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Effect of Arabidopsis extracts on the Status of Liver Histology of Alloxan-induced Diabetic Mice

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Abstract:

Arabidopsis is a member of the Brassicaceae family, which includes important crops. It has no agronomic significance, but offers important advantages for basic research in genetics and molecular biology . Insulin-like growth factor 1 (IGF-1), known as "somatomedin C"," found in *Arabidopsis thaliana* seeds. Anti-diabetic medical plants are general known to exert their beneficial effects on diabetes via various modes and mechanism depending on the phytochemicals and bioactive agents endowed in such plants.

In this study the *Arabidopsis thaliana* ethanolic seed extract has hyperglycemic. In conclusion, the present results showed that Arabidopsis consumption reversed most of the histological changes in the diabetic mice. This effect was due to the hypoglycemic effect of the Arabidopsis and improving the insulin resistance. In addition, in diabetes there was an increase in the oxidative stress which was significantly reduced by Arabidopsis consumption owing to its antioxidant effect.
Key words: *Arabidopsis thaliana*, Brassicaceae family, ethanolic seed extract, Insulin-like, hyperglycemic.

1. Introduction:

Type II diabetes mellitus (also sometimes called adult-onset or non insulin-dependent diabetes) is increasing worldwide and it is the most common form of diabetes Wild *et.al* (2004). Diabetes mellitus is a syndrome characterized by a loss of glucose homeostasis from defects in insulin secretion and its action, both resulting in impaired metabolism of glucose and other energy yielding fuel such as lipids and proteins. The liver is insulin - dependent tissue that plays a vital role in glucose and lipid homeostasis and is severely affected in diabetes Sivajothi *et.al* (2007).

Antidiabetic medicinal plants are in general known to exert their beneficial effect(s) on diabetes via various modes and mechanisms depending on the phytochemicals and bioactive agents endowed in such plants. These mechanisms have been enumerated to include among others, modulation of carbohydrate and lipid metabolism in the liver, influence on beta cell integrity and insulin releasing activity, aldose reductase and antioxidant defense system manipulation, and glucose uptake and utilization Tiwari& Rao (2002). Others include interference with carbohydrate digestion / absorption, insulin-like action and inhibition of insulinase activity Jelodar *et.al* (2005).

Arabidopsis thaliana, a small, annual flowering, dicotyledonous plant, was discovered by Johannes Thal (hence, thaliana) in the Harz mountains in the sixteenth century. Arabidopsis is a member of the Brassicaceae family, which includes important crops. It has no agronomic significance, but offers important advantages for basic research in genetics and molecular biology. Insulin-like growth factor 1 (IGF-1), known as "somatomedin C"," found in *Arabidopsis thaliana* seeds, is a single polypeptide protein hormone consisting of 70 amino acids and having a molecular weight of 7,649 Da. It has three intermolecular disulfide bridges and the molecular structure is similar to insulin Sommerville& Koornneef (2002).

IGF-1 plays a major role in cell growth and differentiation; it is involved in various physiological processes in mammals with the regulation of somatic growth and cellular proliferation both in vivo and in vitro. Because of its structural similarity to insulin, IGF-1 has a high potential as a therapeutic agent for a variety of indications, including growth failure, type 1 or type 2 diabetes, amyotrophic lateral sclerosis, severe burn injury, and myotonic muscular dystrophy by International Union of Biochemistry and Molecular Biology (2011).

2. Materials and methods

2.1 Plant material:

Seeds of *Arabidopsis thaliana* were obtained from Dr. Enas Muhgin in Ebn-Albetar Center-Baghdad and authenticated by Biology Department-College of Science-Baghdad University.

2.2 Preparation of the extract:

The powdered material of seeds (50 gram) added to 250 ml of distilled water, left over night on stirrer, the extract then dried under reduced pressure and was subjected to various chemical tests to detect the presence of different active phytoconstituents like alkaloids, tannins, flavonoids, saponins, terpens and steroids Kokate (1994).

2.3 Detection of some active compounds:

This detection was carried out using chemical reagents to determine the presence of the active compounds only without quantification or determination of their types. Flavonoids were extracted by well established method of Harborne 1984, and the procedure has also been followed by several others authors. The extraction protocol which has been carried out in this investigation is only for detection of flavonoids in seed extract of *A. thaliana.*

2.4 Detection of Tannins:

(10 gram) of plant powder was mixed with 50 ml distilled water in a magnetic stirrer. The mixture was boiled in a boiling water bath for few minutes, then filtered and the filtrate was treated with few drops of 1% lead acetate solution. The development of greenish-blue precipitate is an indicator for the presence of tannins Evans (1989).

2.5 Detection of Saponins:

Saponins were detected by two methods: The first method, aqueous extract of *A. thaliana* seeds powder was shaken vigorously with distilled water in a test tube. The formation of foam standing for a time indicates a positive result. The second method, five milliliters of aqueous extract of the plant was added to 1-3 drops of 3% ferric chloride solution, a white precipitate was developed which indicates a positive result Alsereita& Abu-Amer (1996).

2.6 Detection of terpenes and steroids:

(1 ml) of ethanolic extract was participated in a few drops of chloroform, then a drop of acetate anhydride and drop of concentrated sulfuric acid were added, brown precipitate appeared which representing the presence of terpene, and the appearance of dark blue color after few minutes would represent the present of steroids Harborne (1984).

2.7 Detection of flavonoids:

Ethanolic extract was partitioned with petroleum ether (1:1v/v), the aqueous layer was mixed with the aluminum solution. The appearance of dark color is an evidence for the presence of flavonoids. Flavonoids react with the reagent and give colour reactions. Spraying reagents 5% fehling solution and 1% AlCl3 solution are exclusively used to detect flavonoids Harborne (1984).

2.8 Detection of Alkaloids:

(10 gram) of the extract was boiled with 50 ml of distilled water and 4% of hydrochloric acid was added, then the solution was filtered and cooled. 0.5 ml of the supernatant was tested with Mayer solution , appearance of white precipitate indicates the presence of alkaloids Harborne (1984).

2.9 Experimental animals and Diabetes induction:

Healthy 15 adult male albino mice of Swiss albino strain were obtained from the animal house of the College of Medicine, Baghdad University. The mice age was 8 weeks, and its weight was 25 gram. The animals were housed in clean cages, sterilized weekly with 70% ethanol. The mice were kept in with natural 14 hours light, 10 hours dark, at a controlled temperature (24-28) C^{o}. The animals were fasted for 24 hours, then diabetes was induced by a single intraperitoneal injection of alloxan monohydrated dissolved in distilled water at a dose of 150 mg/kg of mice body weight in volume of 0.1 ml. The diabetic state was confirmed 72 hours after alloxan injection. Blood glucose value was reached 320 mg/dl which indicate hyperglycemia (120-140 mg/dl) as standard before treatment, and there was 4% mortality in animals treated with alloxan.

2.9 Histological investigation:

At the end of the experiment animals were sacrificed under ether anesthesia and the liver tissues were obtained.For light microscopic study, the liver tissue was fixed in 10 % formaline and embedded in paraffin. Five um sections were cut and stained with Hematoxylin and Eosin for general histological study, Periodic Acid Schiff and Diastase techniques for the demonstration of glycogen in the liver sections Weili *et.al* (2011) and methylene blue & eosin for demonstration of endoplasmic reticulum Kiernan (2008).

3. Results:

3.1 Chemical tests for Detection of some active compounds:

Table -1 show the chemical test of the active compounds in A*rabidopsis thaliana* seed extract.

Table-1 the chemical test of the active compounds in A*rabidopsis thaliana* seed extract.

No.	Compound	Result
1	Tannins	+
2	Flavonoids	+
3	Alkaloids	+
4	Saponins	+
5	Terpenes and Steroids	+
6	Glycoside	-

* (+) means positive detection , (-) means negative.

3.2 Hypoglycemic effect:

Table-2 showed that the daily treatment with *Arabidopsis thaliana* ethanolic extract of 200 mg/kg b.w. led to a significant reduction in the blood glucose levels after 3, 6, 9 days of the treatment which recorded 192, 154, 132 mg/dl respectively.

Table (2): Effect of *Arabidopsis thaliana* seed ethanolic extract on reducing blood glucose levels of white albino mice after different periods of time

Group/treat	Dose	0 days	3 days	6 days	9 days	12 days	
Normal mice	0.1 ml distilled H_2O	122	121	119	123	119	p-value= 0.046
Induce diabetic mice (Alloxan)	0.1 ml (150 mg/kg)	314	316	312	309	320	
Diabetic mice (Arabidopsis ethanolic extract)	0.1 ml (200 mg/kg)	278	192	154	132	121	

The effect seems to reach maximum on 12th day of the treatment period (121 mg/dl) with ethanolic extract and then became stable as control treatment. Significant reduction in blood glucose level was observed as compared to the normal group (119 mg/dl) and diabetic group (320 mg/dl) after 12 days with gradually reduction till reached 121 mg/dl at the end of the experimental period (12 days).

3.3 Histological effect:

Examination of H&E stained sections of the control group showed the normal architecture of the classic hepatic lobules. The hepatocytes form branching and anastomosing cords radiating from the central vein. They showed vesicular nuclei and some of them appeared binucleated. The cells appeared to be separated by the blood sinusoids that were seen to be lined by flat endothelial cells (Fig. 1).
H&E stained sections of the untreated diabetic group showed degenerative changes in the hepatocytes. Cells all over the hepatic lobules were observed to have many vacuoles giving them foamy appearance and some of them showed pyknotic nuclei (fig. 2). In addition, liver sections of this group revealed sinusoidal dilations and hyperemia in sinusoids and central veins (Figs. 3).
The Arabidopsis- treated diabetic group showed normal hepatic architecture which was almost similar to that of the control group(Fig. 4).
PAS-stained sections of the control group showed PAS positive granules in most of the hepatocytes (Fig. 5). While those of untreated diabetic group showed few PAS positive granules as compared to that of the control group (Fig. 6). TheArabidopsis- treated diabetic group showed that most of the hepatocytes were studded with PAS positive granules (Fig. 7).
Liver sections of control group stained with methylene blue & eosin showed that hepatocytes contained bluish granules in their cytoplasm(Fig. 8) while those of untreated diabetic group showed fewer bluish granules in their cytoplasm(Fig.9). The Arabidopsis- treated diabetic group showed that hepatocytes were studded with bluish granules(Fig.10).

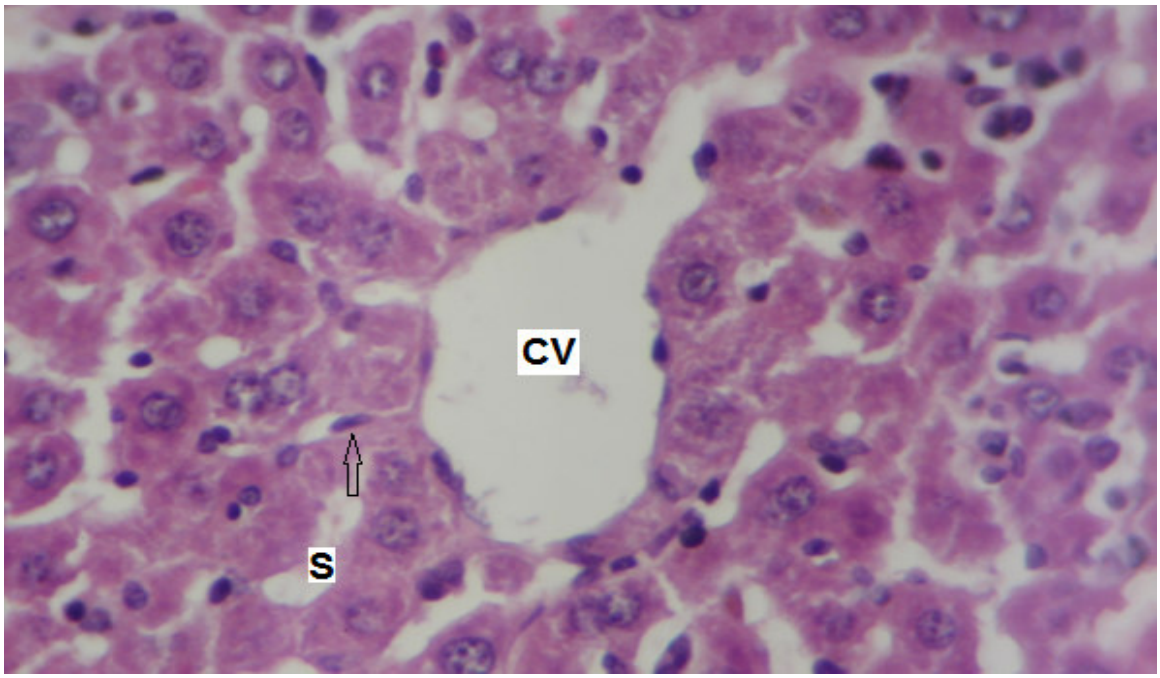

Fig. I: Shows branching and anastomosing cords of hepatocytesradiating from the central vein (C.V.). The hepatocytes have vesicularnuclei and some of them appear binucleated. The cells are separated bythe blood sinusoids (S.) lined by Bat endothelial cells (↑) (H&E X 400) .

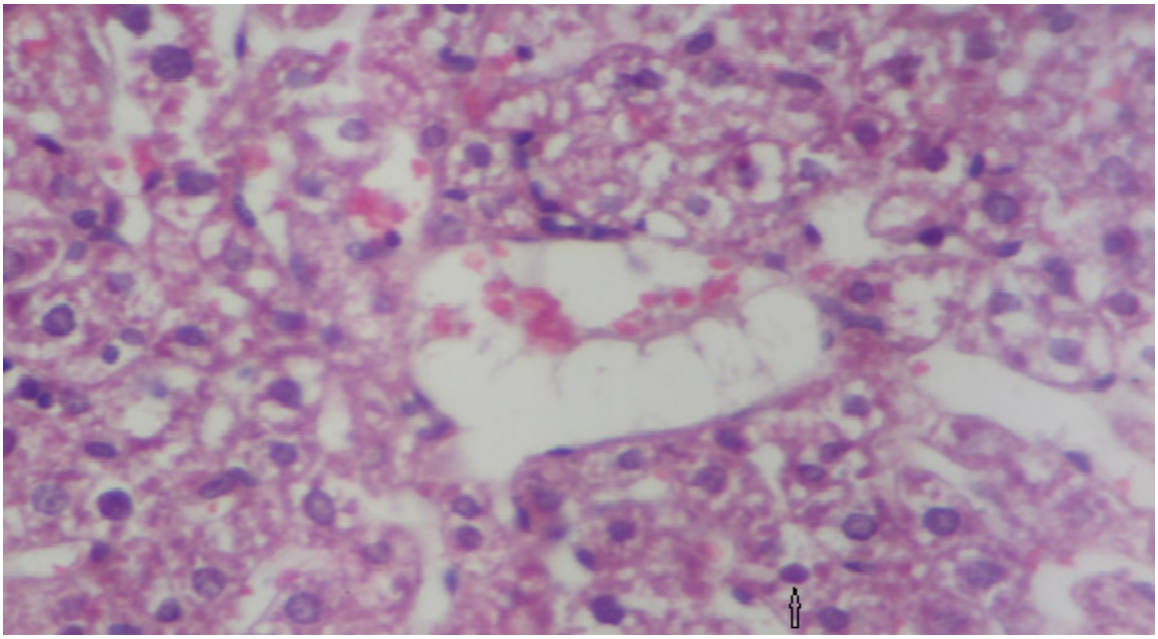

Fig.2: Liver of diabetic group Shows that some hepatocytes have vacuoles giving them foamy appearance. Most of cells have vesicular nuclei and some havepvknotic nuclei (↑) (H&E X400) .

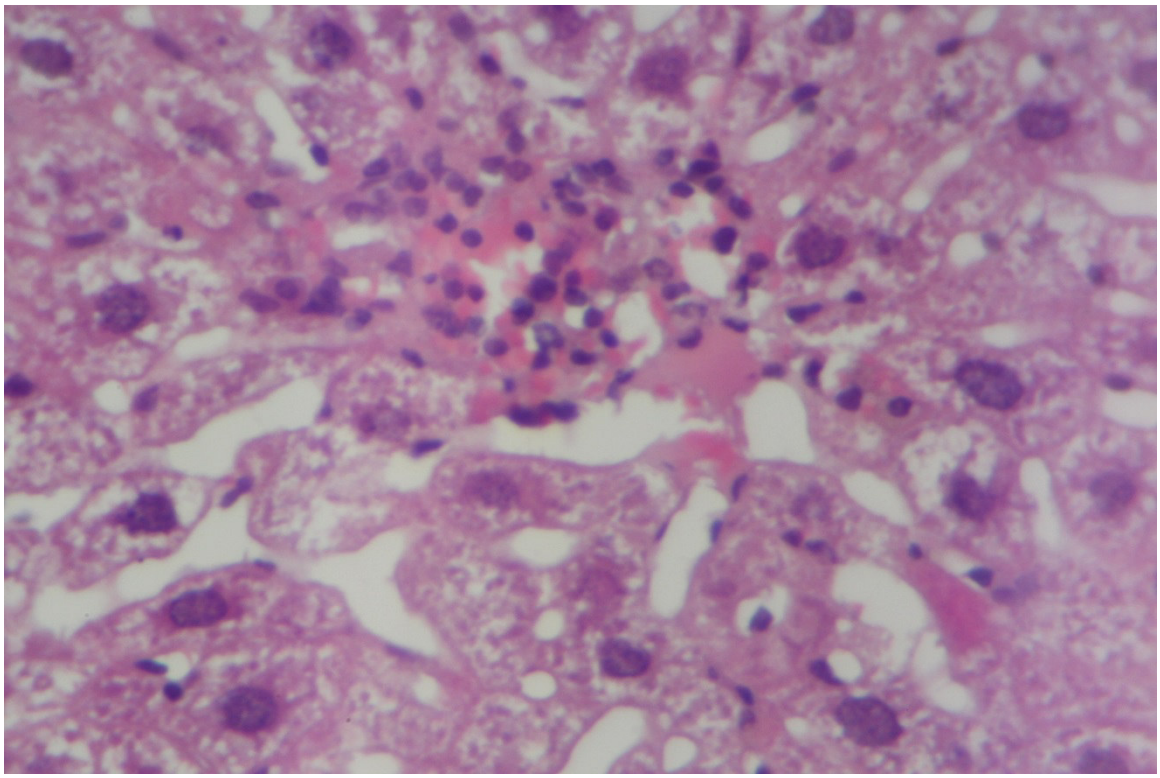

Fig.3: Liver of diabetic group showing foamy appearance hepatocytes with leukocytic infiltration(H&E X 400)

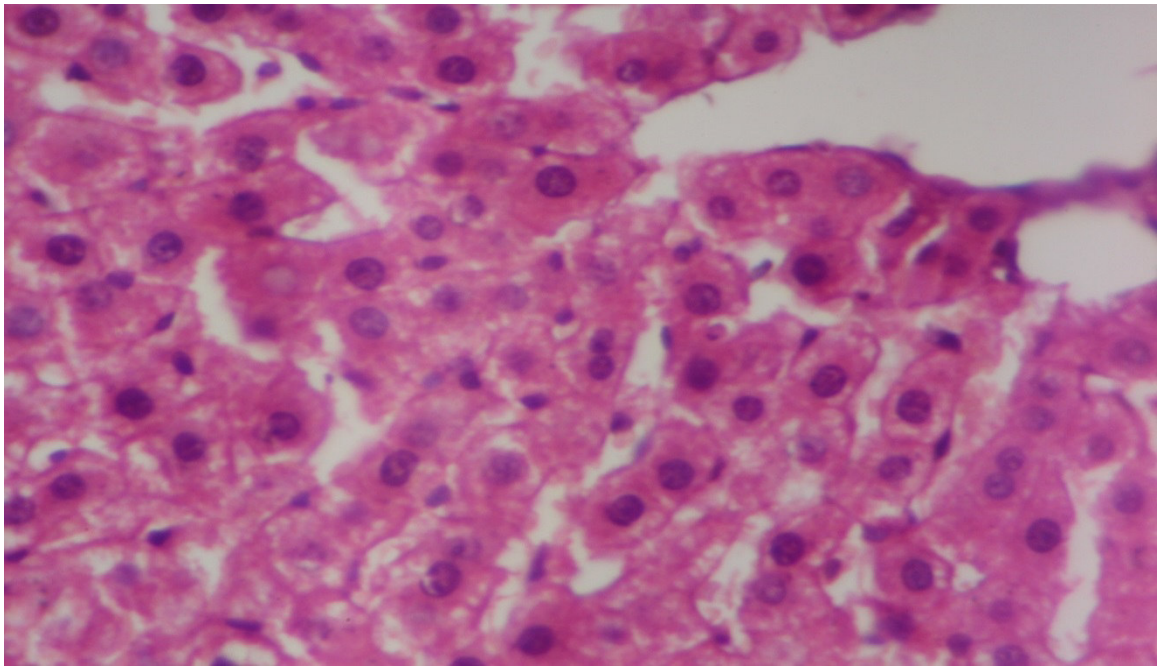

Fig.4: Liver of Arabidopsis- treated diabetic group showed normal hepatic architecture which was almost similar to that of the control group(H&E X 400)

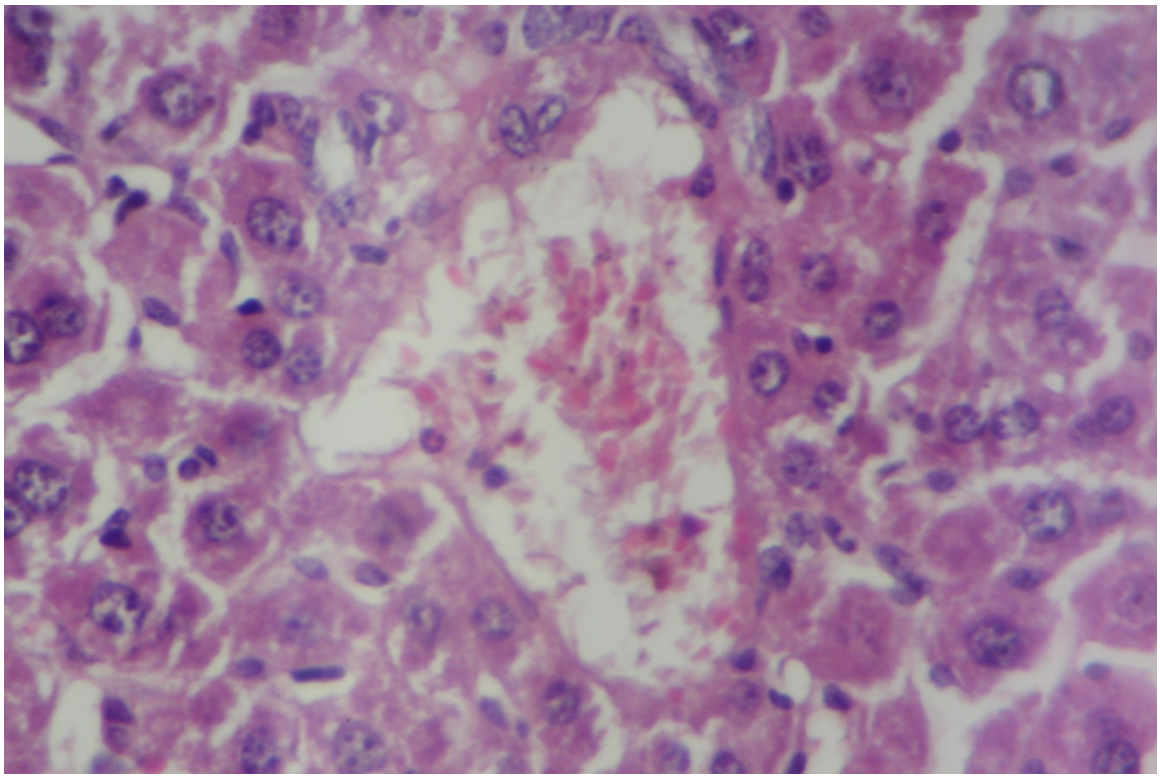

Fig.5: Liver of control rat showing *that most of the* hepatocytes are studded with PAS positive granules(PAS X 400).

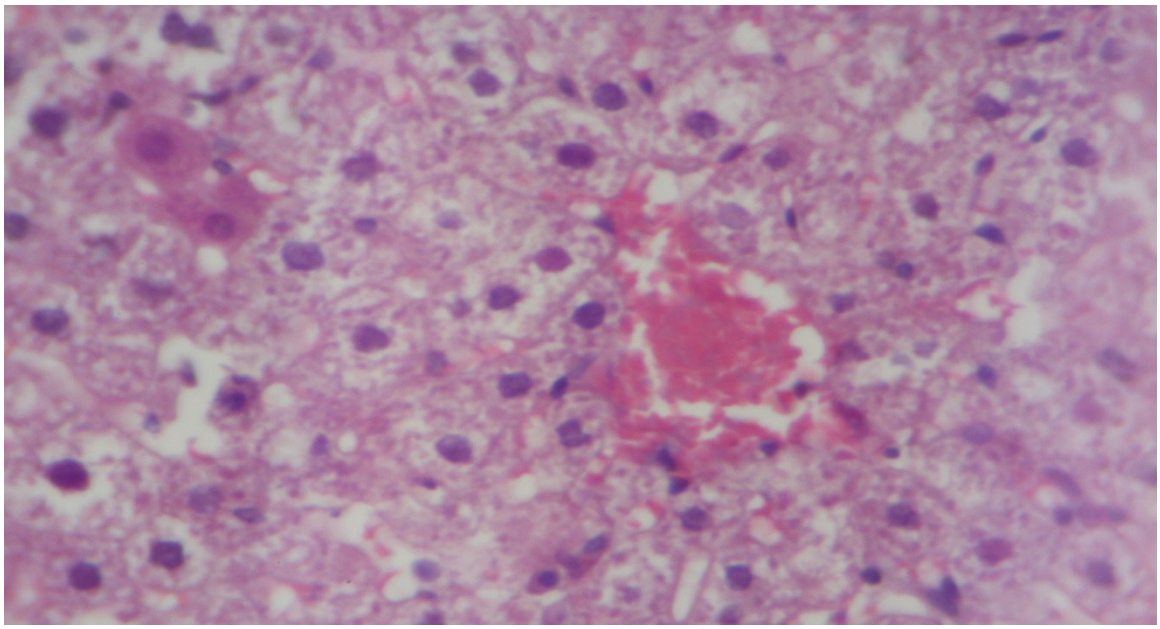

Fig.6: Liver of diabetic group showing that hepatocytes contained few PAS positive granules as compared to that of the control group(PAS X 400)

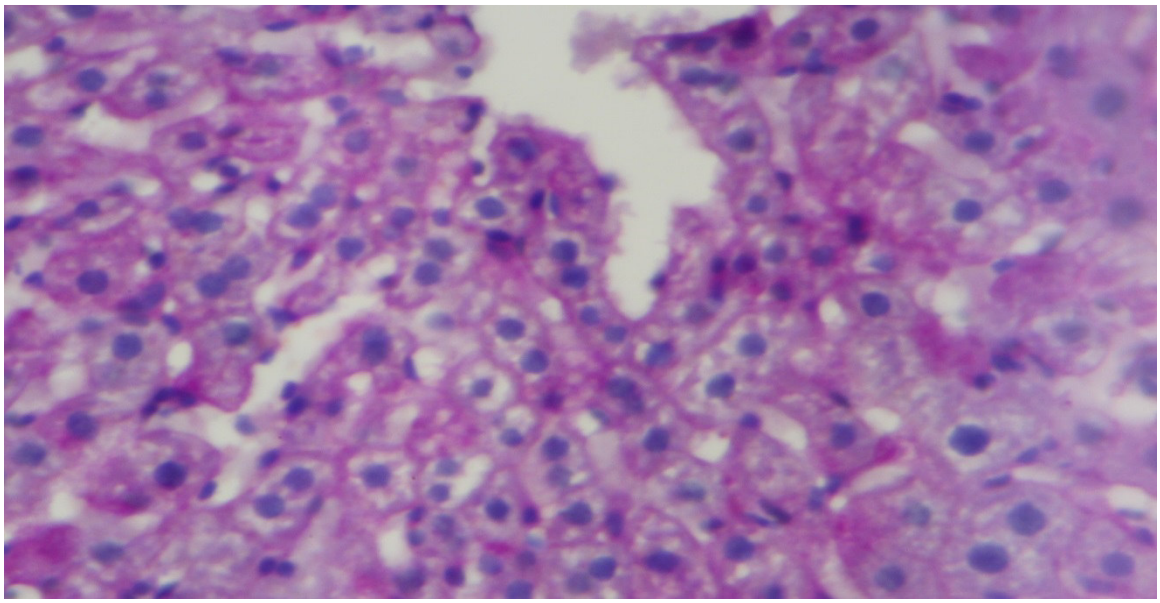

Fig.7: Liver of Arabidopsis- treated diabetic group showed that most of the hepatocytes were studded with PAS positive granules(PAS X 400)

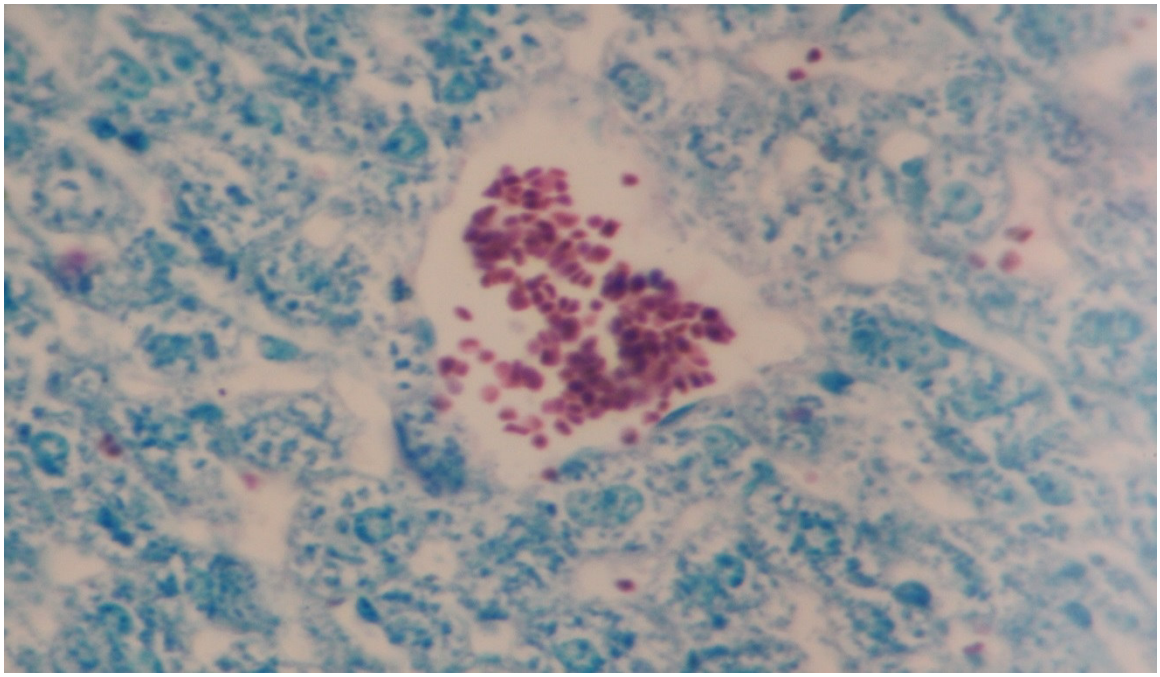

Fig. 8: Liver of control group showed that the cytoplasm of hepatocytes contained positive bluish granules(Methylene blue & Eosin X 400)

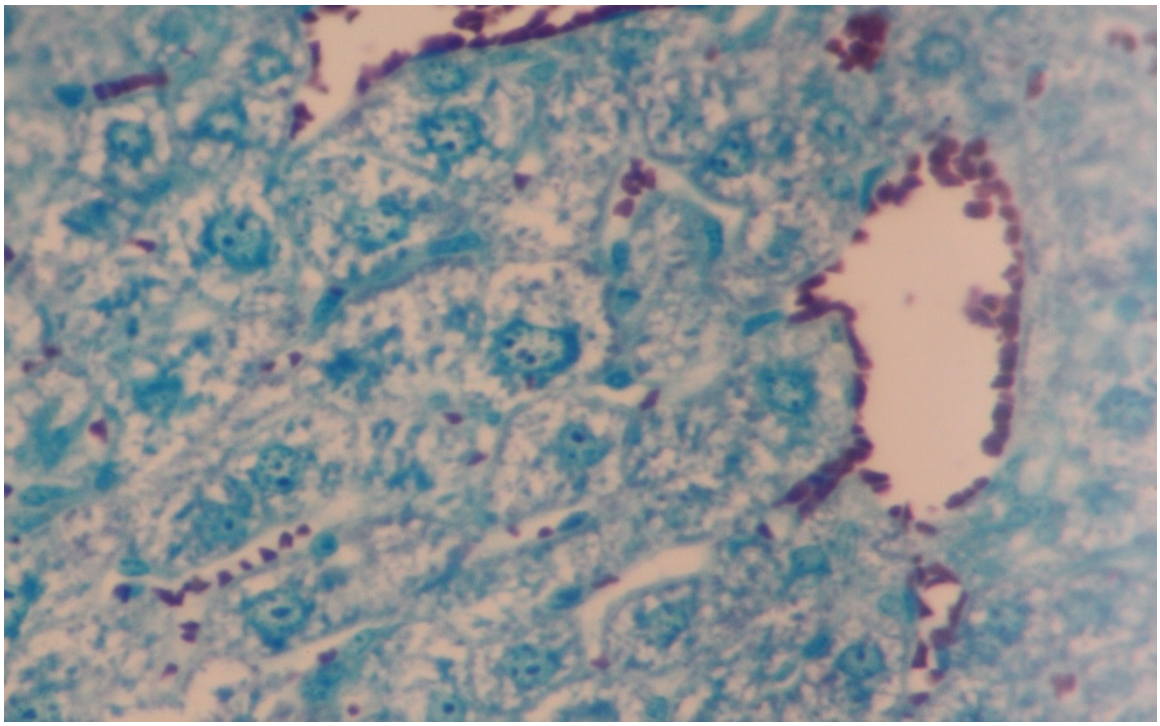

Fig.9 : Liver of diabetic group showing that hepatocytes contained fewer bluish granules in their cytoplasm than those in control group (Methylene blue & Eosin X 400)

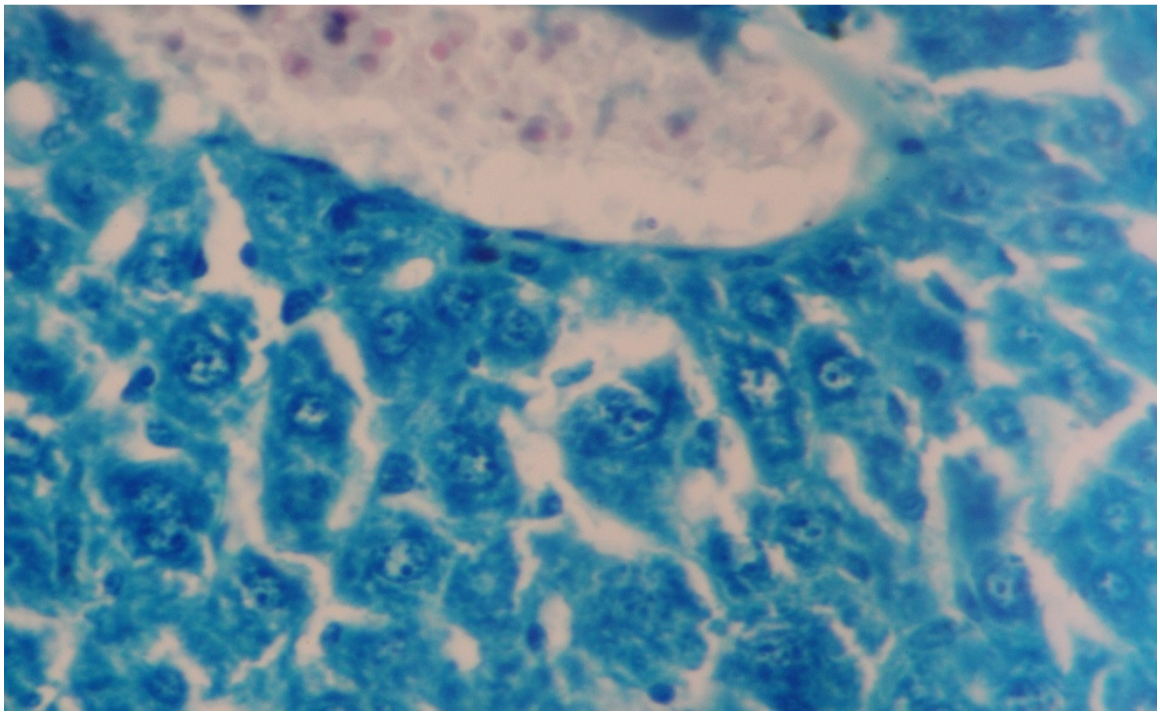

Fig. 10: Liver of Arabidopsis- treated diabetic groupshowing that the hepatocytes were studded with bluish granules(Methylene blue & Eosin X 400)

4. Discussion:

Results showed that *A. thaliana* ethanolic seed extract has hyperglycemic activity as compared to the control treatment. The effect of the insulin-like protein and the active compounds such as flavonoids that found in *A. thaliana* caused a significant decrease in blood glucose levels in diabetic mice similar to that reported on the hypoglycemic activity of *A. thaliana* by Weili *et al*., who reported that an important therapeutic protein human insulin-like growth factor 1(hIGF-1) called somatomedin C, was expressed in *A. thaliana* seeds via oleosin fusion technology Liui (1989).

We observed accumulation of lipid droplets in the cytoplasm of hepatocytes of diabetic group. This change was reminiscent to the formation of fatty liver. It could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis Ohno *et al* (2000) Hyperlipidemia could be another factor for fatty liver formation. Our findings of fatty liverformation are in agreement with the findings of Ohno *et al* (2000) and Merzouk *et al* (2000).

The present study showed few PAS positive granules in the untreated diabetic group indicating a decrease in the glycogen content in the liver . These results were similar to a previous study which showed a marked decrease of glycogen granules in the diabetic mice Welt *et.al* (2004) In diabetes as the activities of glycogen synthase and hexokinase were diminished as a result of insulin deficiency, glucose cannot be transformed into glycogen and glycogenesis was reduced and thus the amount of glucose increased Chaudry *et.al* (1993).

Insulin is considered as an anabolic hormone with a wide range of effects on metabolisms including stimulation of protein synthesis Jefferson *et.al* (1977) and Wool & Cavicchi (1966) and inhibition of protein degradation Ballard *et.al* (1980).It was demonstrated by GehanKhalafand Abdel-Gabbar Mohamed that hepatocytes of diabetic rat exhibited ill defined rough endoplasmic reticulum with dilatationof their cisternae and swollen mitochondria with loss of its cristae Gehan and Abdel-Gabbar (2008). Therefore, in our study, hepatocytes of diabetic group demonstrated fewer bluish granules than in normal group which indicated that ribosomes were decreased in hepatocytes of this group and consequently, protein synthesis was diminished. In Arabidopsis-treated diabetic group, hepatocytes were studded with bluish granules which indicated that Arabidopsis stimulated protein synthesis by increasing the number of ribosomes.

In conclusion, the present results showed that Arabidopsisconsumption reversed most of the histological changes in the liver of the diabetic rats.This effect was due to the hypoglycemic effect of the Arabidopsisand improving the insulin resistance. In addition, in diabetes there was an increase in the oxidative stress which was significantly reduced by Arabidopsis consumption owing to its anti oxidant effect. So, we can say that Arabidopsis had a significant hepatoprotective role in diabetic rats and offers promising perspectives deserve further investigation.

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