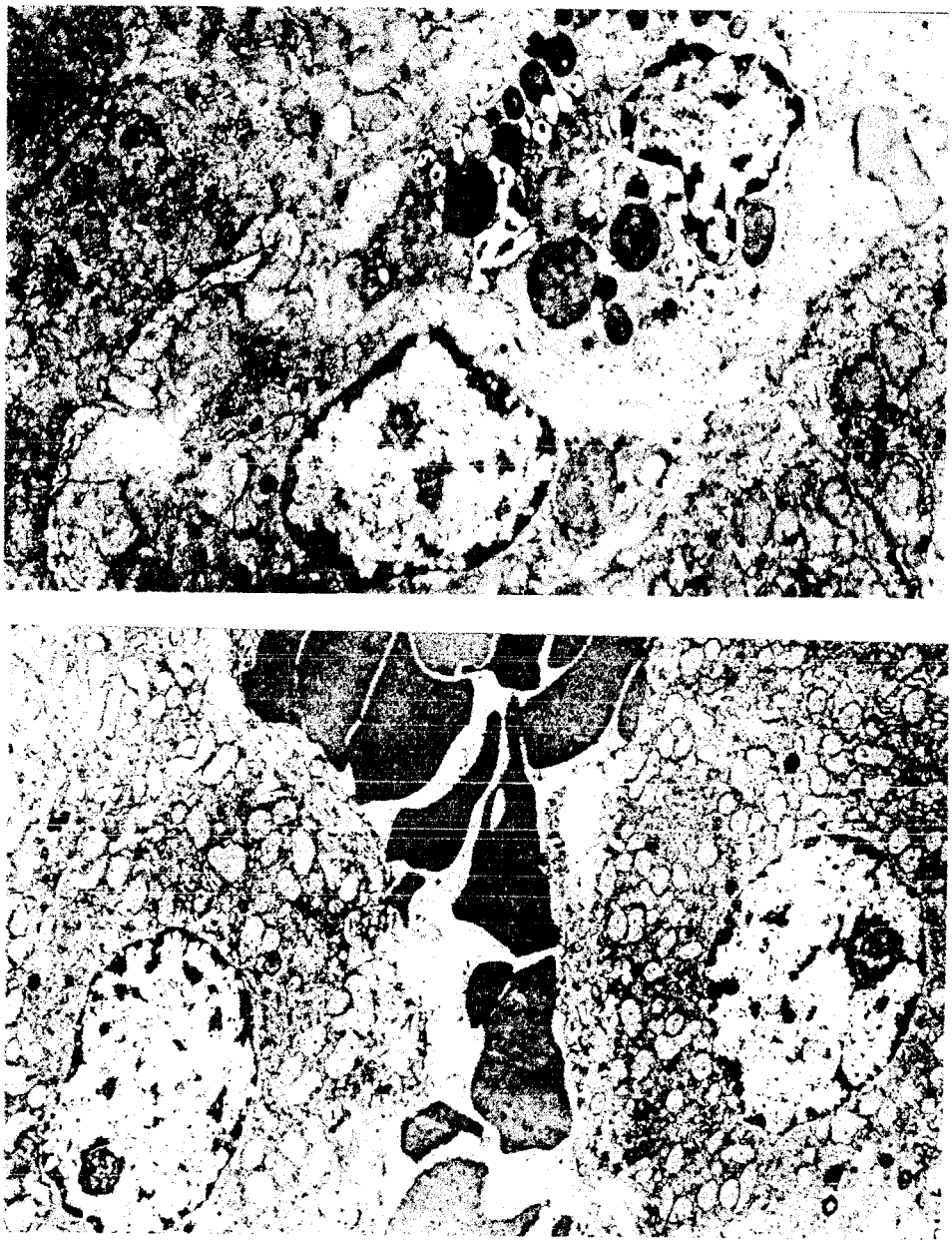


Fig. 15. Liver–diabetic group treated for 3 days with the leaf extract, x 4600.
Fig. 16. Liver–diabetic group treated for 6 days with the leaf extract, x 3800.



Figs. 17, 18. Liver–diabetic group treated for 6 days with the leaf extract.
Fig. 17, x 7030. Fig. 18, x 4750.