

تأثير المستخلص المائي لنبات إكليل الجبل على كلية وكبد ذكور الجرذان المصابة

تجريبيا بداء السكر

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الخلاصة

صممت الدراسة الحالية لمعرفة تأثير المستخلص المائي لإكليل الجبل على بعض الأعضاء مثل الكلية وكبد الجرذان المصابة بداء السكر تجريبيا، قسمت ثمان وعشرون من ذكور الجرذان إلى أربع مجاميع جرعت المجموعة الأولى بالماء المقطر وأعتبرت كمجموعة سيطرة وجرعت المجموعة الثانية بالمستخلص المائي لإكليل الجبل وجرعت المجموعة الثالثة بالألوكسان لإستحداث داء السكر تجريبيا فيما قد أصيبت المجموعة الرابعة بالسكر تجريبيا ثم جرعت بالمستخلص المائي . لوحظت تغيرات نسجية في الكلية تمثلت بإنكماش ونزف وتنخر وتنكس الكبيبات الكلوية وتوسع حيز محفظة بومان وتنكس وتنخر النيببات الكلوية وإختفاء الكبيبات مع تجمع الخلايا الإلتهابية ثم تطورت التغيرات النسجية وصولا لموت الكبيبات وإنسداد تام لقنوات النيببات الكلوية وإحتقان وريدي وفرط التنسج . كما سبب إستحداث داء السكر بعض التغيرات النسجية في الكبد كتجمع الخلايا الإلتهابية والخزب وإحتقان الوريد المركزي وتنكس وتنخر وتفجى الخلايا الكبدية ونزف دموي وتوسع الجيبانيات إضافة إلى التليف . كما بينت النتائج إن معاملة الحيوانات بمستخلص إكليل الجبل سبب إنخفاضا في التأثيرات النسجية لداء السكر التجريبي .

الكلمات المفتاحية : داء السكر ، المستخلص المائي ، إكليل الجبل.

Introduction

Rosemary (*Rosmarinus officinalis*) is a small ever-green bush , belonging to the Labiatae family. It grows principally in the basin of the Mediterranean Sea, while in Poland it is usually cultivated in pots . Active substances present in *Rosmarinus* yield it a series of properties, desirable from the point of view of the food industry and medicinal phytology (Djeddi et al., 2007; Ruminska and Ozarowski,1990) . Its herb and oil are commonly used as spices and flavoring agents in food processing for its desirable flavor and high antioxidant activity (Derwich et al.,2011 ; Woyengo et al.,2009) . Rosemary was stated to act as a mild analgesic and antimicrobial agent in traditional herbal use (Zangana , 2016 ; Trindade et al.,2010) .

Diabetes mellitus (DM) is the most common clinical syndrome characterized by hyperglycemia resulting from defect in insulin secretion, action or both (Rother,2007; Punnose,1997) . There are many classical signs of diabetes mellitus as weight loss, Ravenous appetite, increased water consumption and increased urination (Paresh- Dandona et al.,2003) . Diabetes mellitus this disease is one of the leading causes of blindness, atherosclerosis and kidney failure , these and other pathological conditions are called complications of diabetes, this is can be divided into two type : Acute complications and chronic complications (Myers ,2002) .

Today, diabetes mellitus is the most common of metabolic disorders and it is a major threat care worldwide (Mandrup- Poulsen,2008) . It's considered as the top ten dangerous diseases in the whole world but it is in the first five orders of the dangerous diseases .

The aim of the present study was to examine the effect of aqueous extract of rosemary on histological changes in diabetic male rats .

2- Materials and Methods

Plants Samples Collection

Rosemary leaves were obtains from some-one lawns in local markets in Thi-qar province , the plant was grind by electric grinder and saving the powder in clean plastic bags .

Preparation of Aqueous Extract

Aqueous extract was prepared by weight (6.25) gm from powder of leaves plant that dissolvent in (250) ml of distilled water on heat . The mixture were filtered by two layer of gauze and centrifuged at 3000 cycle/ minute for 10 minute . The extract again filtered by buchner funnel and using filter paper (Kuo et al.,2011; Rashan et al.,1992) .

Induction of Diabetes in Animals

Experimental diabetes was induced in rats by intraperitoneal (IP) injection of the concentration (125mg/kg) body weight (that prepare before indirect using by dissolvent 125 mg

alloxan in 1ml distilled water) about 0.5ml for any animal (Alarcon-Aguilara et al.,2002) . Again intraperitoneal injection 5ml glucose (20%) ,the water displaced by glucose (5%) for one day to inhibition happening acute deficiency diabetic resulted from destroy pancreas that lead to bane it . Diabetes was occur after seven days from injection .

Laboratory Animals

Twenty eight male rats (*Rattus norvegicus*) were (8-10) weeks old . The rats put in plastic cages respective of breeding it and the rats were randomly selected and divided into four equal groups (7rats/cage) :

- 1-**The first group** :They were gulped with distilled water which considered as the control group.
- 2- **The second group** : Administered (0.2mg/ml) for animal every day from aqueous extract of rosemary, that was continued to end of the experiment period .
- 3- **The third group**: (Infected group) experimentally diabetic by alloxan ,that received food and water to end of the experiment period .
- 4- **The fourth group** : diabetic group and treatment by (0.2mg/ml) for animal every day from the aqueous extract of rosemary.

All the animals were gulped orally by stomach tube , after 30 days of administered , the animals were sacrificed by cervical dislocation, (small pieces of tissues of kidney and liver were removed for histological study by fixing in 10% formaldehyde , the specimens were dehydrated in ascending grades of ethanol, cleared with xylene and embedded in paraffin wax, sectioned to get 5 μ m sections and stained with haematoxylin- eosin stain) (H&E) according to the method of Bancroft and Gamble (2008) .

Results

1-Kidney

Histological examination of the kidney of control rats or rosemary group treated ones revealed entirely normal histological features that showed normal glomeruli and patent capsular space surrounding proximal and distal convoluted tubules Figure (1) , Figure (2) . Examination of the kidney sections of diabetic group showed contraction of glomeruli , enlargement of space Bowman's capsule and most of the renal tubules were damaged by degeneration and necrosis of their Figure (3) . Diabetic caused some histological damages to the kidneys such haemorrhage , absence of glomeruli and aggregation of inflammatory cells Figure (4) , degenerative and necrosis of glomeruli and renal tubules Figure (5) . Figure (6) showed death of glomerulus , perfect closed to canal of renal tubules while Figure (7) showed hyperplasia , necrosis of renal tubules and venous congestion .

Treating animals with diabetic and aqueous extract of rosemary revealed an improvement in the histological appearance of the kidney with venial contraction of glomeruli , most of the renal tubules appeared normal but few tubules were damaged by degenerative and necrosis Figure (8) , natural glomeruli , simple haemorrhage and venial infiltration of lymphocytes Figure (9) , Figure (10) respectively .

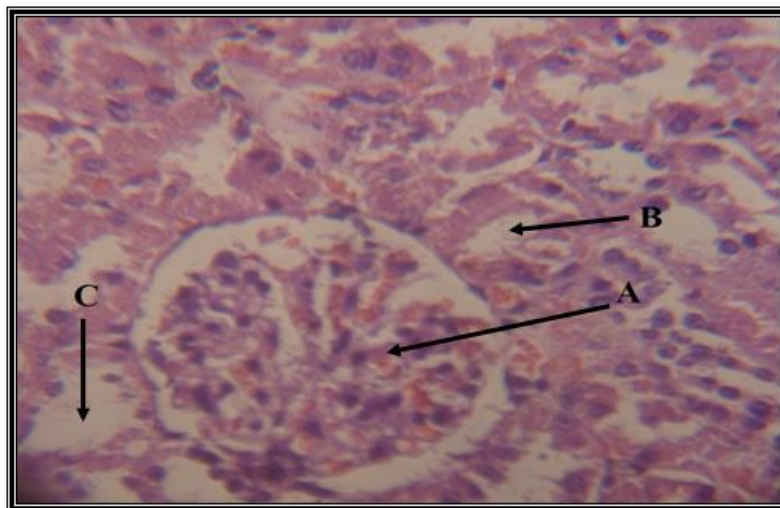


Fig.(1): Section in the kidney cortex of **control** group showing glomerulus (A) renal tubules proximal (B) and distal (C) (H&E) (760 X) .



Fig.(2) : Section in the kidney cortex of **aqueous extract of Rosemary** group showing normal glomeruli (A) and renal tubules (B) (H&E) (400 X) .

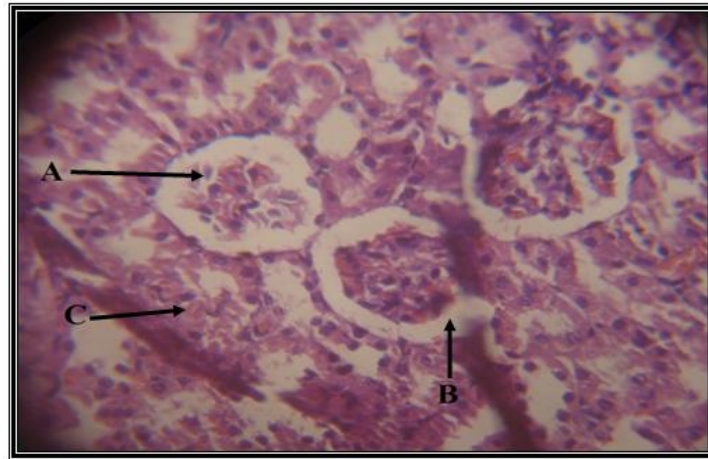


Fig.(3): Section in the kidney cortex of **diabetic** group showing contraction of glomerulus (A) enlargement of space Bowman's capsule (B) and degeneration of renal tubules (C) (H&E) (640 X) .

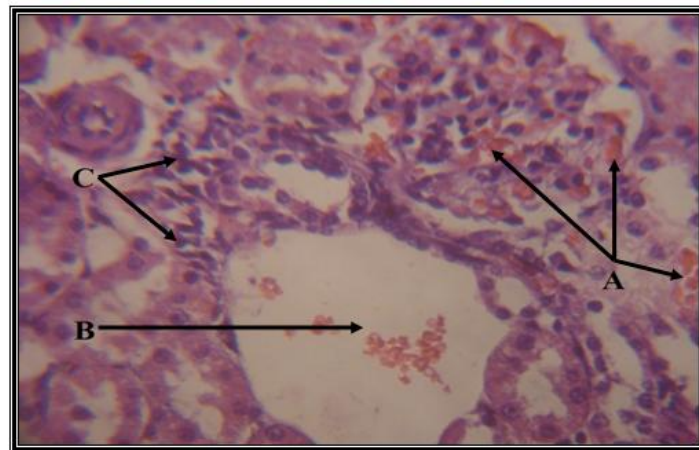


Fig.(4): Section in the kidney cortex of **diabetic** group showing haemorrhage (A) absence of glomerulus (B) aggregation inflammatory cells (C) (H&E) (840 X) .

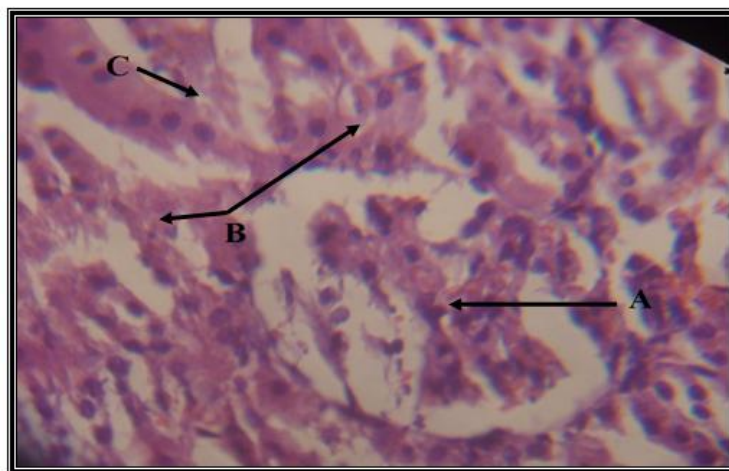


Fig.(5): Section in the kidney cortex of **diabetic** group showing degenerative and necrosis of glomerulus (A) degenerative of renal tubules (B) necrosis (C) (H&E) (1200 X) .

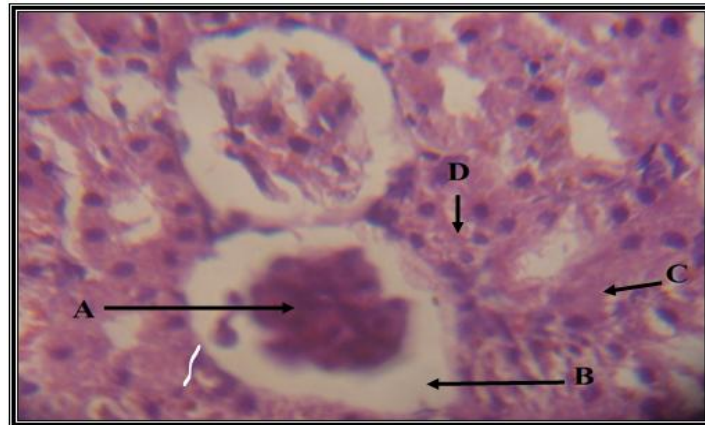


Fig.(6): Section in the kidney cortex of **diabetic** group showing death of glomerulus (A) enlargement of space Bowman's capsule (B) degenerative of renal tubules (C) and perfect closed to canal of renal tubules (D) (H&E) (1200 X) .

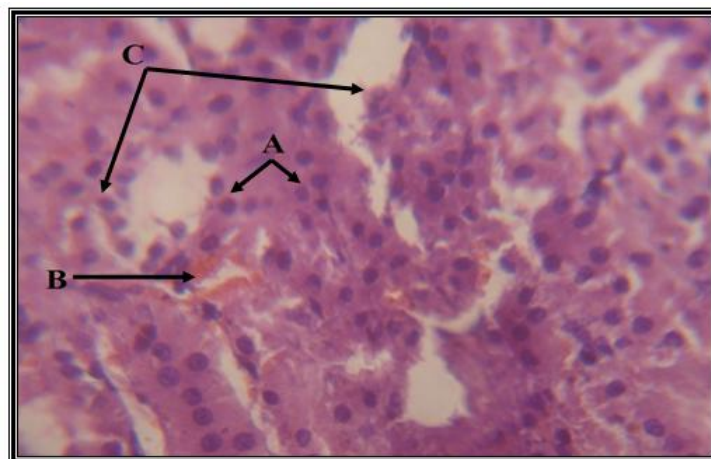


Fig.(7): Section in the kidney cortex of **diabetic** group showing hyperplasia of renal tubules (A) venous congestion (B) necrosis of some renal tubules (C) (H&E) (600X) .

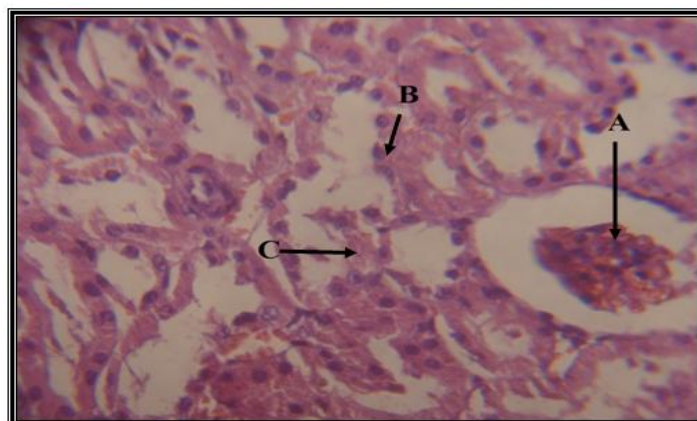


Fig.(8): Section in the kidney cortex of **diabetic group and treatment from aqueous extract of Rosemary** showing venial contraction of glomerulus (A) some statuses of degenerative (B) necrosis in renal tubules (C) (H&E) (800X) .

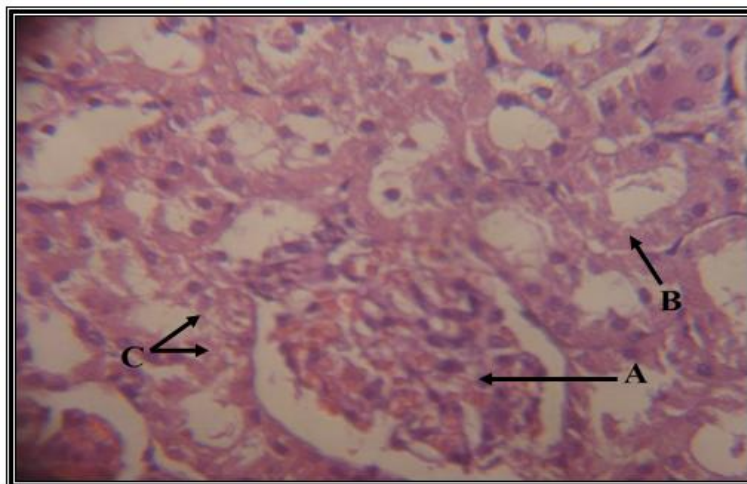


Fig.(9): Section in the kidney cortex of **diabetic group and treatment from aqueous extract of Rosemary** showing natural glomerulus (A) venial necrosis (B) degenerative in renal tubules (C) (H&E) (1040X) .

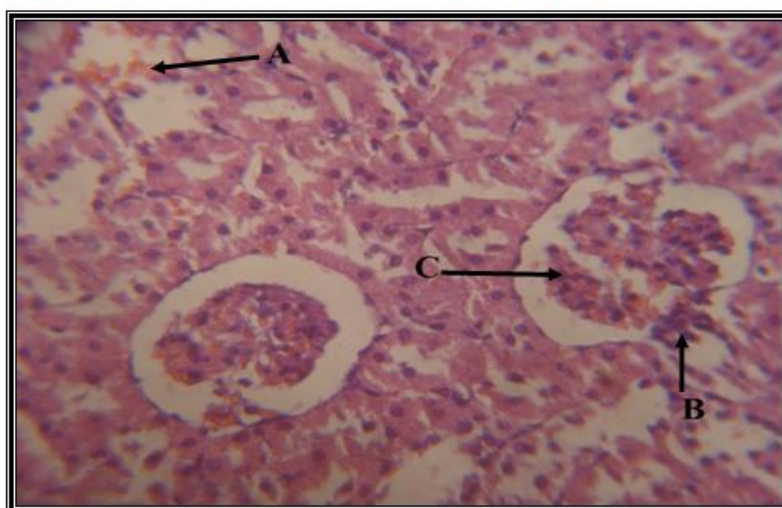


Fig.(10): Section in the kidney cortex of **diabetic group and treatment from aqueous extract of Rosemary** showing simple haemorrhage (A) venial infiltration of lymphocytes (B) noticing natural glomerulus (C) (H&E) (1040X) .

2- Liver

When viewed microscopically , the liver of the control rats or rosemary group only showed normal structure of hepatocytes radiating from the central vein to the periphery of the lobule , hepatocytes were separated by narrow blood sinusoids Figure (11) , Figure (12) .

The histological examination of liver sections of diabetic group showed aggregation inflammatory cells , edema in central vein , necrosis and degeneration of hepatocytes Figure (13) . Figure (14) showed haemorrhage and enlargement of sinusoids while Figure (15) showed congestion

of the central vein , vacuolation of hepatocytes . Figure (16) revealed to clearly edema, necrosis , degeneration and noticing fibrosis Figure (17) .

The treatment with rosemary was improved in histopathological changes and the severity of histopathological changes was decreasing that caused by diabetic , the sections of livers of diabetic group and treatment from aqueous extract of rosemary showed simple haemorrhage in central vein , degeneration , necrosis and aggregation of inflammatory cells Figure (18) , Figure (19) while the cells appeared healthy with natural central vein Figure (20).



Fig.(11): Section in the liver of **control** group showing central vein (A) radial form of hepatocytes (B) (H&E) (1320X) .



Fig.(12): Section in the liver of **aqueous extract of Rosemary** group showing normal structure of tissue central vein (A) natural hepatocytes and sinusoid (B) (H&E) (1400 X) .

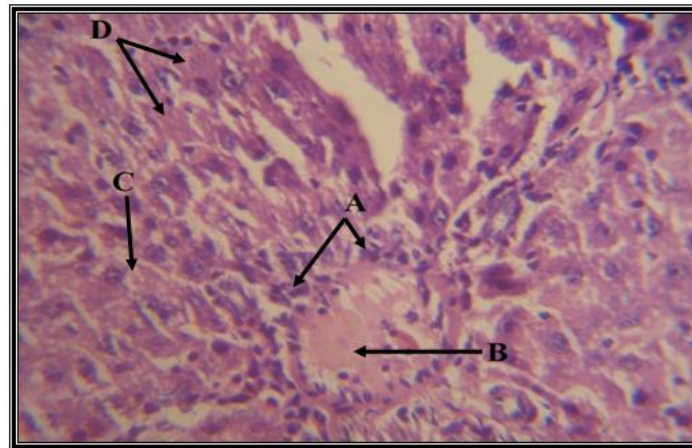


Fig.(13): Section in the liver of **diabetic** group showing aggregation inflammatory cells (A) edema in central vein (B) necrosis (C) degeneration of hepatocytes (D) (H&E) (920 X) .

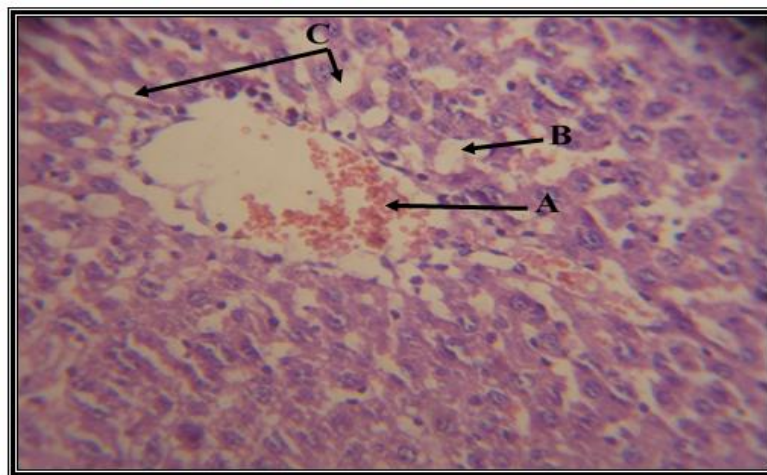


Fig.(14): Section in the liver of **diabetic** group showing haemorrhage (A) necrosis of cells (B) enlargement of sinusoids (C) .(H&E) (720 X) .

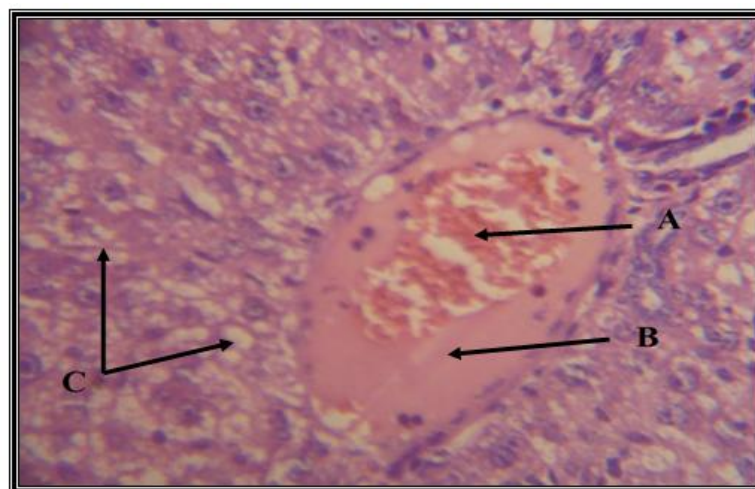


Fig.(15): Section in the liver of **diabetic** group showing congestion of the central vein (A) edema (B) vacuolation of hepatocytes (C) (H&E) (1040 X) .

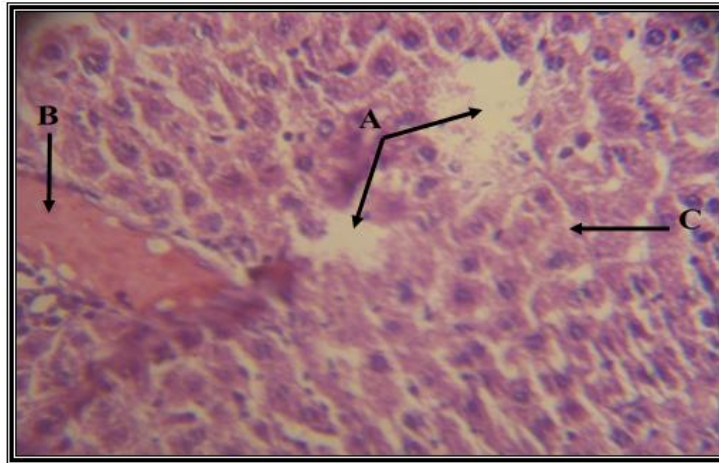


Fig.(16) : Section in the liver of **diabetic** group showing atrophy (A) congestion (B) degeneration (C) (H&E) (960 X) .

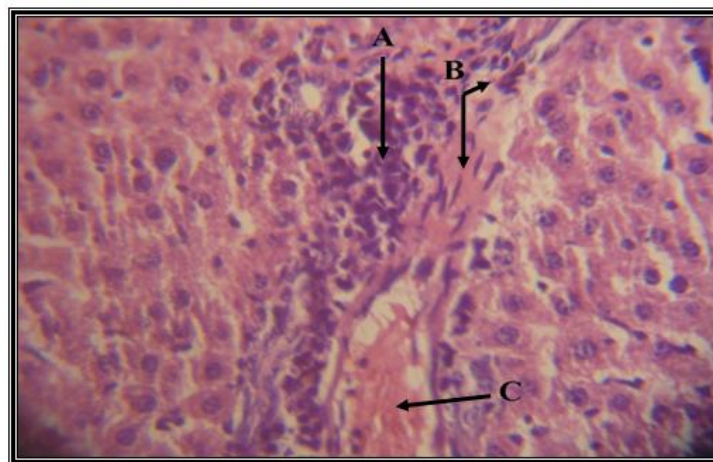


Fig.(17) : Section in the liver of **diabetic** group showing acute aggregation inflammatory cells (A) fibrosis (B) haemorrhage (C) (H&E) (960 X) .

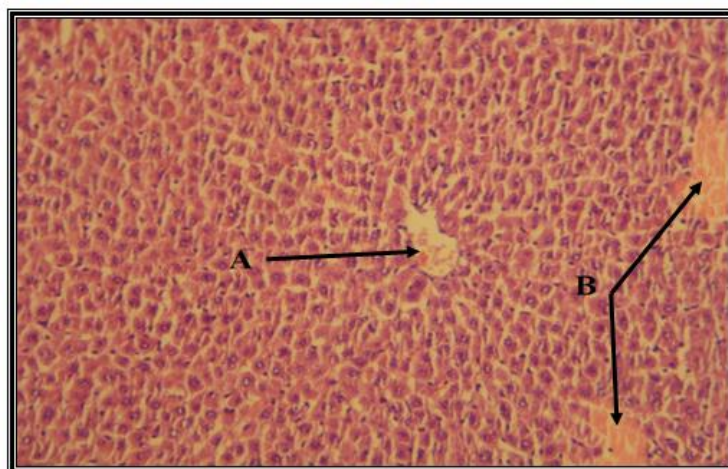


Fig.(18) : Section in the liver of **diabetic group and treatment from aqueous extract of Rosemary** showing simple haemorrhage in central vein (A) venial degenerative (B) (H&E) (1120 X) .

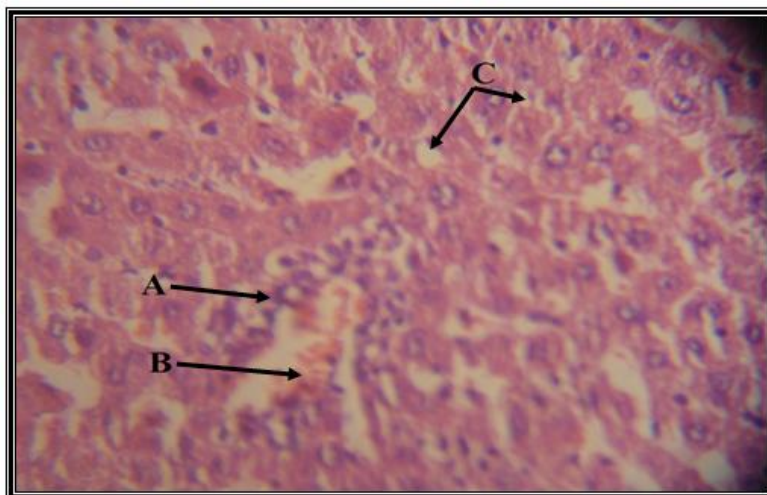


Fig.(19) : Section in the liver of **diabetic group and treatment from aqueous extract of Rosemary** showing aggregation inflammatory cells (A) simple haemorrhage (B) necrosis (C) (H&E) (880 X) .

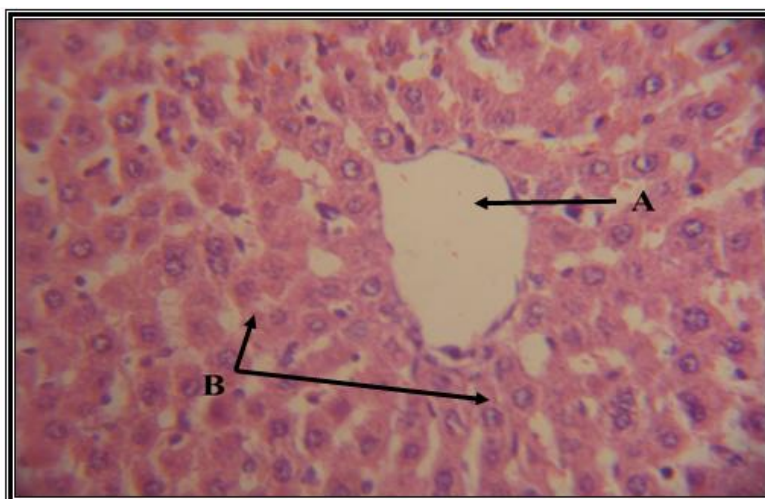


Fig.(20): Section in the liver of **diabetic group and treatment from aqueous extract of Rosemary** showing natural central vein (A) return of tissue to natural status (B) (H&E) (720 X) .

Discussion

1-Kidney

The histological changes in kidney after induced diabetic showed contraction and absence of glomeruli , enlargement of space Bowman's capsule , degeneration the renal tubules . That effect of glomeruli by diabetic mellitus may be back to continuance effect high pressure of filtration on elastic of glomeruli consequently to happen interstices in glomeruli (Stadler et al.,1960) . Diabetic caused some histological changes to the kidneys such as haemorrhage because of increasing of arterial blood pressure and happening disturbances in lipoproteins that lead to effects in blood vessels (Ali et al.,2002) . Degenerative, necrosis and death of glomeruli , necrosis and perfect closed to canal of

renal tubules that histological changes caused by diabetic . Bailes (2002) and Varghes et al . (2001) showed diabetic lead to damage in organs as kidney caused destroying and failure in kidney functions . Dubois et al.(1997) and Bailes (2002) noted that diabetic caused damages in organs and disturbance in their functions as kidney because of intransigency in glomeruli and increasing thickness to the internal membranes of it by chronic hyperglycemia . Perhaps there is explanation of these changes related to free radicals formation in diabetic group , Moussa (2008) reported in diabetes patients glucose level in blood is arises that causes lipid peroxidation and oxidative stress by increasing production of free radicals especially reactive oxygen reactive (ROS).

The histological changes of kidney after treatment by aqueous extract of rosemary in present study showed venial contraction of glomeruli , most of the renal tubules appeared normal form with natural glomeruli and simple infiltration of lymphocytes and aggregation inflammatory cells , the result can belong to leaves of rosemary possess a variety of bioactivity including anti-inflammatory actions (Altinier et al., 2007) or may be because the rosemary is important in melioration action of kidney and protect it from diseases by dismissal of toxins , McCord (2000) showed that rosemary contains many antioxidants which strafes free radicals resulted from diabetes . The current study revealed that rosemary alleviated the renal change that caused by diabetic .This was manifested by normal appearance of kidney tissue.

2-Liver

The results indicated that diabetic caused many histopathological changes in liver . These changes included aggregation inflammatory cells , edema in central vein , necrosis and degeneration , vacuolation of hepatocytes and noticing fibrosis. These results belong to diabetes because the liver is among organs that more sensitive to high levels of glucose (Abdollahi et al., 2010) . The effects on hepatocytes may be happen by free radicals that resulting from alloxan exposure , the free radicals have responsible of appearance and development the infection of liver which lead to necrosis and apoptosis of hepatocytes (Vitagliona et al., 2004) and this agreement with Zrustova and Rostlapil (1966) who reported that diabetic has effect in liver and caused fibrotic in some tissues of liver . Diabetic group showed haemorrhage and enlargement of sinusoids and congestion of the central vein that may be back because of disturbance blood vessels by diabetic cause change in coagulation and liquid of blood (McMillan and Barbara ,1975) . All upper histopathological that observed in liver accepted with Mahdi and Abdul-Latif (2014) who found that induction diabetes led to many changes in liver as necrosis , pyknosis of hepatocytes and congestion in central vein .

The treatment with rosemary was improved in histopathological changes in liver that showed natural hepatocytes. This good result belongs to rosemary and is accepted with Faixova and Faix (2008) who found that rosemary extract has importance in hepatoprotective. Most experimental studies showed rosemary has protective role against liver diseases because this plant has antioxidant properties due to the presence of carnosic, carnosol, rosmanol, epirosmenol, methyl carnosate and rosmarinic acids in it (Al-sheyab et al., 2012; Gonzalo et al., 2012). Rosemary is rich in phytochemical derivatives such as triterpens, flavonoids or poly phenols and these compounds were present in leaves while the rosmarinic acid found in all organs of plant (Del Bano et al., 2003). Many studies reported that the preventive effects of rosemary and its extracts are attributed to its antioxidant activity (Zeng and Wang, 2001). Similarly, Ahmed and Abdella (2010) reported that rosemary prevents histopathological lesions and oxidative stress in liver of mice.

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