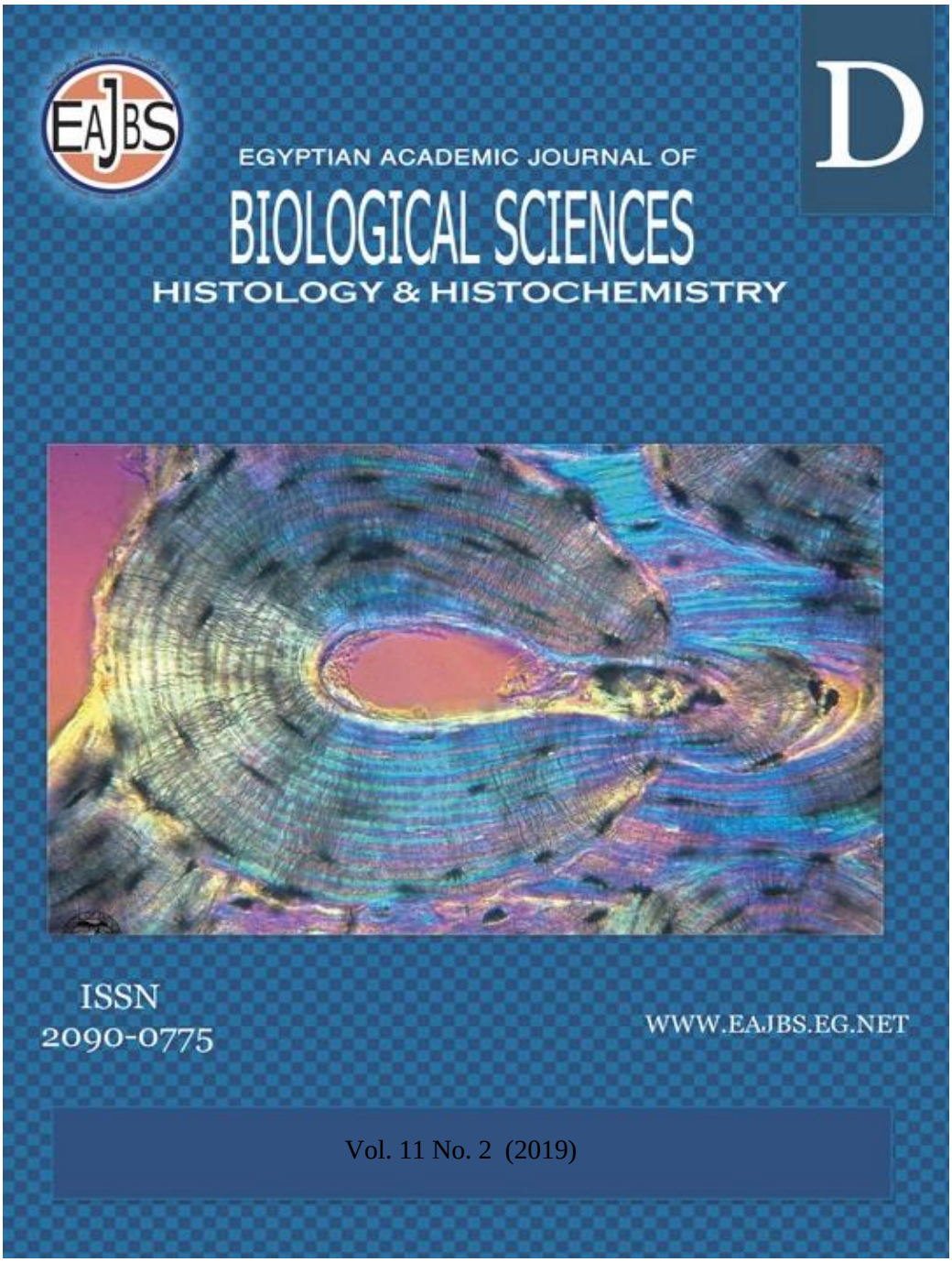

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The Enhancing Effect of Chamomile on Histological and Immunohistochemical Alterations in Diabetic Rats

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ABSTRACT

Background: Herbal medicine showed an important role in diabetes and other disorders treatment. Using natural products in animal diabetic models is still unclear so that in this study we aimed to investigate the possible effect of Chamomile extract on the diabetic dysfunctions-induced by streptozotocin (STZ) through the histopathological and immunohistochemical point of view.

Result: Our experiment was carried out on 4 rat groups, five rats in each to study the effect of Chamomile water extract on STZ -induced diabetes .The animals were classified into four groups. A negative control group (G1). A positive diabetic control group (G2) STZ (45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to rats after all overnight fasting (all animals exhibited hyperglycemia within 36 to 48 hr). Chamomile treatment group (G3) normal rats administered water extract of Chamomile, orally at a dose of 200 mg/kg body weight for 21 successive days. Diabetic-Chamomile treated group (G4) diabetic rats received 1 ml water extract of Chamomile for 21 days 36 h post- STZ injection.

Conclusion: Histopathological investigations of our result of pancreas, kidneys, spleen and liver besides pancreatic anti-insulin and caspase-3 monoclonal antibody marker were carried out, the collected data revealed protective, and enhancement effect of Chamomile against STZ- induced diabetes in rats.

INTRODUCTION

Nowadays, diabetes is more seen in society, and show prevalence in about 382 million people around all the world (Shi and Hu 2014). It is also considered as a metabolic disorder, which is characterized by many, disorders such as hyperglycemia, glucosuria and negative nitrogen balance these all due to inhibition of insulin secretion, which occurs in beta cells inside pancreas, and desensitization of insulin receptors to the insulin. Vats *et al.* (2005) found that, it is the most prevalent disease in the world, which affects 25% of the population, afflicts 150 million people, and is predicted to rise to 300 million by 2025. In addition, it caused different disorders like retinopathy, neuropathy, cardiovascular diseases, peripheral vascular insufficiencies, ulcers and amputations (Wrighten *et al.*, 2009).

Many experimental diabetic models of animals used and for the past 30 years these drugs, alloxan, streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3- nitrosoureido)-D-glucopyranose), high-fat diet-fed and nicotinamide are for establishing it (Islam and

Loots 2009). Streptozotocin also still the most commonly used as an agent to induce diabetes in rats and mice (Ngubane *et al.,* 2015), and its cause a decrease in the plasma insulin levels in animal models after Streptozotocin administrated which is used for diabetes induction (Patel *et al.,* 2015).

Streptozotocin-antibiotic produced naturally from Streptomyces achromogenes, which cause impairing for glucose oxidization, and thus causing reduction in insulin biosynthesis, secretion and abnormalities of B-cells, it induced hyperglycemia and widely used in the experimental model by screening the activity of hypoglycemic agents (Szkudelski, 2001).

Beltramini *et al.* (2006) found that, hyperglycemia arise because of b-islet cells irreversible destruction in pancreas by Streptozotocin which leads to a reduction of insulin secretion, and also the generated reactive oxygen species (ROS) and the subsequent increase of local oxidative stress, DNA methylation, and protein modification all are considered as the pathophysiological mechanisms of STZ-induced diabetes. Ramesh and Pugalendi (2006) found that the antioxidant agents acted against STZ-induced diabetes because they are made a diminishing for the oxidative stress by inhibiting ROS generation and lipid peroxidation. ROS is also involved in the etiology and pathogenesis of diabetes and the development of diabetic complications (Altan *et al.,* 2006).

In spite of the use of many hypoglycemic agents, diabetes and its health complications are still an important medical problem. And also it is considered of the drugs that have limited efficacy and certain adverse effects, which causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea. Anorexia, brain atrophy and fatty liver in many chronic treatments (Weidmann et al., 1993).

Many investigations found that herbal drugs effect on the treatment of diabetic mellitus (Pari *et al.,* 1999). Present anti-diabetic agents for diabetes is still not made a complete cure. Insulin therapy is the only satisfactory approach in diabetic mellitus, despite it has several drawbacks like insulin resistance (Piedrola *et al.,* 2001). The efficacy of herbal medicine is the low incidence of side effects, and also the low cost (Manal, 2012).

Hyperglycemia long-run exposure caused oxidative stress and reduced the efficiency of the endogenous antioxidant defense system by the production of several reducing sugars (through glycolysis and the polyol pathway) (Robertson and Harmon, 2006). The reducing sugars produced during glycolysis react easily with lipids and proteins (nonenzymatic glycation reaction), which caused an increase in the production of ROS (Jay *et al.,* 2006). On the other hand, it has been found that these ROS generation contributes to streptozotocin (STZ) and thus induced destruction of pancreatic b-cells (Szkudelski, 2001).

Chamomile tea (*Matricaria chamomile L.)* is widely used from the native old world and also well known as a medicinal plant (Astin *et al.,* 2000) .Chamomile tea is prepared from dried flowers and also has been used as herbal medicine in Europe. Especially, it also has been used in the treatment of many inflammations, irritations, and many pains such as skin diseases, wounds, eczema, ulcers, gout, neuralgia, and rheumatic pains. On the other hand, many studies found that Chamomile plant extract suppressed the growth of human cancer cells

(Srivastava and Gupta, 2007). The ameliorative effect of Chamomile on hyperglycemia and diabetic complications induced suppression of blood sugar levels, increased liver glycogen storage, and also the inhibition of sorbitol in the human erythrocytes (Kato *et al* ., 2008).

The pharmacological activity of Chamomile showed an independent effect on insulin secretion (Eddouks *et al.,* 2005). Also, different studies showed its protective effect on pancreatic beta cells which leads to diminishing hyperglycemia-related oxidative stress (Cemek *et al.,* 2008). So that many studies are required to evaluate the useful effect of Chamomile in managing diabetes.

MATERIALS AND METHODS

Collection and Extraction of Plant Material:

The fresh leaves of the plant *Matricaria chamomile L* (Chamomile) were obtained from the local herbal market present in Egypt. Then 10 gm of plant leaves of Chamomile was taken and soaked in 100 ml of boiled water in a glass jar, then shaken and stirred for 4 hr. The container content was left overnight at room temperature and then was filtered through double filter paper (Double Rings filter paper 102, 11.0 cm). Finally, the filtrates were concentrated by using a vacuum pump rotary evaporator to afford a greenish mass of leaves extracts (manal, 2012).

Experimental Animals:

The experiment was conducted on adult male albino rats with the weights of 150 - 200gm obtained from Animal House of Faculty of Science, Menoufia University, Egypt. All rats were fed normally in Labe a chow food containing (16% protein, 66% carbohydrate, 8% fats and water). They were housed at a (12:12) hrs. Light and Dark cycle at $24^0C \pm 3^oC$ and with relative humidity (60-70) %. The guidelines followed for the animal experiment were approved by experimental procedures was approved by the animal ethical committee in accordance with the guide for the care and use of laboratory animals with approval No (MUFSS/F/HI/2/19).

Experimental Design and Treatment Schedule:

The experiment was carried out on 4 groups, five rats in each group, to study the effect of plant water extract on STZ -induced diabetes as follows:

1-The Negative Control Group (G1): Healthy control rats, received distilled water ad-libitum.

2-The Positive Diabetic Control Group (G2): Received a freshly prepared solution of Streptozotocin or STZ (45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) by injection intraperitoneally to rats after overnight fasting (deprived of food for 16 h but allowed free access to water). STZ injected animals exhibited hyperglycemia within 36 to 48 hr (Olfat, 2012). Rats having hyperglycemia with fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.

3-Chamomile Treatment Group (G3): Normal rats administered water extract of Chamomile, orally at a dose of 200 mg/kg body weight (dosage determined earlier, (Seelinger *et al,* 2008), for 21 successive days.

4-Diabetic-Chamomile Treated Group (G4): After induction of diabetes, rats received 1 ml water extract of Chamomile for 21 days 36 hr post- STZ injection. 200 mg/kg/day Chamomile (Tavafi et *al.,* 2011) was given daily for 21days to the diabetic rats, using gastric channel (Makino et *al.,* 2002).

No detectable irritation or adverse effect such (respiratory distress, abnormal locomotion or catalepsy) was observed in any animals after Chamomile administration.

Study Protocol and Collection of Samples:

In the present study increase consumption of drinking water by rats insure induction of diabetes mellitus (DM) , and after confirmation of diabetic status , a blood drop was taken from the distal end of the tail of fasted animals and applied to a blood strip and analyzed using a blood glucose monitoring device (Acu-Check, Performance, Germany) for both control and diabetic animals, and FBG (Fasting blood glucose) level was measured in every 7th day using blood strips over the course of the treatment, and rats receive injection of STZ exhibiting hyperglycemia with (FBS) 250 mg/dl. And from the 1st day (3rd day of STZ-injection) of Chamomile extract administration to diabetic rats (Mallick *et al.,* 2007). On the 21st day of extract administration, all the animals were anesthetized (Nesdonal 50 mg/kg, i.p.), the pancreas was removed for the histological and immunohistochemical analysis. Kidney, liver, heart and spleen were excised immediately and thoroughly washed in saline and used for histological experiments.

Histological Study:

After blood sampling, the kidney, spleen, pancreas and liver were removed with minimum handling and fixed in 10% neutral buffered formalin for 24 hr, washed the specimen in tap water then dehydrated through serial dilutions of alcohol (ethyl and absolute ethyl) were used always for dehydration. Specimens were cleared in hydrocarbon (xylene), infiltrated in paraffin at 56 degrees in an oven, and embedded outside the oven. Paraffin bees wax tissue blocks were prepared for *sectioning* at 4 microns by slide using microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examination through the electric light microscope (Suvarna *et al.* , 2018).Then all slides were viewed by using Labomed , Labo America, Inc. USA microscope and images were captured by a digital camera (Sony DSC_S5000). The severity of inflammation was recorded using a grading scale of 0 to 4, as follows:

0 = indistinguishable from controls.

1 = minimal, ≤25% of cells affected.

2 = mild, 25% ≥ 50% of cells affected.

3 = moderate, 50% ≥ 75% of cells affected.

4 = marked, 75% cells affected.

Scale bars were measured by using Image-J software**.**

Immunohistochemical Study:

Principle

The standard immunohistochemical methods were adopted (Eissa and Shoman, 1998). The tissue sections were routinely microwave-treated to unmistaken the epitopes of antigen (Cattoreti *et al.,* 1992). The demonstration of antigen in tissues by immunostaining is a two-step process. The first step is binding of the primary antibody to insulin and Caspase 3 (Biogenex Laboratories, San Ramón, CA, U.S.A.), followed by visualization of this reaction by a secondary or link antibody to which are attached different enzyme systems. The primary antibody determines the specificity of the reaction; whereas, the secondary antibody, with its linked enzyme, causes amplification of the reaction, hence, increase of the sensitivity of the test. The Biotin-Streptavidin (BSA) system was used to visualize the markers (Hsu *et al.,* 1981). Diaminobenzidine (DAB) was used as chromogen since it allows a permanent preparation. Hematoxylin counterstain was done using Mayer's hematoxylin

(Hx). The specimens were mounted using mounting medium Canada balsam and examined by a microscope (Olympus DP 71), the percentages of the relative staining areas were measured by using Image-J software.

RESULTS

1. Histopathologic Finding:

Pancreas:

Histological examination of pancreas sections of negative control group rats showed normal islets cells with prominent beta and alpha cells beside normal pancreatic parenchymal and stromal cells. The positive diabetic control group showed congested pancreatic blood vessels, focal degeneration of the pancreatic acini and cystic dilatation of pancreatic ducts were seen. Some of the islets cells showed decrease cellular population with degeneration and necrosis of some of the beta cells. Other islets cells showed a normal population of cells including alpha, beta and delta cells. The chamomile treatment group showed normal pancreatic tissue with apparently normal islets and their cellular contents. In Diabetic, Chamomile treated group most of the pancreatic islets were large in size or of normal sizes with apparently normal active cellular contents. In some sections, the pancreatic blood vessels were mild to moderately congest with mild interstitial lymphocytic infiltration. Some of the pancreatic ducts were cystically dilated and filled with secretion (Fig.1& 2).

Liver:

Liver sections of the negative control group showed normal histomorphology with preserved hepatic cords, central veins, portal area and stromal structures. Positive diabetic control group revealed mild portal congestion and mixed inflammatory infiltrates with round cells infiltration central-portal fibrous septa. The chamomile treatment group showed normal hepatic parenchyma, central veins, sinusoids and portal triads. Diabetic, Chamomile treated group revealed apparently normal histomorphological structures (Fig.3).

Kidney:

Histological examination of a kidney of the negative control group showed normal nephron units with preserved glomerular and tubular structures with a normal appearance of a tuft of capillaries, and glomerular basement membrane surrounded by a double-walled epithelial capsule. The positive diabetic control group showed shrinkage of the Malpighian corpuscles and mild vacuolated of epithelial lining renal tubules also dilated congested vascular spaces. Chamomile treatment group showed normal nephron histomorphology and, there is no histopathological alterations were observed. Diabetic, Chamomile treated group revealed apparently normal nephron histomorphology (Fig.4).

Heart:

Rats of the negative control group showed normal cardiac muscles with preserved coronary, intermuscular blood vessels, normal cardiomyocytes and Purkinje fibers. Positive diabetic control group revealed interstitial and perivascular edema beside degenerative changes in some cardiomyocytes. Chamomile treatment group revealed normal cardiac parenchymal and stromal structures with mild congestion of the coronary and intermuscular blood vessels. Diabetic, Chamomile treated group showed apparently normal cardiomyocytes with mild congestion of the coronary and intermuscular blood vessels (Fig.5).

Spleen:

Spleen sections of the negative control rats showed normal white pulp lymphoid structures, red pulp sinusoidal, and reticuloendothelial

structures. The positive diabetic control group showed a decrease in the size of white pulp and congestion inside red pulp. The chamomile treatment group showed preserved white pulp, red pulp histomorphology keeping the normal lymphoid population, sinusoidal and reticuloendothelial structures. Diabetic, Chamomile treated group showed normal structures with mild to moderate reactivity of the white pulp lymphocytes and increased infiltration of mature lymphocytes in the red pulp (Fig.6).

Table (1): Histopathological scoring obtained by using light microscopic analysis of pancreas, Liver, Kidney, Heart and Spleen in Tissue sections of all treatment groups

Organ / Grade/group	Pancreas					Liver					Kidney					Heart					Spleen				
Negative control group (n=5)	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
	5	0	0	0	0	5	0	0	0	0	4	1	0	0	0	4	1	0	0	0	5	0	0	0	0
Positive diabetic control group (n=5)	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
	0	0	0	2	3	0	0	0	4	1	0	0	0	0	5	0	0	0	2	3	0	0	0	3	2
Chamomile treatment group (n=5)	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
	4	1	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
Diabetic, Chamomile treated group (n=5)	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
	0	0	0	2	3	1	2	2	0	0	1	2	2	0	0	2	1	2	0	0	2	2	0	1	0

2. Immunohistochemical Findings:

Insulin:

Immuno-histochemical examination of non-diabetic non treated rats for detection of insulin marker revealed active positive cytoplasmic stainability of almost all β cells of islets of Langerhans, the stained cells appeared large , rounded with centrally located nuclei and granular orange-red to brown cytoplasm . Alpha and delta cells were negatively stained. Alpha cells (α-cells) were smaller in size, round or ovoid in shape and arranged peripherally and delta cells (δ-cells) were the smallest, rod shape and were randomly distributed inside the islets. While, the pancreas of diabetic rats (45 mg STZ/kg BW (Single IP)) showed a decrease on positively reacted and stained cells with insulin diabetic marker (25-30% /HPF), the remaining

cells 70-75 %/HPF were negatively stained. Other islet cells (α and δ-cells) were also unstainable**.**

Most of the β cells of the islets of Langerhans (90-95%/HPF) of non-diabetic rats treated with Chamomile 200 mg /kg BW (daily oral dose for 3weeks) were positively stained for the diabetic marker insulin in most of the examined sections. Moreover, sections of pancreas of Diabetic rats (45 mg STZ /kg BW, single IP) treated with Chamomile (200 /kg BW, daily oral dose for 3 weeks), showed an enhanced ameliorative effect of Chamomile, as the positively stained cells for insulin marker in most of the examined sections were ranged from 85-90 %HPF cells, a few cells (10-15%/HPF cells appeared weakly positive or unstained.

Caspase 3:

Examined serial sections from pancreatic islets cells treated with anti-caspase 3 monoclonal antibodies revealed that almost all the islets cells including Beta, alpha and delta cells were negatively stained for the apoptotic marker caspase 3 in the negative control group. Very few cells not exceeding 1-1.5% of alpha and beta cells were weakly stained (Fig.8). In the positive diabetic control group, most of the examined sections showed dramatic changes regarding the apoptotic marker caspase 3, as about 45-55% of beta cells and a few alpha cells were reactive to caspase 3 and showed deep brown cytoplasmic reactivity (Fig.9). While, almost all sections revealed caspase 3 negatively reacted islets cells of Chamomile treatment group (Fig.10). Examined sections of diabetic, Chamomile treated group demonstrated that, about 10-15% of beta cells were weakly reactive to caspase 3 as represented by a light brown stainability of their cytoplasm. Most of the alpha cells (peripherally located) appeared degenerated and a few were apoptotic (Fig.11).

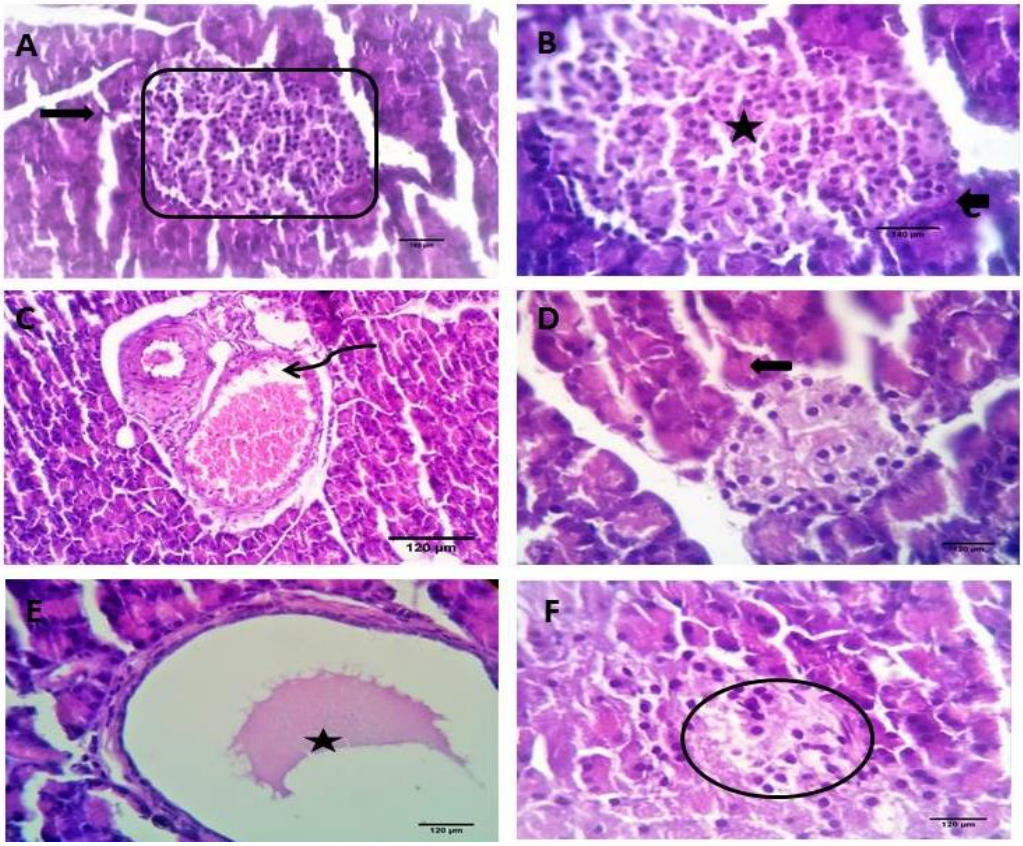

Fig. 1: Photomicrograph of (A &B , G1) pancreas showing normal islets cells (star) and pancreatic parenchymal and stromal cells (arrowhead),(C ,G2) showing congested pancreatic blood vessels (curved arrow), (D ,G2) focal degeneration of the pancreatic acini (arrowhead) , (E .G2) cystic dilatation of pancreatic ducts (star) with (F ,G2) reduction of cellular population , degeneration and necrosis of some islets cells (Circle).Scale bars correspond A =180 µm , B=140 µm ,C=120 µm,D=120 µm,E=120 µm,F=120 µm.

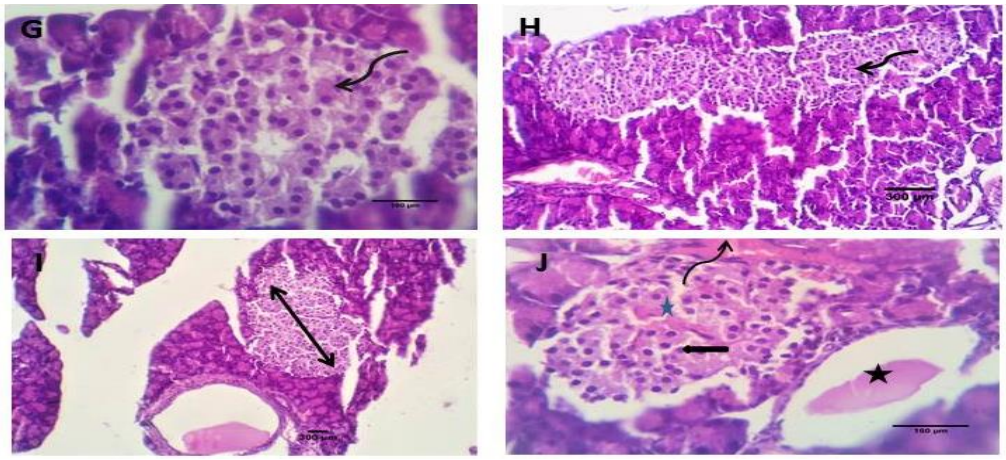

Fig. 2: Photomicrograph of Pancreas (G&H , G3) showing normal pancreatic tissue with apparently normal islets and their cellular contents (curved arrow).(I&J ,G4) showing large size pancreatic islets (double-headed arrow) with apparently normal active cellular contents (arrowhead), mild to moderate congestion of pancreatic blood vessels (curved arrow) and cystically dilated pancreatic ducts filled with secretion (stars) ,Scale bars correspond G =160 µm , H=300 µm , I=300 µm, J=160 µm.

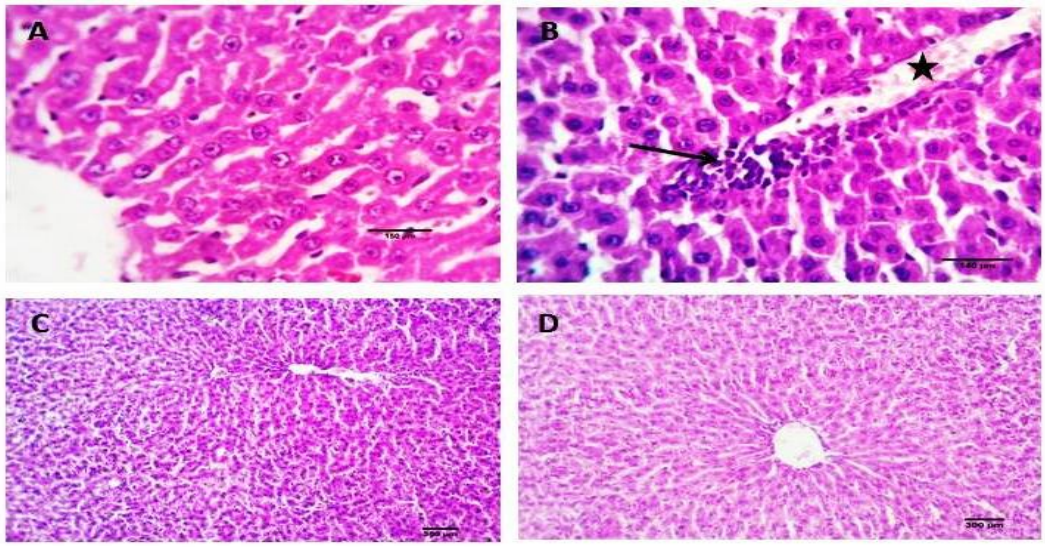

Fig. 3: Photomicrograph of the liver (A, G1) showing preserved hepatic cords, central veins, portal area and stromal structures, Liver (B, G2) showing mild portal congestion (star) and round cells infiltration (arrow). (C, G3) liver showing normal histomorphological structures, (D,G4) liver showing normal histomorphological structures. Scale bars correspond A =150 μm , B=140 μm , C=300 μm, D=300 μm.

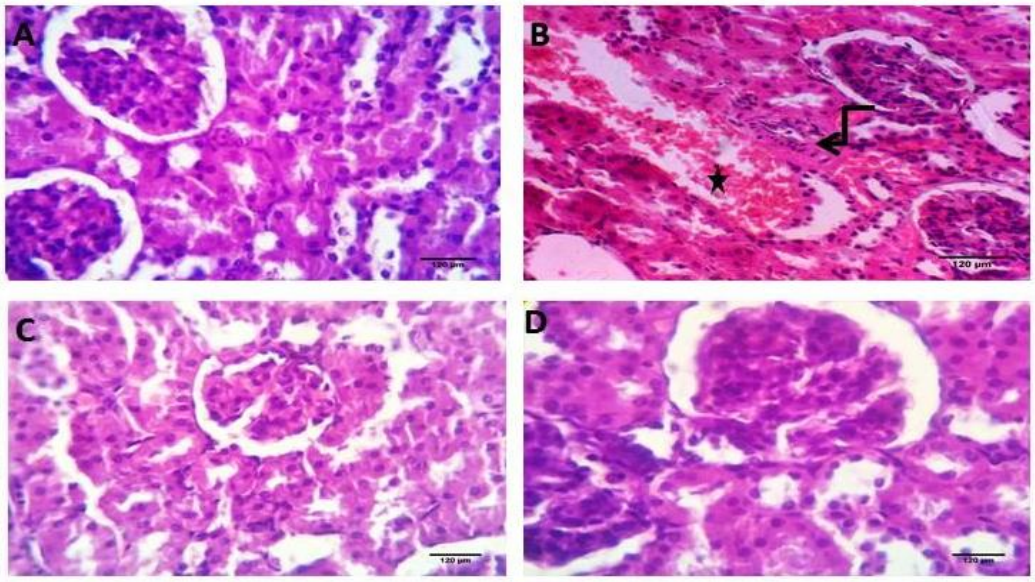

Fig. 4: Photomicrograph of Kidney showing (A G1) preserved glomerular and tubular structures. (B G2) showing distal congested vascular spaces (star) and milled aggregates of inflammatory cells in between the renal tubules and hydropic degeneration of the renal tubular epithelial cells (curved arrow),(C G3)Kidney showing normal histomorphological structures ,(D ,G4)Kidney showing normal renal tubules and glomerular structures. Scale bars correspond A & B&C&D μm =120.

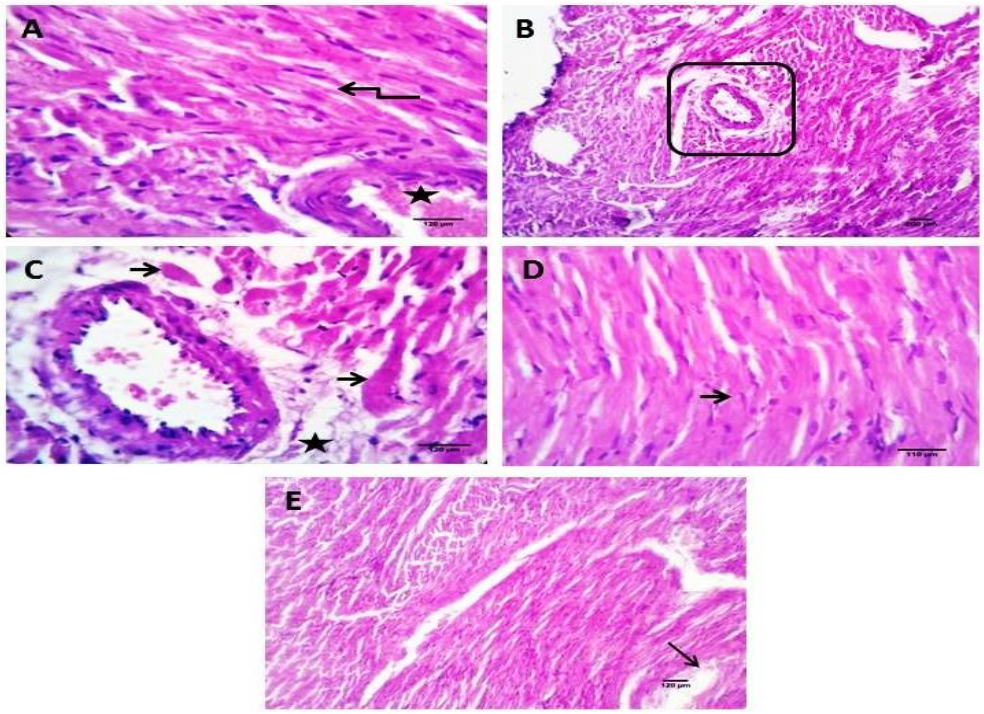

Fig. 5: Photomicrograph of Heart (A, G1) showing normal cardiac muscles (Curved arrow) with preserved coronary blood vessels (star). (B & C, G2) Heart showing interstitial and perivascular edema (star) beside degenerative changes in some cardiomyocytes (arrowheads). (D, G3) Heart showing normal cardiac parenchymal (arrowhead) with mild congestion of intermuscular blood vessels. (E,G4)heart showing apparently normal cardiomyocytes with mild congestion of coronary blood vessels (arrow).Scale bars correspondA =120 µm , B=200 µm , C=120 µm, D=110 µm,E=120 µm.

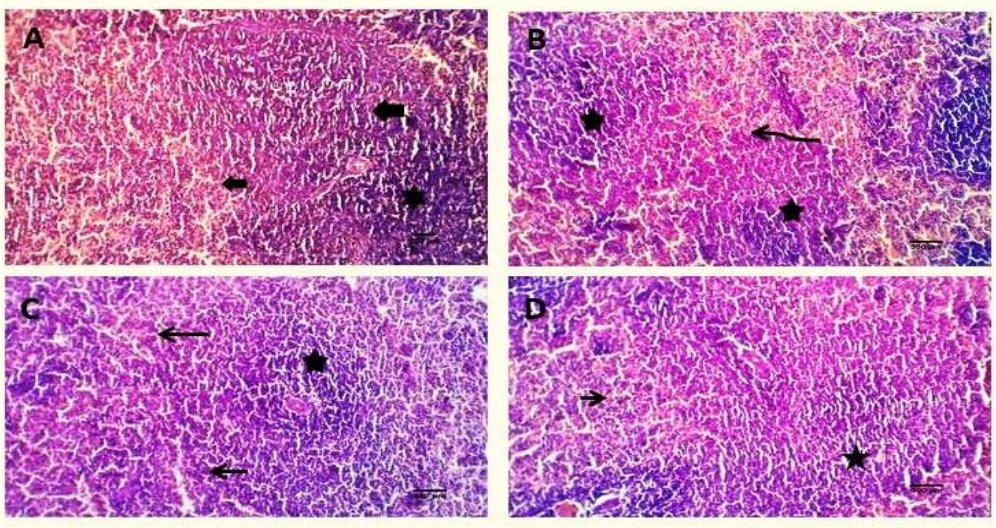

Fig. 6: Photomicrograph of spleen (A, G1) Spleen showing normal white pulp (star), red pulp and Sinusoidal structures (arrowhead). (B,G2) Spleen showing decrease in the size of white pulp and congestion inside red pulp (star),(C, G3)Spleen sections showing preserved white (star) and red pulp structures (closed arrows). (D,G4) Spleen showing moderate reactivity of the white pulp (star) and increased infiltration of mature lymphocytes in the red pulp (closed arrow).Scale bars correspond A =120 µm , B=200 µm , C=120 µm, D=110 µm, E=120 µm.

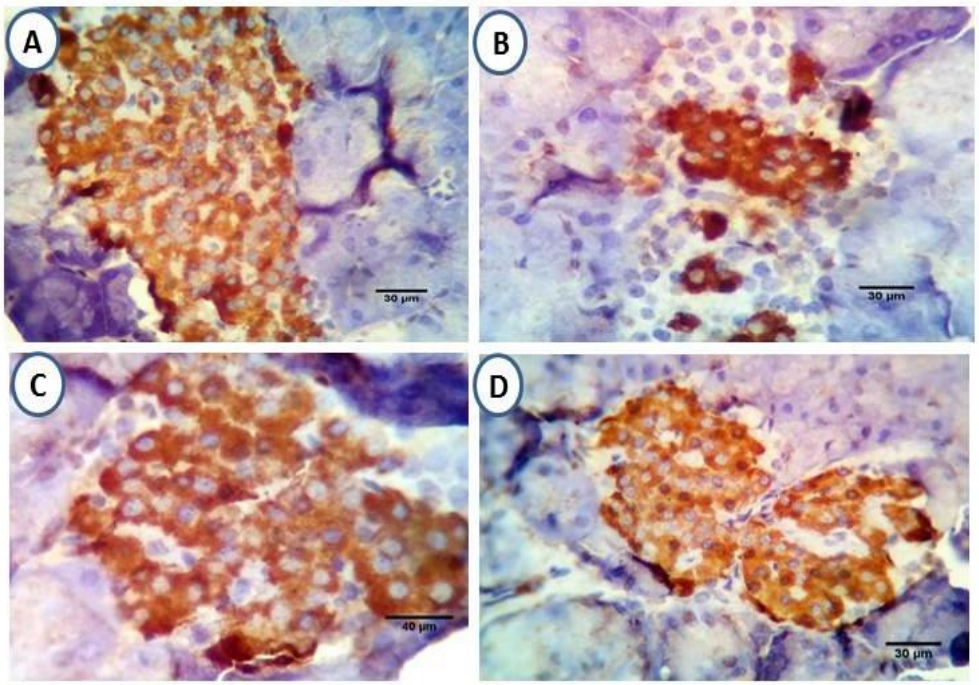

Fig. 7. Photomicrograph of Pancreas (A &B,C,D) showing, (A)rat's pancreas, G1 (Negative control rats) immune-stained with Diabetic marker, insulin showing active positive cytoplasmic stainability of almost all **β cells** of islets of Langerhans, the stained cells appear large , rounded with centrally located nuclei and granular orange-red cytoplasm.(B) G2,Positive diabetic control group, immune-stained with the diabetic marker, insulin showing characteristic changes in the islets cells , particularly the **β cells**. The later showing 25-30% /HPF cells positively reacted and stained cells, the remaining cells 70-75 %/HPF cells are negatively stained. Other islet cells are also unstainable.(C) Non diabetic rats treated with Chamomile, 200 mg/kg BW (daily oral dose for 3weeks), immune-stained with the diabetic marker, insulin showing most of **β cells** of the islets of Langerhans (90-95%/HPF) positively stained for the diabetic marker insulin in most of the examined sections (D) Diabetic treated rats with Chamomile 200 mg/kg BW (daily oral dose for 3weeks) immune-stained with the diabetic marker, insulin showing, about 85-90% %/HPF positively stained **β cells**, some with weak staining reaction .Scale bars correspond A =30 µm , B=30 µm , C=40 µm, D=30 µm.

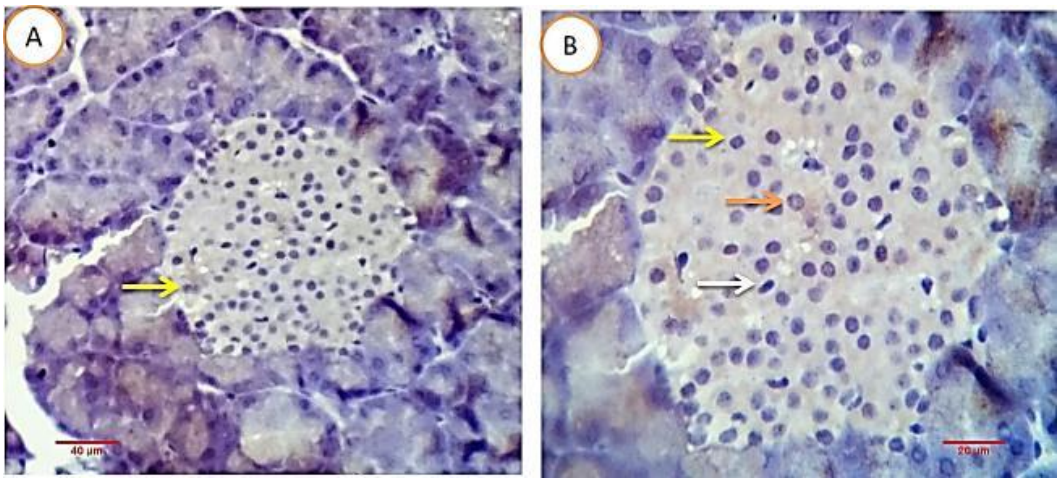

Fig. 8: Photo-micrograph demonstrating caspase 3 reactivity in islets cells. Very few cells not exceeding 1-1.5% of alpha and beta cells are weakly stained. Scale bars correspond A =40 µm , B=20 µm .

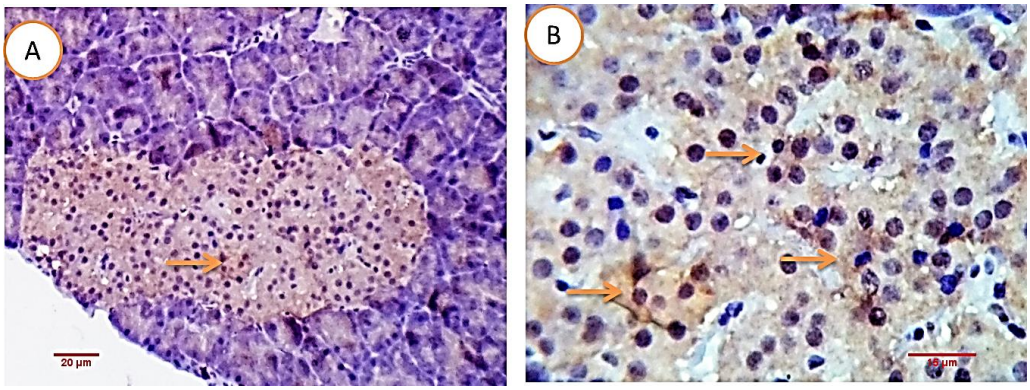

Fig. 9: Photo-micrograph demonstrating caspase 3 reactivity in islets cells. About 45-55% of beta cells and a few alpha cells are reactive to caspase 3 and showed deep brown cytoplasmic reactivity.Scale bars correspond A =20 µm , B=18 µm

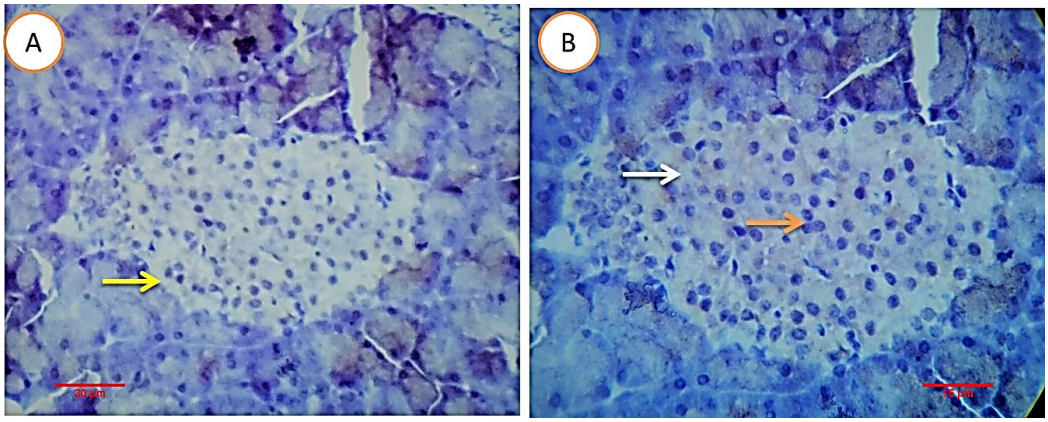

Fig. 10: Photo-micrograph demonstrating caspase 3 reactivity in islets cells. Almost all sections revealed caspase 3 negatively reacted islets cells. Scale bars correspond A =20 µm, B=15 µm

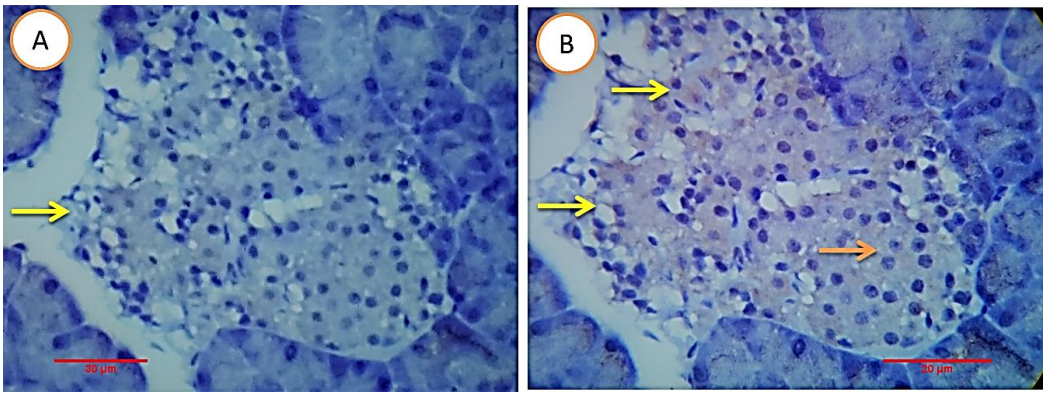

Fig. 11: Photo-micrograph demonstrating caspase 3 reactivity in islets cells. About 10-15% of beta cells are weakly reactive to caspase 3 as represented by a light brown stainability of their cytoplasm. Most of the alpha cells (peripherally located) appear degenerated and a few are apoptotic. Scale bars correspond A =30 µm , B=20 µm.

DISCUSSION

Diabetes was induced in this study by intraperitoneal injection of Streptozotocin (STZ) (45 mg/kg BW freshly prepared in normal physiological saline solution), to the overnight fasted rats. Animals had blood glucose values above 250mg/dl on the third day after STZ injection, were considered as diabetic rats (Gurudeeban *et al.,* 2016). Then the third day of STZ injection the treatment was started and it was considered as a first day of treatment. In general, several studies have demonstrated that streptozotocin has a β-cell cytotoxic and slight carcinogenic effect, which significantly induced diabetes by damaging the cells that cause a reduction in insulin release. The single high dose streptozotocin-induced diabetic rat, is one of the animal models of human Insulin dependent Diabetes Mellitus (IDDM) or type I diabetes mellitus.

The major findings of the present study is that Chamomile recutita flowers aqueous extract is considered highly safe plants not only for the human being , but also for animals and its administration caused significant reduction in blood glucose level in diabetic rats. In addition, Chamomile recutita ameliorated the liver and kidney damage in streptozotocin-induced diabetic rats and improved the lymphoid tissue immune reactivity of hematopoietic organs, particularly spleen. Our results have provided that extract of Chamomile flowers possess a potent significant hypoglycemic effect comparable to that of diabetic control positive rats. Studies suggest that Chamomile extract pharmacological activity has shown to be independent of insulin secretion (Eddouks et al., 2005). As the hypoglycemic effect of Chamomile is the result from increased peripheral uptake of glucose, inhibition of hepatic glucose synthesis so that it's not accompanied by the increase in serum insulin levels (Hamden *et al.,* 2008). Chamomile has an ameliorative effect on hyperglycemia and diabetic complications by suppressing blood sugar levels, increasing liver glycogen storage and inhibition of sorbitol in the human erythrocytes (Kato *et al.,* 2008). Different studies revealed the protective effect of Chamomile on pancreatic beta cells by diminishing hyperglycemia-related oxidative stress (Cemek *et al.,* 2008).

A strong antioxidant effect of Chamomile was supporting to the opinion that, oxidative stress have the effect in the Glucose Transport Protein (GLUT) or at insulin receptor increasing serum glucose levels and scavengers of oxidative stress and thus may have an effect in reducing the increased serum glucose level in diabetes (Jacqueline *et al.,* 1997; Dallak *et al.,* 2009 a &b). All these findings agree to previous researches carried out on different Chamomile species or extracts. Kato *et al.,* (2008) found that drinking Chamomile tea has an ameliorative effect on the hyperglycemia and diabetic complications via suppressing blood sugar levels and increasing liver glycogen storage. Besides, Eddouks *et al.* (2005) have reported a potent hypoglycemic effect of Chamomile extract in diabetic rats, an effect that is independent on insulin secretion. Moreover, on other hand studies revealed that the protective effect of Matricaria chamomilla extract is on pancreatic β cells by diminishing hyperglycemia-related oxidative stress (Cemek *et al.,* 2008). Chamomile is considered as one of the richest

sources of dietary antioxidants and its hepatoprotective effect on hepato-renal damage can be seen in diabetic rats. Also, there is evidence that the oxidative damage occurs in the skins, membranes, proteins and DNA can be managed by these compounds which have suppressive activity by inhibiting free radical scavenging activity and thus make protection against chronic health disorders such as atherosclerosis and hypertension (Emam, 2012).

Histopathologic and immunohistochemical investigations in the present work supported the above-mentioned results and observations as diabetic rats revealed that some of the islets cells showed decrease cellular population with degeneration and necrosis of some of the beta cells, immune–histochemically, Pancreas of diabetic rats (45 mg STZ/kg BW, Single IP) showed 25-30% /HPF positively reacted and stained cells with insulin diabetic marker, the remaining cells 70-75 %/HPF were negatively stained. Other islet cells (α and δ-cells) were also unstainable. STZ is diabetogenic through its toxic effects in pancreatic β cells and as a potential inducer of oxidative stress. It inhibits the production of insulin by destroying the insulin-producing β cell and thus inducing necrosis and so that leads to hyperglycemia (Eleazu *et al.*, 2013).

Though remarkable progress achieved in the management of diabetes mellitus using synthetic drugs, still management of diabetes and its complication is an unsolved problem. Many studies are trying to find the substances that naturally effective in the treatment of diabetes and its complication, as certain compounds present in plants can modulate β-cell apoptosis and enhance the action of insulin (Modak *et al.*, 2007). On Other hands, there studies shown that there are several natural compounds can suppress the activity of certain enzymes involved in glucose production and absorption (Schmidt *et al*., 2008)

In our investigation, most of the pancreatic islets in diabetic, Chamomile treated rats were large in size or of normal sizes with apparently normal active cellular contents. Sections of pancreas of diabetic rats (45 mg STZ /kg BW, single IP) treated with Chamomile (200 /kg BW, daily oral dose for 3weeks), showed an enhanced ameliorative effect of Chamomile, as the positively stained cells for insulin marker in most of the examined sections were ranged from 85-90 %HPF cells, a few cells (10-15%/HPF cells appeared weakly positive or unstained. Our data agree with the results obtained by many investigations which have reported that Chamomile is a popular herb and has been used for thousands of years in ancient Egypt, Greece, and Rome and its phytomedicinal application is on its antioxidant, anti-inflammatory, antiseptic, antispasmodic and wound-healing effects Heinle *et al.* (2006), especially in gastrointestinal disorders Hoheneste et al . (2004), and other few studies showed antidiabetic effects of diverse, mainly from water Chamomile extracts, and the underlying mechanisms of action are still poorly understood (Singh *et al.*, 2011).

Moreover, our results are in harmony with Khan *et al.* (2014) as they postulated that Chamomile is considered as one of the oldest and considered as a popular medicinal plant. It has shown to be an anti-inflammatory and antioxidant especially in floral part since ancient time. Its potential activity appears to lower blood sugar levels in hyperglycemia. Actually, the role of the oxidative stress in inducing the inflammation and apoptosis may have an important role which causing

histological deteriorations since the STZ-induced diabetic rats and their results exhibited a remarkable increase in the expression of immunohistochemically-detected pro-inflammatory 331 cytokine, TNF-α and apoptotic markers including P53 and caspase-3 (Osama *et al.*, 2019).

In the present study it was indicated that, the expression caspase-3 and insulin were remarkably increased in diabetic rats and were decreased as a result of treatment with Chamomile, and this proved the fact that the use of Chamomile with other plant extracts induced a more synergistic enhancing effects of the aqueous extracts of Chamomile and oregano and made it useful in treating hyperglycemia and related diabetic complications. The mixture of Chamomile and Oregano extracts produced a medical product with a potent anti-diabetic activity (Rajagopalan *et al.*, 2016). Finally, our results showed high expression of the apoptotic marker caspase 3 Further studies are necessary to elucidate the exact molecular mechanism involved in anti-diabetic action of Chamomile.

Conclusions

Chamomile extract used as popular medicinal plant many studies indicate it's anti-inflammatory and antioxidant activity, this study showed a remarkable antdiabetic effect through elevation of histological parameter in kidney, liver and immunohistochemical parameter pancreas against STZ- induced diabetes in rats**.**Altan N., Sepici-Dinc¸el A., Koca C. (2006). Diabetes mellitus and oxidative stress.Turk J Biochem 31:51–56

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