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PREVENTIVE EFFECTS OF *Arabidopsis thaliana* EXTRACT ON LIVER DAMAGE OF DIABETIC MICE INDUCED BY ALLOXAN

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Abstract- This study was carried out in March/2013 in the College of Medicine - Baghdad University to determine the effects of *Arabidopsis thaliana* seed extract on blood glucose levels and liver damage of induced diabetic mice by alloxan. Results showed that the treatment with 200 mg/kg of body weight led to significant reduction in blood glucose levels after 3 to 12 days of treatment. This reduction revealed that the seed extract of *Arabidopsis thaliana* have hyperglycemic activity as compared to the control (untreated) animals. On the other hand, the Arabidopsis-treated diabetic group of mice showed normal hepatic architecture which was similar to that of untreated group (control) and they have few PAS positive granules, and most of the hepatocytes were studded with PAS positive granules. The Arabidopsis-treated diabetic group showed that hepatocytes were studded with bluish granules, while those of untreated diabetic group showed fewer bluish granules in the cytoplasm. It was observed the accumulation of lipoid droplets in the cytoplasm of hepatocytes of diabetic group. This change was reminiscent to the formation of fatty liver due to the increased of fatty acids into the liver induced by hypoinsulinemia. Also it was observed a reduction in glycogen in the liver cells of diabetic group as compared to the Arabidopsis-treated group which indicates that Arabidopsis extract stimulated protein synthesis by increasing the number of ribosomes, and improving the insulin resistance. Finally, Arabidopsis consumption have hypoprotective role in diabetic mice and offers promising perspective deserve further investigation.

Keywords- *Arabidopsis thaliana*, alloxan, diabetic mice

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Introduction

Type II diabetes mellitus (sometimes called adult-onset or non insulin-dependent diabetes) is increasing worldwide and it is the most common form of diabetes [1]. 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 [2].

Antidiabetic medicinal plants are in general known to exert their beneficial effects 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 [3]. Others include interference with carbohydrate digestion/absorption, insulin-like action and inhibition of insulinase activity [4].

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 [5]. It has no agronomic significance, but offers important advantages for basic research in genetics and molecular biology [6]. 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 [7].

Materials and Methods

This study was carried out in March 2013 in the college of medicine laboratories- Baghdad University and the Center of Biotechnology-Al-Nahrain University in Baghdad.

Plant Material

Seeds of *Arabidopsis thaliana* were obtained from the laboratories of Ministry of Science and authenticated by Biotechnology center-Al Nahrain University.

Extraction Method

The seeds of *Arabidopsis thaliana* plant were reduced to powder using grinding machine. In order to prepare the extracts, 25 g of the powder was separately extracted with 125 ml of distilled water, stirring for 24 hours, and then the solution was evaporated in vacuum pressure at 40°C [8].

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 age of mice was 8 weeks, and the 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. The animals were fed chow and water. The protocol was proved by Institutional Animal Ethical Committee [9]. 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 5% mortality in animals treated with alloxan. Surviving mice with fasting blood glucose level 250 mg/dl or higher were included in this study [10].

Experimental Groups and Blood Samples Collection

The animals were divided into three groups (five mice per each group), and the groups were treated as following: First group, control, normal mice administrated with 0.1 ml distilled water. Second group, diabetic mice administrated with 0.1 ml of alloxan. Third group, diabetic mice administrated with 0.1 ml of Arabidopsis seed extract (200 mg/kg b.w). For 12 days after alloxan injection, blood samples were collected every three days (0, 3, 6, 9, 12) days from the tail vein of the mice and glucose level was assayed using glucometer apparatus.

Microscopic 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% formalin and embedded in paraffin. Five um sections were cut and stained with Hematoxylin and Eosin (H and E) for general histological study, Periodic Acid Schiff and Diastase techniques for the demonstration of glycogen in the liver sections and methylene blue and eosin for demonstration of endoplasmic reticulum [11,12].

Results

Antiglycemic Activity

[Table-1] 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. 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.

Table 1- 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
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

Dependent Variable: Data

Treatment	Mean	Std. Error	95% Confidence Interval L. B.	95% Confidence Interval U. B.
Normal	120.8	16.365	83.062	158.538
Induced (Alloxan)	314.2	16.365	276.462	351.938
Ethanolic ext.	175.4	16.365	137.662	213.138

Pairwise Comparisons

Dependent Variable: Data

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Sig.	C.S.
Normal	Induced (Alloxan)	-193.4	0	HS
	Ethanolic ext.	-54.6	0.046	S
Induced (Alloxan)	Ethanolic ext.	138.8	0	HS

Based on estimated marginal means

**The mean difference is significant at the .05 level.*

Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Biological Effects

Examination of Hematoxylin and Eosin (H and 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 and 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 [Fig-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]. The Arabidopsis-treated diabetic group showed that most of the hepatocytes were studded with PAS positive granules [Fig-7].

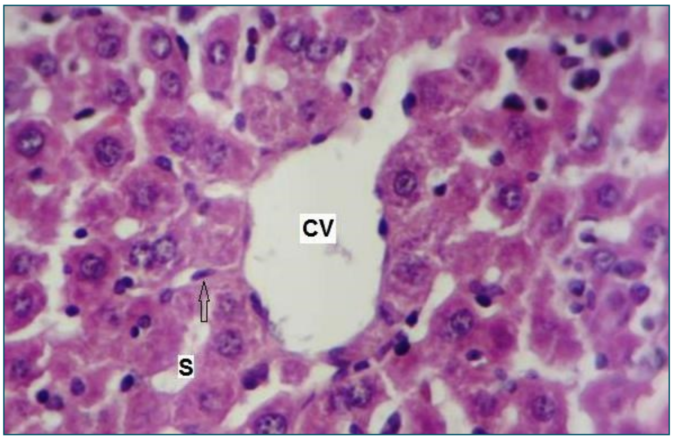

Fig. 1- Shows branching and anastomosing cords of hepatocytes radiating from the central vein (C.V.). The hepatocytes have vesicular nuclei and some of them appear binucleated. The cells are separated by the blood sinusoids (S.) lined by Bat endothelial cells (↑) (H&E X 400)

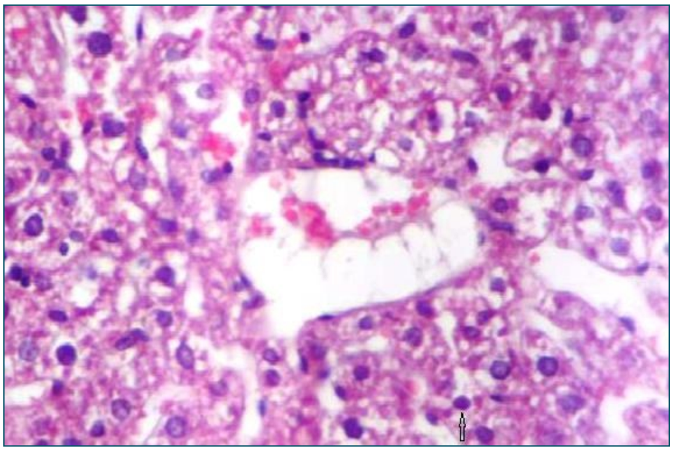

Fig. 2- Liver of diabetic group Shows that some hepatocytes have vacuoles giving them foamy appearance. Most of cells have vesicular nuclei and some have pvknotic nuclei (↑) (H&E X400).

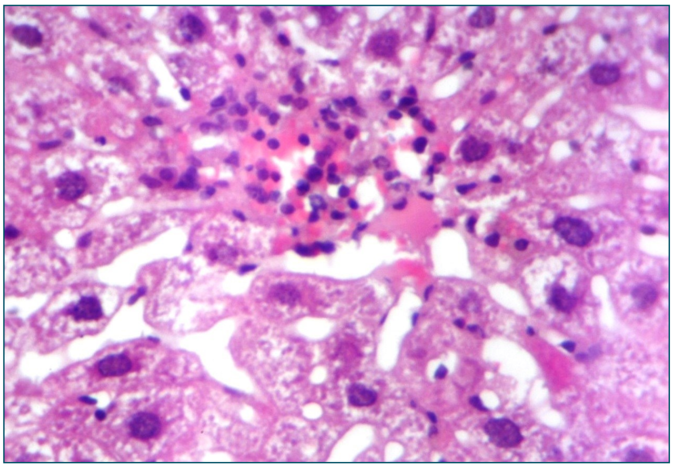

Fig. 3- Liver of diabetic group showing foamy appearance hepatocytes with leukocytic infiltration (H&E X 400)

Liver sections of control group stained with methylene blue and 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].

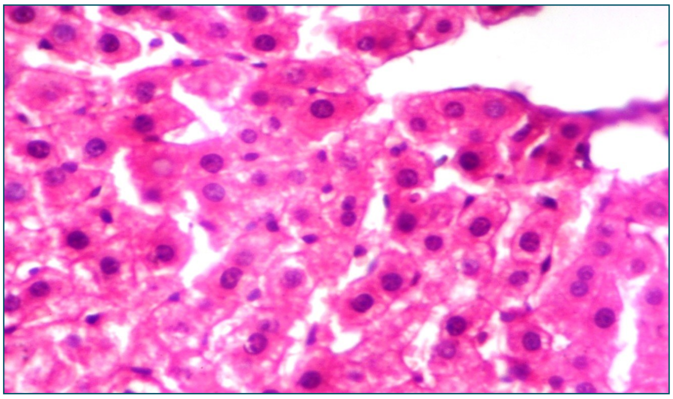

Fig. 4- Liver of Arabidopsis- treated diabetic group showed normal hepatic architecture which was almost similar to that of the control group (H&E X 400)

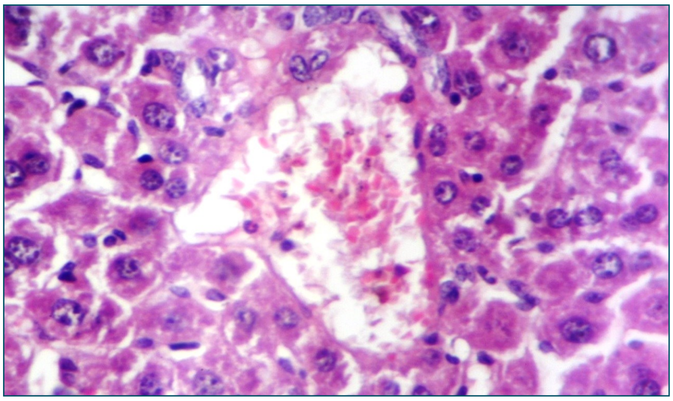

Fig. 5- Liver of control rat showing *that most of the* hepatocytes are studded with PAS positive granules (PAS X 400)

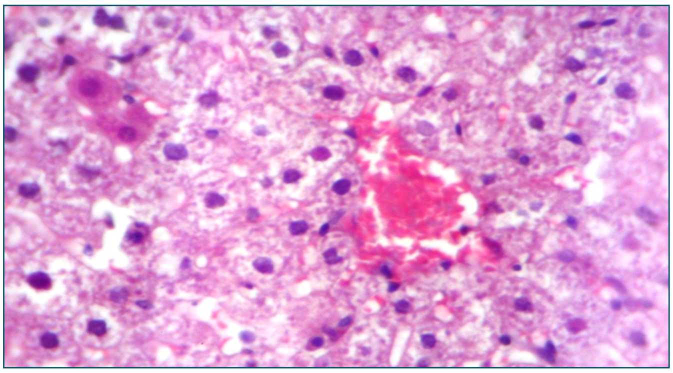

Fig. 6- Liver of diabetic group showing that hepatocytes contained few PAS positive granules as compared to that of the control group (PAS X 400)

Discussion

Significant reduction in the 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 test period (12 days). These results showed that *A. thaliana* ethanolic seed extract have 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* on glucose levels in the serum of diabetic mice 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 [13].

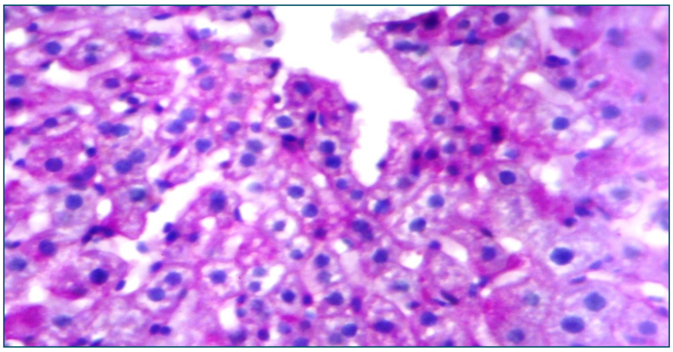

Fig. 7- Liver of Arabidopsis- treated diabetic group showed that most of the hepatocytes were studded with PAS positive granules (PAS X 400)

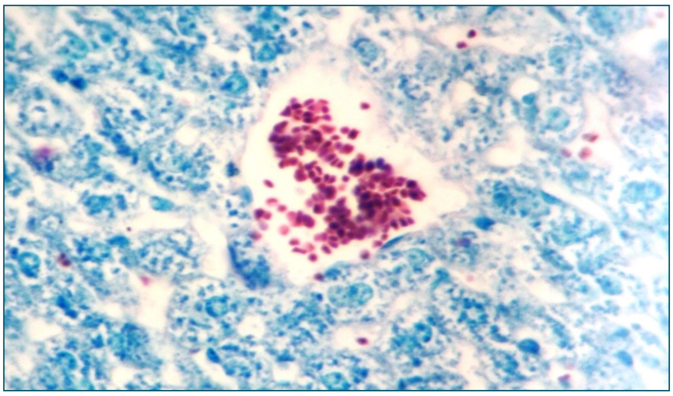

Fig. 8- Liver of control group showed that the cytoplasm of hepatocytes contained positive bluish granules (Methylene blue & Eosin X 400)

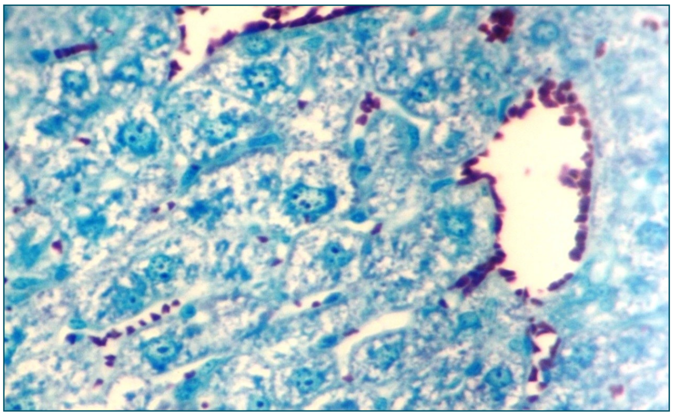

Fig. 9- Liver of diabetic group showing that hepatocytes contained fewer bluish granules in their cytoplasm than those in control group (Methylene blue & Eosin X 400)

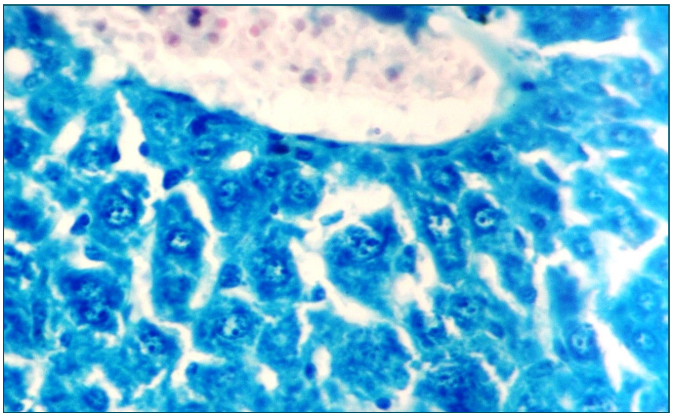

Fig. 10- Liver of Arabidopsis- treated diabetic group showing that the hepatocytes were studded with bluish granules (Methylene blue & Eosin X 400)

The hypoglycemic activity shown in this study may be related to the presence of flavonoids compounds which had very pronounces effect in the seed extract of this plant. Flavonoids may preserve β-cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes. A prospective study in Finland showed that the intakes of some specific types of flavonoids including quercetin and myricetin were inversely associated with risk of incident type 2 diabetes. In addition, emerging evidence shows that oxidative stress may be involved in the pathogenesis of chronic inflammation underlying insulin resistance, diabetes and cardiovascular disease [14,15].

It was observed that 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. Hyperlipidemia could be another factor for fatty liver formation. Our findings of fatty liverformation are in agreement with the findings of [16,17]. 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 [18].

In diabetes as the activities of glycogen synthase and hexokinase were diminished as a result of insulin deficiency, glucose can not be transformed into glycogen and glycogenesis was reduced and thus the amount of glucose increased [19]. Insulin is considered as an anabolic hormone with a wide range of effects on metabolisms including stimulation of protein synthesis [20,21] and inhibition of protein degradation [22]. It was demonstrated by Gehan Khalaf and Abdel-Gabbar Mohamed that hepatocytes of diabetic mice exhibited ill defined rough endoplasmic reticulum with dilatationof their cisternae and swollen mitochondria with loss of its cristae [23].

Therefore, in this 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 Arabidopsis consumption reversed most of the histological changes in the liver of 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. So, can say that Arabidopsis had a significant hepatoprotective role in diabetic mice and offers promising perspectives deserve further investigation.

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Conflicts of Interest: None declared.

References

[1] Wild S., Roglic G., Green A., Sicree R. and King H. (2004) *Diabetes Care*, *27*(5), 1047-1053.

[2] Sivajothi V., Dey A., Jayakar B. and Rajkapoor B. (2007) *Journal of Medicinal Food*, *10*(2), 361-365.

[3] Tiwari A.K. and Rao J.M. (2002) *Current Science*, 83(1), 30-37.

[4] Jelodar G., Maleki M., Motadayen M.H. and Sirus S. (2005) *Indian Journal of Medical Sciences*, 59(2), 64-69.

[5] Hoffmann M.H. (2002) *Journal of Biogeography*, 29, 125-134.

[6] Sommerville C. and Koornneef M. (2002) *Nature Reviews Genetics,* 3, 883-889.

[7] Li W., Li L., Li K., Lin J., Sun X. & Tang K. (2011) *Biotechnology and Applied Biochemistry*, 58(3), 139-146.

[8] Akueshi C.O., Kadiri C.O., Akueshi E.U., Agina S.E. and Gurukwem B. (2001) *Nigeria. J. Bot*., 15, 37-41.

[9] Institutional Animal Ethical Committee (2008) *Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research / PhD*.

[10] Mukhtar H.M., Ansari S.H., Ali M., Naved T. and Bhat Z.A. (2004) *J. Nat. Remed*., 4(2), 150-154.

[11] Kiernan J.A. (2008) *Histological and Histochemical Methods, Theory and Practice*, 4th ed., Bloxham, UK, Scion. Chapter 11, 274-306.

[12] Liui K.K.S. (2012) *An Alcoholic Eosin and Methylene-Blue Staining Method*, Department of Physiology, Edinburgh University.

[13] Li W., Li L., Li K., Lin J., Sun X. and Tang K. (2011) *Biotechnology and Applied Biochemistry*, *58*(3), 139-146.

[14] Knekt P., Kumpulainen J., Järvinen R., Rissanen H., Heliövaara M., Reunanen A. and Aromaa A. (2002) *American Journal of Clinical Nutrition*, *76*(3), 560-568.

[15] Esposito K., Nappo F., Marfella R., Giugliano G., Giugliano F., Ciotola M. and Giugliano D. (2002) *Circulation*, *106*(16), 2067-2072.

[16] Ohno T., Horio F., Tanaka S., Terada M., Namikawa T. and Kitoh J. (1999) *Life sciences*, *66*(2), 125-131.

[17] Merzouk H., Madani S., Sari D.C., Prost J., Bouchenak M. and Belleville J. (2000) *Clinical Science*, *98*(1), 21-30.

[18] Welt K., Weiss J., Martin R., Dettmer D., Hermsdorf T., Asayama K. and Fitzl G. (2004) *Experimental and Toxicologic Pathology*, *55*(5), 331-345.

[19] Chaudry A.R., Alam M., Ahmad M., Khan F.Z. and Nomani N. (1993) *Fitoterapia*, 64(6), 510-515.

[20] Jefferson L.S., Li J.B. and Rannels S.R. (1977) *Journal of Biological Chemistry*, *252*(4), 1476-1483.

[21] Wool I.G. and Cavicchi P. (1966) *Proceedings of the National Academy of Sciences of the United States of America*, *56*(3), 991-998.

[22] Ballard F., Wong S.S.C., Knowles S.E., Partridge N.C., Martin T.J., Wood C.M. and Gunn J.M. (1980) *Journal of cellular physiology*, *105*(2), 335-346.

[23] Khalaf G. and Abdel-Gabbar M. (2008) *The Egyptian Journal of Histology*, 31(2), 245-255.