Original Article

Structural Changes of the Pancreas in Female Albino Rats Treated with the Atorvastatin and the Alleviating Effect of Propolis : Histological and Biochemical Study

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ABSTRACT

Introduction: Atorvastatin is "HMG-COA" reductase inhibitors" that is widely used for management of hyperlipidemia particularly in patients with risk of cardio and cerebrovascular stroke. Similar to other medications, atorvastatin has adverse effect and recent studies showing that atorvastatin may affect the function of the pancreas .The aim of this work is to assess the functional and the structural changes of endocrine and exocrine parts of pancreas of female rats that were treated with atorvastatin and to assess the possible defensive role of propolis.

Methods and Result: Four groups of female albino rats (ten animals in each) were treated as follow: Group I regarded as control and received nothing , group II received 100 mg/ kg propolis by oral gavage, Group III treated with atorvastatin at a dose of 80 mg/kg orally while group IV was treated with propolis and atorvastatin as same dose as GII and GIII respectively . After three months, blood was drown from tail's vein for biochemical assessment , then animals were sacrificed and pancreas were removed and processed for light microscopic examination. The results indicated that atorvastatin significantly elevate blood glucose , pancreatic enzyme as well as oxidative stress biomarkers of pancreas these finding were confirmed by histological changes in both endocrine and exocrine portion which include degeneration, ,vascular congestion, hemorrhage and inflammatory cells infiltration of pancreatic tissue. However, administration of propolis concomitantly with atorvastatin improved both the biochemical and the histological changes.

Conclusion: Propolis has protective effect against atorvastatin induced pancreatic dysfunction.

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Key Words: Diabetes, pancreas, propolis, statin.

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INTRODUCTION

Hyperlipidemia"high lipid levels in blood" is a common medical condition particularly in modern developed countries, it is increasing around the world as a consequence of lifestyle factors, including low physical activity, smoking and an unhealthy food[1]. Hyperlipidemia is responsible for atherosclerosis which consider as the main cause for heart disease, stroke and other peripheral arterial disease[2]. Statin drugs as Atorvastatin, simvastatin and others are prominent and most effective lipid-lowering agents, they are "HMG-COA reductase inhibitors" and regard as first choice therapy for treatment of hyper-lipidemia in most of the patients hence, it is lowering low density lipoprotein level in blood and decrease the incidence of heart and cerebral strok[3]. Additionally, this drug has pleiotropic activity, it affects the endothelial function and has anti-inflammatory-action[4].

Although statins are goodly tolerated with few unwanted effects, recent study has been reported that the use of statin medication as atorvastatin may affect the mitochondrial function and decreases the enzyme activity of respiratory chain of mitochondria[5]. Some researchers connect the apoptotic activity of atorvastatin to this mitochondrial dysfunction and attribute the myopathy which consider as a side effect of statin treatment to this dysfunction[6,7]. Another author suggested that the apoptotic action of atorvastatin responsible for its beneficial effect on pancreatic cancer[8] although others not confirm this effect[9]. Additionally, clinical studies revealed that statins may predispose to acute pancreatitis as one of its serious side effect[10] while others suggested that atorvastatin could affect the glucose homeostasis and may induce diabetic state[11].

Propolis is usually obtained from beehives. It is a "resin-like material" prepared by bees from the sprouts of poplar and cone-bearing plants. Propolis appears to have action against microbial infections, it also acts as anti-inflammatory and assists in skin healing[12]. Propolis and its extracts are used frequently by many people for control of diabetes and for treating mouth sores as well as for burns, herpes and in treating various disease depending on its hypoglycemic effect, anti-inflammatory, antioxidant, anticancer, and immunomodulatory action[13] despite this, the scientific evidence to support these uses are few. So the aim of the presented study is to determine if atorvastatin has structuarl and biochemical effect on pancreas of female rats and to evaluate the effect of propolis.

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METHODS

chemical and drugs

Atorvastatin and propolis were purchased from local pharmacy in Mosul city in Iraq. All other chemical used for analytic purpose were acquired from Sigma chemical co. (Sigma-St Louis Mo-USA).

Animal treatment and protocols

Female albino rats, their weights about 200-225 gm, were acquired from veterinary collage, university of Mosul/Iraq. All rats were held under normal situations of controlled light, temperature and humidity with free access to water, a d libitum standard food . This experiment were done in agreement with nationwide rules for the usage and care for research laboratory animals according to the Ninevah medical collage's ethical committee. The doses of atorvastatin and propolis were determined on bases of the previous studies[14,15] Atorvastatin and propolis were given in a dose of (80 mg/ kg, 100mg/ kg b.w respectively) by oral gavage. Rats were divided into 4 groups (10 animals in each): control (group I) received distilled water; propolis (100 mg/kg) treated rats (group II) ; atorvastatin (80 mg/ kg) treated rats (group III) ; and lastly (group IV) in whom the animals treated with atorvastatin (80mg/kg) and propolis (100 mg/kg). Blood samples has been withdrawn from tail's vein then all rats were killing under anesthesia, the pancreas were removed for further processing .

Histological preparation and evaluation

Pieces of pancreas were fixed in 10 % buffer formalin for approximately 24 hours. Further processing, embedding in paraffin wax , sectioning and finally staining were done on the basis of Hegazy *et al*., (2015)[16]. The following stains were used in this study:

1. Hematoxylin and Eosin (H&E) stain, this stain demonstrated a broad range of cytoplasmic, nuclear, and extracellular matrix structures. All sections were examined and the pathological changes of the pancreas were assessed by identification of any changes in the acini, duct, if there was congestion of blood vessels, edema, inflammatory cells infiltration and degeneration of islets of Langerhans.
2. Periodic acid-Schiff (PAS): This technique depends on principle of libration of aldehyde group from carbohydrate material when oxidize by 0.5% periodic acid. With Schiff 's reagent, the aldehyde group gives magenta color, the density of the color depends on quantities of carbohydrate in the tissue. The intensity of the magenda color was graded from + to ++ as quantitative measurement of carbohydrate[17].

Then all sections were inspected under a light microscope using Olympus microscope. High resolve photos of sections (×100 and ×400) were taken.

Morphometric Analysis

Parameters were calculated by means of the color USB 2.0 digital image camera omax (A3590U) which was prepaird with image processing software (TuopView 12.5). The software of camera was adjusted to all objective lenses of Microscope-Olympus-CX31 by aid of 0.01mm stage micrometer (ESM-11 / Japan). It was used for calculation of the number and size of islet of Langerhans in each section. The measurements was done on ten randomly chosen felids from each group by using low magnification. Depending on Noor *et al*. The number of pancreatic endocrine islets was expressed as N/10 mm2 of the pancreatic parenchyma[18].

Blood sampling

All blood samples were collected in the morning about 9-10 AM. The blood were centrifuged at 3000r/m for 15 mint, then serum was separated and stored at −20°c.

Blood sugar was measured depending on O-Toluidine method by using a modified chemical[19]. Amylase and lipase which are pancreatic exocrine enzyme were estimated by using colorimetric enzyme assays (Bio Systems Assay, USA).

Biochemical Assays

Preparation of tissue homogenates

A piece of pancreatic tissue not less than one gram were washed in cold saline, presser -ved in aluminum foil and stored at -70°c. Then at pH 7.0 and in cold saline of 50-m M phosphate buffered the tissues were homogenized, centrifuged at 10 000 r/m at over 10 minutes. The supernatant are separated to be used for determination of:

A- Evaluation of the antioxidant status

1. Superoxide dismutase (SOD) enzyme activity. It is regarded as first line of defense against free radicles in cells[20]. It was measured by using Spectrometer (UV-1700, Shimadzu, Kyoto, USA). The SOD activity was expressed as U/g of tissues
2. Catalase (CAT) activity. It is a current antioxidant enzyme that exist approximately in all living cells the level of this enzyme was detected by using A novel kinetic method the result was expressed as micromole per second[21].
3. Reduced Glutathione (GSH) it is an important enzyme that inhibit the lipid peroxidation and protects the cells from oxidative damage[22]. It was assessed pursuant to the method of Beutler *et al*. (1963) the GSH activity was expressed as mg/g tissue weight[23].

B- Determination of oxidative damage biomarker

Malonaldehyde (MDA) level. It is a final toxic product of lipid peroxidation of cell membrane. Since, many organelles as mitochondria, endoplasmic reticulum and

others contain membrane so the lipid peroxidation of these membranes affect the viability and the function of the cells and asset in pathogenesis of many disease[24]. MDA level was measured depending on thiobarbituric acid (TBA) test, the result was expressed as nmol/mg.

C-Determination of the caspase-3 Activity

Caspase-3 "cysteine–dependent aspartic acid protease" is a lysosomal enzyme that considered as a marker for cells apoptosis, it cleaves the cellular targets as DNA and performs cell death[25]. The activity of caspase-3 in supernatant of cellular lysates was evaluated by enzyme linked immune sorbent assay (ELISA) kit, It has a fluorogenic substrate "N-Acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin or AC-DEVD-AMC" for caspase-3.

Statistical-Analysis

All data were expressed as mean ± SE (standard error of the mean). Comparisons between different groups have been done by using one-way analysis of variance (ANOVA) and Tukey s multiple comparisons test. Statistically significant level was considered at $P<0.05$. Statistical analysis was performed by using Graph pad prism program.

RESULT AND OBSERVATION

Change of biochemical parameters

A. The serum glucose levels was significantly ($p < 0.05$) raised in GIII and GIV compared to other groups . The elevation of blood sugar in GIV was significantly less than that of GIII (Table 1).

B. Lipase and amylase serum levels were significantly upraised mainly in GIII followed by GIV as compared to control groups (Table 1).

C. According to tissue homogenate, the biochemical assessment revealed a significant ($p < 0.05$) elevation of MDA with significant reduction of SOD, CAT and GSH levels in GIII followed by GIV compared to GI and GII (Table 2). Additionally, there is a significant difference between GIII and GIV.

D. Caspase-3 activity revealed a significant difference between atorvastatin treated group and other groups with higher level of caspase-3 activity was observed in atorvastatin treated group (Figure 1).

Histopathological changes

The H& E stained sections of pancreas from control and proplis groups showing normal structure with exocrine and endocrine parts. The exocrine part form the bulk of pancreatic tissue, it consists of highly packed acini and ducts. Each acinus has a cluster of pyramidal cells with rounded, basally located nucleus and deeply eosinophilic apical cytoplasm and basal basophilic stained. The ducts are lined by simple epithelium. The islets of Langerhans which form the endocrine part of pancreas have regular outline and embedded with in pancreatic exocrine tissue. Endocrine cells have rounded to polygonal shape with lighter staining to slightly eosinophilic cytoplasm. The beta cells in the center of islet are pale with oval nuclei while the α cells are small with deep nucleus at the peripheral (Figures 2,3,4). Pancreatic histopathology of atorvastatin treated rats showed distortion of acinar architecture, degeneration of acinar cells, congestion of blood vessels, periductal edema with hyaline proteinaceous material in the lumen of the duct. Widening of interlobar space may be due to edema (Figures 5,6,7). The islet of Langerhans become smaller with ill -defined boundary and reduced number of their cells associated with vacuolar degeneration of some endocrine cells, other cells appear with pyknotic nucleus (Figures 8,9). However, the sections from atorvastin and propolis (GIV) showed improvement in the hisopatholgical changes that observed in GIII, the acinar cells and islet of Langerhans looked near to normal with less degenerative changes (Figures 10,11).

Sections from pancreas of atorvastatin treated group showed accumulation of glycogen material in the cells of islet of Langerhans compared to control group , on the other hand there is a notable decrease come to be nearly normal in atorvastatin treated with propolis group (Figure 12). The intensity of the magenta color was ranging from weak reaction (+) to moderate reaction (++) as shown in (Table 3) .

Morphometrical analysis

The histmorphometric study of H&E sections of different groups reveals a significant decline ($p\leq 0.01$) in in Langerhans islet's diameter of atorvastatin treated rats compared to other groups . However the number of the islet / pancreas was non significantly reduced ($p> 0.05$) in atorvastatin treated group (Table 4) .

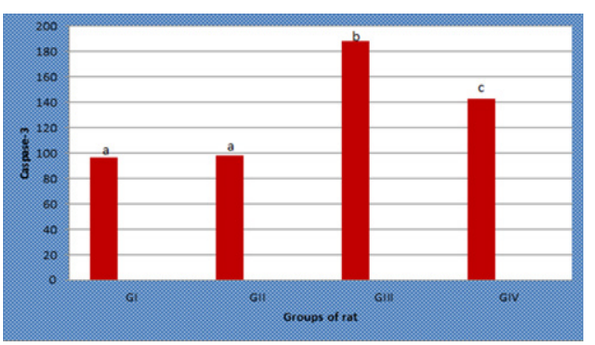

Fig. 1: caspase-3 activity. Data are the Mean±Stander error . Different letters in line mean significant difference at ($p \leq 0.05$) while similar letters indicate non-significant variation at ($p > 0.05$).

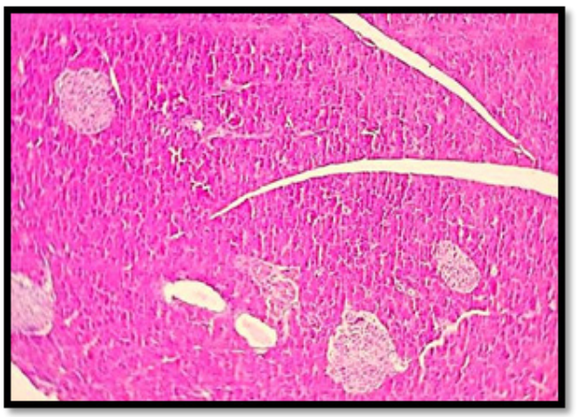

Fig. 2: photomicrograph of pancreatic tissue from control rat showing normal appearance of pancreas with lobule(L) separated by connective tissue septa (C) , each lobule consists of highly packed acini (AC)and inter lobular duct (D). The endocrine part (arrows) appears as pale rounded area .H&E X100.

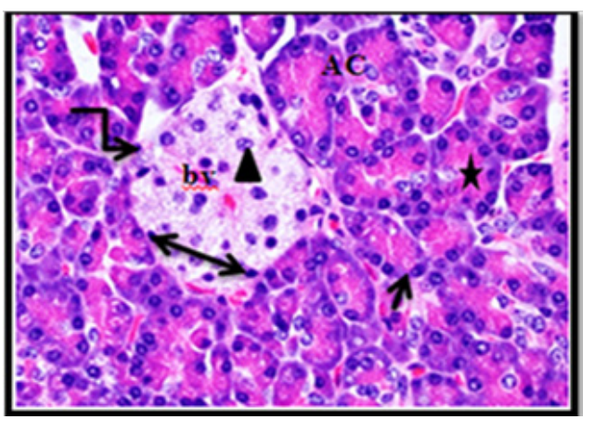

Fig. 3: photomicrograph of pancreatic tissue from control rat showing acini (AC) consist of pyramidal cells with eosinophilia of apical area and(star) basal basophilia (arrow). The islet of pancreas (curved arrow) appear as pale stained area with B cells (head arrow) in the center while the α cells at the peripheral (bihead arrow) and blood vessel (bv). H&E X400

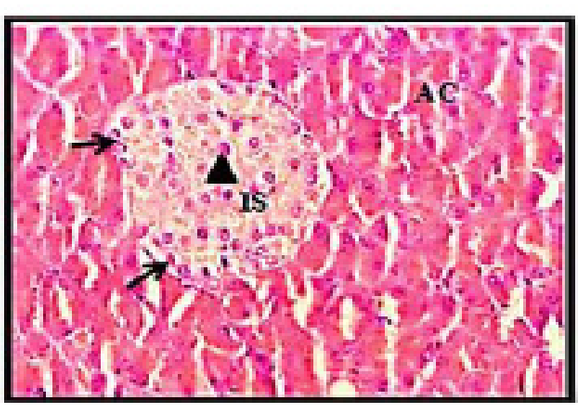

Fig. 4: photomicrograph of rat p ancre as from propolis treated group showing normal highly packed acini (AC) and Langerhans islets (IS) with B cells (head arrow) in thecenter while a cells (arrows) at the peripheral H&E stain. 400X

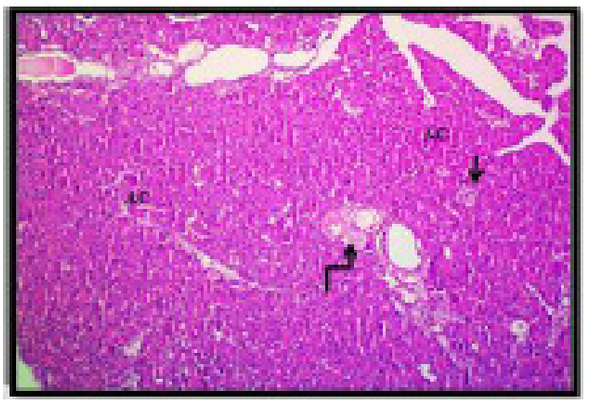

Fig. 5: photomicrograph of rat p ancre as from atorvastatin treated group showing degeneration and necrosis of pancreatic acini cells (AC) and Langerhans islets cells (arrow) with perivascular edema (curved arrow) H&E stain, 100X

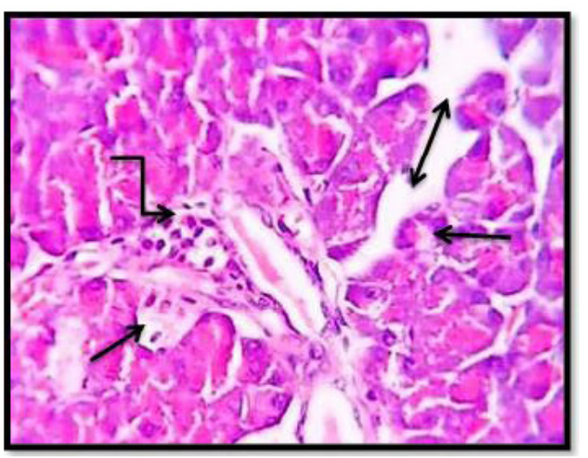

Fig. 6: photomicrograph of pancrestic tissue from group treated withatovastatin revealed distortion with vacuolar degeneration ofacini (arrows) some ofacinar cells appear with pyknic nucleus and eosinophilic cytoplasm (curved arrow) Widening of interlobar septa (bi heads arrow) H&E X400

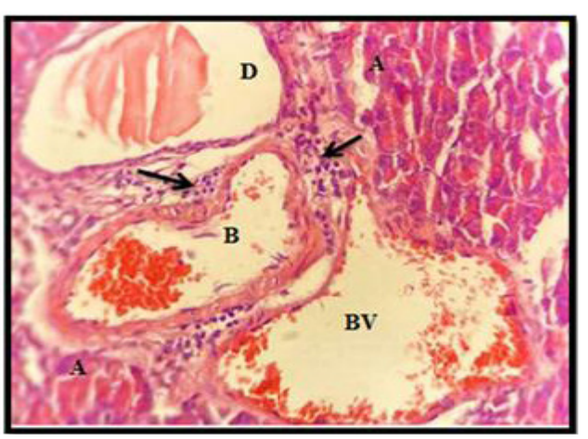

Fig. 7: photomicrograph of pancreatic tissue from group treated with atorvastatin showing acini (A) Dilated blood vessels (BV) inflammatory cells (aarows) dilated duct (D) with retained secretion H&E X400

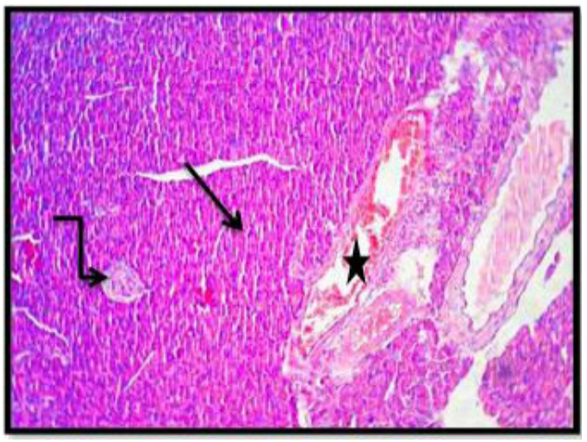

Fig. 8: photomicrograph of rat pancre as of atorvastatin treated group showing degeneration of pancreatic acini cells (arrow) degeneration of Langerhans islets cells (curved arrow) and dilated blood vessel (star) H&E 200X

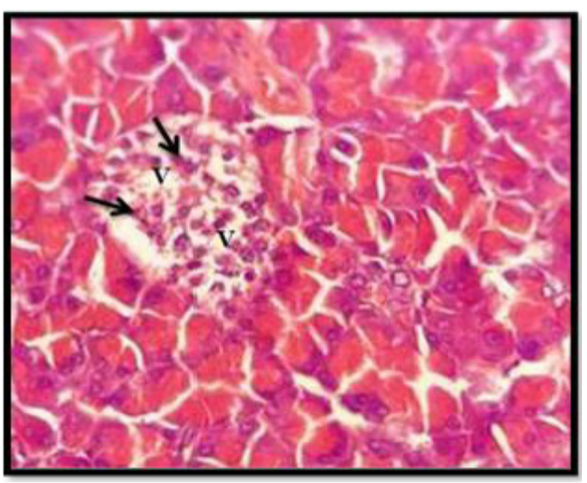

Fig. 9: photomicrograph of pancreatic tissue from atorvastatin group showing cells with pyknic nucleus (arrows) and vacuolar degeneration (v) of cells in silet of Langerhans H&E X400

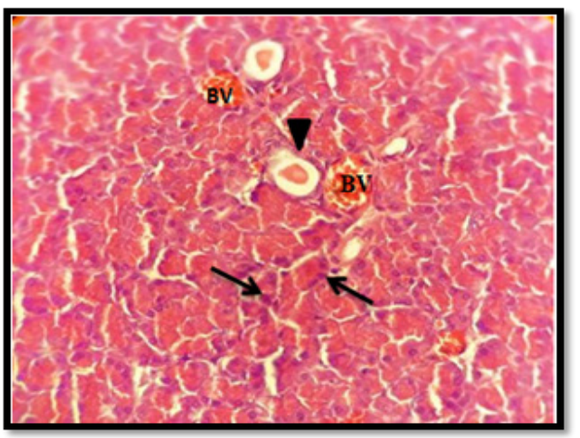

Fig. 10: photomicrograph of pancreas from atorvastatin + propolis treated group showing some acinar cell with pyknotic nuclei with eosinophilic cytoplasm(arrow) . still there are congested blood vessel (BV) , dilated intra lobular duct (head arrow) .H& E X400.

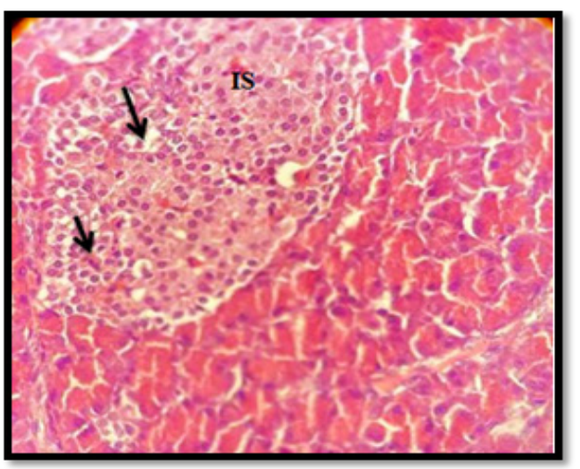

Fig. 11: photomicrograph of pancreas from atorvastatin and propolis treated group showing islet of Langerhans(IS) which look like normal except few degenerated cells (arrows) . H&E X400.

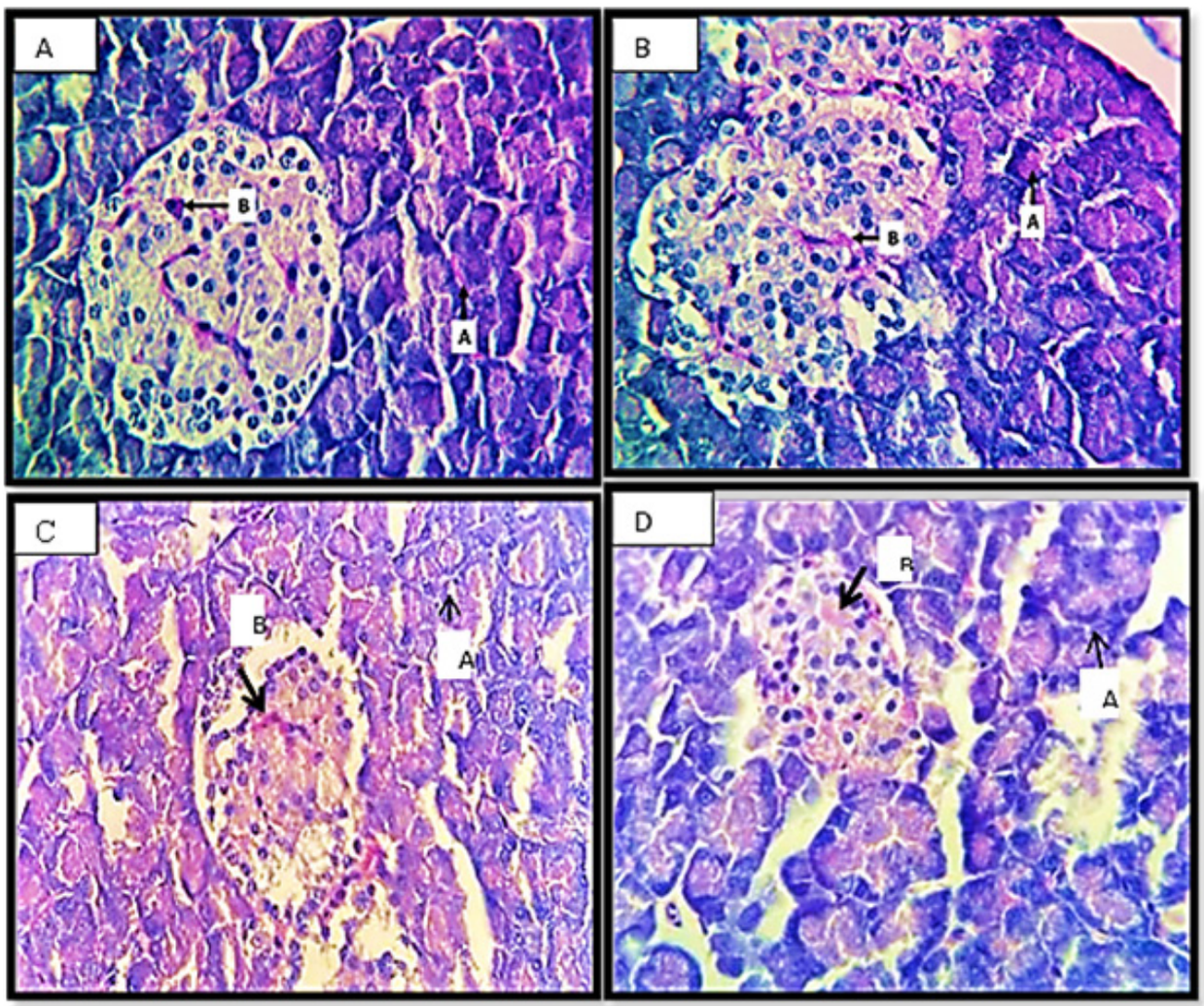

Fig. 12: photomicrograph of pancreas from control group (A), propolis treated group (B), atorvastatin group(C) and atorvastatin + propolis treated group(D) showing +ve reaction with Periodic Acid-Schiff (PAS) stain in the pancreatic acini (A) of all groups and accumulation of glycogen in cells of Langerhans islets (B) of atorvastatin group compared to other group . PAS stain, 400X.

Table 1: Serum level of glucose and pancreatic enzymes (lipase, amylase) in different groups. Data expressed as mean ± stander error of means

	Blood glucose mg/dL	Serum amylase U/L	Serum lipase U/L
GI (control)	85.2 ± 2.6	40±1.6	37.2±1.2
GII (propolis)	80.4 ± 3.1	46±2.1	40.1±1.5
GIII(Atorvastatin)	200.1± 2.1[a]	147.9±.1.3[a]	207.4±1.7[a]
GIV (Ator+ prop)	112.2±1.5[a,b]	92.3±.1.5[a,b]	115.1±1.2[a,b]

Turkey s multiple comparisons test had been used to compare between the groups. a: indicate significant difference from GI and GII , $P \leq 0.05$; b: indicate significant difference from GIII(Atorvastatin) group, $P \leq 0.05$.

Table 2: level of MDA, SOD, CAT and GSH in different groups Data expressed as mean ± stander error of means

	MDA nmol/g	SOD U/g	CAT µmol /g	GSH µmol/g
GI (control)	30.2± 4.5	15.2± 1.6	274±3.1	9.8± 2.3
GII (propolis)	25.0± 3.5	17.4±2.3	295 ± 2.8	10.1 ±1.3
GIII (Atorvastatin)	90.8 ± 3.4[a]	7.6 ±1.5[a]	185 ± 3.45[a]	7.5 ± 1.5[a]
GIV (Ator+ prop)	65.7±4.1[a,c]	10.2±2.1[a,c]	201±2.5[c]	9.5±2.1[a,c]

Turkeys multiple comparisons test had been used to compare between the groups. a: indicate significant difference from GI and GII , $P \leq 0.001$; c: indicate significant difference from GIII , $P \leq 0.001$

Table 3: Histochemical evaluation for PAS stain

Groups	Result
Control Group	+
Propolis Treated Group	+
Atorvastatin Treated Group	++ a
Atorvastatin + Propolis Treated Group	+

Periodic Acid-Schiff reaction. += weak reaction .++= moderate reaction. Turkeys multiple comparisons test had been used to compare between the groups. a: indicate significant difference at $P \leq 0.05$.

Table 4: Morphometric assessment of the pancreatic islet of different groups Data expressed as mean ± stander error of means.

	control group	Propolis group	Atorvastatin group	Atorvastatin+ propolis group
Number of islet N/ 10 mm^2	10.4±1.4	11.6±2.1	8.9±1.3	9.2±3.1
Diameter of islet (µm)	131±6.5	135±5.4	71.3±7.3[a]	95.6±5.3[a,c]

Turkeys multiple comparisons test had been used. a: indicate significant difference from GI and GII , $P \leq 0.01$; c : indicate significant difference from GIII, $P \leq 0.01$.

DISCUSSION

Atorvastatin is one of the most recommended statin medication that is widely used as prophylactic and management of cardiovascular disease that caused by hyperlipidemia. Though, some unwanted effect as myopathy, hyperglycemia and renal toxicity had been reported[26,27]. Moreover , Incidence of acute pancreatitis and pancreatic damage have been noticed with statin treatment10. So the purpose of this study is to explore the effect of atorvastatin on pancreatic tissues and to assess the role of propolis.

The present study showed degenerative changes of some pancreatic acini, the acinar cell appeared with vacuolar degeneration and dark pyknotic nucleus. Other acinar cells had ill-defined cells boundary which may be due to destruction of the their plasma membrane. Additionally, tissue edema, congested and enlargement of blood vessels with inflammatory cells infiltration were observed. These finding are similar to those observed in acute pancreatitis[28]. However, the elevation of pancreatic enzyme as amylase and lipase observed in the present work confirm the suggestion of pancreatitis. As the elevation of these enzymes regard as markers of acute pancreatitis[29]. Other researchers reported that acute pancreatitis may be caused by atorvastatin and other statin group as simvastatin[30]. Controversially , Ghorbani M, *et al* said that treatment with atorvastatin had beneficial effect in acute pancreatitis induced experimentally in rats[31]. The present data revealed that atorvastatin provoked pancreatic caspase-3 activity while propolis significantly reduced this activity. Caspases have critical role in apoptosis[32]. This finding suggest that atorvastatin induced apoptosis of pancreatic cells so impair their function. Yassien and El-ghazouly found that pancreatic sections from atorvastatin treated rats had strongly positive caspase-3 immunoreactivity[33] which is an agree with our result. Additionally, the edema and congestion of blood vessels observed in this study may be due to impairment of microcirculation and extravasation of blood and fluid to the tissue. Some authors regarded the free radicle generated as a result of oxidative stress as the main aspect in the pathogenesis of pancreatitis whether acute or chronic[34]. The current data showed a significant elevation of MDA level which regard as oxidative stress biomarker while antioxidant enzymes were significantly reduced. This result was in accordance with other researchers who attributed the pathological changes of pancreas to the accretion of free radicals and augmented oxidative stress[35].

The current study showed irregular outline of islet of Langerhans of atorvastatin treated group with degenerative changes of their cells and decreased their diameter as manifested by morphometrical study. Yassien and El-ghazoul used an electron microscope to study the effect of atorvastatin on the islet of Langerhans they found that most of beta cells rather than alpha cells appeared with small condense nucleus, dilated endoplasmic reticulum and few granule in their cytoplasm[33]. This may explain the vacuolar degeneration observed in present work. The biochemical assessment of blood glucose in the current work showed significant elevation in atorvastatin group and this may be due to reduction of insulin secretion as a result of beta cells degeneration. The present study revealed a moderate PAS reaction of cells of islet of Langerhans in atorvastatin treated group. Malaisse *et al* reported that accumulation of glycogen in pancreatic acini and cells of pancreas were increased in case of hyperglycemia[36]. This may be due to increase of gluconeogenesis. Accumulation of glycogen lead to apoptosis of β-cell[37]. Cho eta al observed a positive correlation between atorvastatin and development of diabetes mellitus in many patients treated with this drug and they stated that the dose of drug had an important role[38]. Sattar eta al attributed the diabetogenic effect of statin either to increase in insulin resistant or to the reduction of insulin secretion[39]. Insulin secreting cells " beta-cells "have low antioxidant expression that is why they are more susceptible to oxidative stress damage[40]. Urbano eta al demonstrated that mitochondrial dysfunction in β-cell models caused by oxidative stress induced by atorvastatin treatment are the main pathogenesis in statin –induced diabetes[41]. The co-administration of propolis with atorvastatin in group IV in this experiment showed amelioration in the histological changes observed in pancreatic tissue of atorvastatin treated rats. The blood level of pancreatic enzymes and blood sugar were significantly reduced compared to atorvastatin groups. Both exocrine and endocrine parts of pancreas showed low degenerative changes with reduced edema and blood congestion. Al-Hariri eta al confirmed the defending effect of propolis in contradiction of the streptozotocin -induced diabetes in rat model and attributed this effect to the antioxidant activity of propolis and to the ability of this material to improve

insulin sensitivity in peripheral tissue[42]. Elumalai *et al* studied the anticancer activity of propolis and highlighted its inhibitory effect on carcinogenic cells proliferation and metastasis[43], other researchers reported that propolis could be used as protective agent of liver and kidney against toxicity induced by anticancer drug[44,45].

CONCLUSION

Our study reveal that atorvastatin induces histological and biochemical changes in pancreas of treated rats and concomitant administration of propolis with atorvastatin may protect the pancreas and improve these changes.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التغيرات الهيكلية للبنكرياس في إناث الجرذان البيضاء المعالجة بالأتورفاستاتين والتأثير المحسن للبروبوليس

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المقدمة : أتورفاستاتين هو مثبط اختزال HMG-COA يستخدم على نطاق واسع لعلاج ارتفاع مستوى الدهون في الدم خاصة في المرضى الذين يعانون من مخاطر الإصابة بالسكتة القلبية والدماغية . وعلى غرار الأدوية الأخرى ، فإن له تأثيرات جانبيًة ، وقد أظهرت الدراسات الحديثة أن أتورفاستاتين له آثار ضارة على البنكرياس.

الهدف من ا العمل : هو تقييم التغييرات الوظيفية والهيكلية لأجزاء الغدد الصماء والغدد الافرازية في البنكرياس لاناث الجرذان التي تم علاجها بالأتورفاستاتين وتقييم الدور الدفاعي المحتمل للبروبلس (العكبر).

المواد والطريقة: أربع مجموعات من إناث الجرذان البيضاء (عشرة حيوانات في كل منها) تم علاجها على النحو التالي: المجموعة الأولى اعتبرت كمجموعة سيطرة ولم تتلقى اي علاج ، المجموعة الثانية اعطيت ١٠٠ ملجم / كجم من البروبوليس عن طريق الفم ، والمجموعة الثالثة تم اعطائها الأتورفاستاتين بجرعة ٨٠ مجم / كجم ، بينما عولجت المجموعة الرابعة باللبروبوليس والأتورفاستاتين بنفس جرع المجموعات الثانية والثالثة على التوالي. بعد ثلاثة أشهر, تم سحب الدم لأجراء القحص الكيميائي ، ثم ذبح الحيوانات وأزالة البنكرياس وتهيئتها لغرض فحصها بواسطة المجهر الضوئي .

النتائج : أشارت النتائج إلى أن أتورفاستاتين يرفع بشكل كبير من نسبة الجلوكوز في الدم وإنزيم البنكرياس وكذلك مؤشرات الاكسدة في البنكرياس وقد تأكدت هذه النتائج من خلال التغيرات النسيجية في كل من جزء الغدد الصماء والغدد الافرازية والتي تشمل التنكس والنخر واحتقان الأوعية الدموية والنزيف وتسلل الخلايا الالتهابية. ومع ذلك ، فإن إعطاء البروبوليس بالتزامن مع أتورفاستاتين أدى إلى تحسين كل من التغيرات الكيميائية والنسيجية للبنكرياس .

الخلاصة: البروبوليس له تأثير وقائي ضد الاختلال الوظيفي للبنكرياس الناجم عن العلاج بالأتورفاستاتين.