International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.12, pp 489-500, 2016

Iraqi Propolis as antioxidant and protective therapy for kidney failure treatment of diabetic rats

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Abstract : Abstract :Seventy five rats were divided into five groups (n=15),namely control group, group stimulating diabetes after 3 weeks of the beginning of the experiment, a group of natural drenched ethanolic extract of local propolis (EEP) daily for 6 weeks, a group drenched (EEP) for 3 weeks then is stimulated diabetes for 3 more weeks, and the fifth group is stimulated diabetes after 3 weeks of the beginning of the experiment and then drenched (EEP) for 3 weeks after stimulation.

At the end of the experiment, collected samples of blood serum were used to evaluate the oxidative status by assessment the Superoxide Dismutase(SOD), Catalase(CAT), Glutathione-s-Transferase (GST), Malondialdehyde(MDA), Nitric Oxide (NO), as well as the extent of the injury was appointed renal failure of cells to measure the level of uric acid(UA) and serum total protein(STP). Observed results were shown a significant decrease in body weight, effectiveness of the antioxidant system, and protein level in the serum, a significant increase in the level of glucose in the blood, an increase in the concentration of malondialdehyde, Nitric Oxide, and Uric acid in the serum for the diabetic group . On the other hand, the EEP has had a significant effect in reducing the level of glucose, MDA, NO, Uric acid and the occurrence of a significant increase in the effectiveness of the system antioxidant and the concentration of total serum protein($P < 0.05$), and improve the structure of the tissue of the kidney.

It can be concluded that the extract has antioxidant effectiveness and reduce the level of sugar in the blood and thus protect the overall impact of its complications such as kidney failure.

Keywords: Iraqi Propolis, antioxidant, protective therapy, kidney failure, diabetic rats.

Introduction

Diabetes is a metabolic disease characterized by a high level of sugar in the blood as a result of several reasons, including a lack of insulin secretion or in the effect of insulin, or both .

Continuous exposure to high blood sugar as a result of this disease for a long time cause many serious complications including damage or dysfunction or failure of various organs of the body, especially the eye, kidney, nerve, heart and blood vessels. Renal failure is one of the major complications of any type of diabetes [1], where there are several mechanisms causing the evolution of kidney failure caused by diabetes, such as:

Increase the formation of Advanced glycation end products (AGEs), increase the effectiveness of protein kinase (PKC), increase the effectiveness of Polyol pathway mechanism, as well as Autoxidation, all these mechanisms stimulating oxidative stress, and thus the incidence of complications causing renal failure [2],soincrease the proportion of glucose causing increase the oxidative stress inside the vesicles in the kidney where the effect will be direct because the vesicles are the target cell in the case of kidney failure, also the

oxidative stressis consider the reason of oxidation of DNA and stimulate mRNA to increase in gene expression for NFK-Bgene (Nuclear Factor Kappa-Beta gene), which stimulates the production of inflammatory proteins such as TGF-B(Tumor growth factor-B) , Laminin, fibronectin, (interlukine1,6) IL-6, IL-1) [3].

There are many plants and natural products have the ability to influence on the defense system, whether antioxidant or immune system because they contain many active compounds, such as flavonoids and phenols and esters and important elements such as Cu, Mg, Ca, Na, Which owns the effectiveness of a biological and physiological as well as the positive and characterized by non-toxicity [4].

In this study, Iraqi propolis was used of to demonstrate the effectiveness as antioxidant and its ability to protect the kidney from failure.

Materials and Methods :

Experimental animals :

Seventy-five rats type (Sprague-Dawley), weighting between 150 ± 10 gm used during this study .Allowed rats adaptation in the House of animal before the experiment for a week where fed standard food and water under conditions of temperature 22-25C° underwent medical tests before the beginning of the experiment to make sure of the safety of them healths .

Ethanolic extract preparation :

Collectedpropolis from different areas in the city of Diwaniyah / Iraq and cut into small pieces and then grind to a powder, then sift the powder to get thesuitable size, then getting pure raw propolis depending on the way of[5],and then conducteddescriptive tests depending on the way [6], to make sure the presence of biologically active compounds and antioxidant.

PreparationStreptozotocin :

STZ was prepared by method of (Sachin et al, 2009)[7].

Stimulation diabetes :

Stimulating diabetes by injection fasting rats for more than 16 hours with a single dose of STZ (ip) concentration of 60 mg / kg of the weight of the rat. STZ prepared in buffer solution of citrate (PH 4.5) and prepared immediately before injection .Been confirmed by high levels of glucose in the blood after 72 hours after injection using a device (Accu-Check), and the result depends on the fifth day after the injection is when the glucose levels in blood was fixed, where is the Diabetic Rats at the rate of 200mg / dl .

Ethanolic extract of propolis is used as a preventive and curative against diabetes.

Sort experimental animals:

All rats were divided randomly into five groups, fifteen rats per group were distributed as follows:

1. Control group(G1): intact rats drenched orally with drinking water (10 ml / kg.b.w) once daily for six weeks, including stopped at the end of the third week for five days after single injected of citrate buffer (3ml / kg.b.w) .
2. Diabetic rats group(G2): rats were drenched drinking water ((10ml / kg.b.w) for three weeks, then injected with a single dose of a solution of STZ for rats after fasting for the whole night, and then make sure to stimulate diabetes after five days, re-dosing with drinking water (10ml / kg.b.w) for three weeks as well .
3. Treatment intact rats(G3): drenched with EEP at dose of (200mg /kg.b.w) for three weeks, then injected of citrate buffer (3ml / kg.b.w) once, then stopped for five days continued in drenching EEP for other three weeks .
4. Diabetic with pre-treated of EEP(G4): rats were pre-treated with local EEP (200 mg/kg. b.w) for three weeks, then induced diabetes by injected with a solution of STZ (60mg / kg.b.w) and then after induction of the disease (after five days) was continued drenched orally with drinking water for three more weeks .

5. Post-treated diabetic rats(G5): rats were drenched with drinking water (10ml / kg.b.w) for three weeks and then after five (5) days of induction of the disease, post treated with EEPat a dose of (200 mg / kg.b.w) daily for other three weeks .

Every three days body weights have been registered for all groups, and blood glucose level of the diabetic rats groups (G2, G4, G5) were measured every three days .

After 49 days from the beginning of the experiment,all groupswithheld from food for 12 hours before blood samples collection.All rats were anesthetized by injection xylazine and Ketamine (10mg and 50 mg / kg, respectively), then collected blood samples andsacrificed animals, samples from kidney in all groups have been quickly and fixed in 10%neutral buffered formalin to microscopic examination then were separated serum to hold the following tests:

1. Appointment Serum SOD effectiveness depending on the method (Misra andFridovich, 1972) [8].
2. Activity of Serum CAT depending on the method (Aebi, 1974)[9].
3. Activity of Serum GST depending on the method of (Habig et al, .1974) [10]
4. concentration Serum MDA depending on the way (Guide and Shah, 1989) [11].
5. Serum NO concentration depending on the method of (Hortelano et al,. 1995) [12].
6. Serum UA depending on the method (Fossati et al, 1980) [13].
7. Conc. of STP depending on (Biurets et al,. 1999)[14].

As well as the samples were tested microscopically where adopted sections depending on the preparation method (Lee, 1968) [15] and the formulation of microscopic sections depending on the method (Wood and Eilis, 1994)[16].

Statistical analysis :

All results totals read statistically by the program SPSS (2010) Version 17Soft wave, method of choice included (ANOVA) for comparison between groups, followed by (LSD) for comparison between groups, the value of ($P < 0.05$), as well as ± (standard error) (SE) Standard error .

Results and Discussion :

1-Body weight :

At the beginning of the experiment ,the weights of rats are convergent (150 + 10gm) until the twenty one day, but after stimulation diabetes, in the day (21), there is a discrepancy in the weight gain, the first group (98.42 gm), second (22.17 gm), third(100.01 gm), forth (45.2 gm) and the fifth Group (75.41 gm) after another twenty days (as shown in Figure 1). Through the results did not prove that the propolis has any effect on weight gain in rats natural (G1, G3) and this corresponds with the results of the researchers (El-Nahrawy et al,. 2012)[17], but contrasts with the results of researchers (Denliet al,.2005)[18], whom shown increase in body weight when propoliswas used in coturnix birds, but in treatment diabetic groups (G4, G5) found significant amelioration in the body weight compared to untreated diabetic group (G2) and this can considering that propolis is antioxidant and free radicalsscavenging[19], and thus is considered as regulator for any metabolic defect accompanies diabetes.

The level of sugar in the blood :

Blood sugar level in G2(diabetic group) was the highest compared to all groups at the end of the experiment ($P < 0.05$), but there was no significant difference between the first group and the third(G1,G3), the groups (4,5) (protective and treatment) were significant differences, including treated group (5) a significant decrease in the level of blood sugar relative to the preventive group($P < 0.05$), as shown in table (1).

The results show that the dosage propolis for the diabetic rats either before or after the induction of the disease has helped in reducing the level of sugar in the blood, especially for the treatment group were more effective than the group preventive, but stayed there significant differences($P < 0.05$) between groups (G4, G5) with natural groups (G1, G3), where not up to the normal limit.

Conformed these results with (Matsue et al,. 2004)[20] where shown results that the propolis effective countermeasures to rising diabetes, where that flavonoids and poly phenols which is one of the key elements of propolis, which owns the effectiveness of anti-oxidant and is considered as a catalyst for the defense system in the tissues of the pancreas [21,22,23]. There are also studies conducted by the researcher (Gray et al,.2000) [24] proved that tannic acid is the catalyst for the secretion of insulin and this component has proved its existence by descriptive statements of local propolis .

The level of antioxidant enzymes (SOD, CAT, GST):

The study showed that there was a significant decrease in the effectiveness of these enzymes in the diabetic group to nearly 50% for SOD, and 22% for CAT and 61% for GST, while the treatment patient groups,the activity of SOD reached to double the activity of the diabetic group, so CAT is almost up to increase in the treatment of diabetic groups (G4,G5)nearly forth times in diabetic group, and for GST, there was an increase of more than a third of the value of the effectiveness of the enzyme in the case of illness,that shown in table (2).

The decline in SOD may be due toautomaticoxidative of hemoglobin, which leads to generate free radicals such as superoxide , which leads to the inhibition of SOD[25], as well as could have been the loss of important elements such as zinc and copper, which is found in a few ratio in the case of diabetes, so consequently this leads to decrease the effectiveness of SOD[26], as for Catalase, Increasing the proportion of glucose in the blood as well as free fatty acids formed in diabetes, since the peroxisome and mitochondria play a key role in the fatty acid oxidation and because of Free radicals, which cause to decline of Performance of mitochondria and peroxisome stationed CAT in them, leads to a lack of activity of CAT[27]. So may be the interaction of the protein with glucose, which in turn leads to the formation of compounds containing amino acids could be associated with Active site of enzyme (CAT), which leads to changes in synthetic and process, and this in turn inhibits the effectiveness of the enzyme[28], as for the GST, which is an important factor in the removal of toxins through the inhibition of linked cellular protein, as well as its ability to reduce free radical damage by helping to link for electrophilic site of Glutathione [29], for a decrease of this enzyme leads to a decrease Glutathionereductase because they are interrelated in their work for the analysis of hydrogen peroxide or anyorganic Hydroperoxide .

Therefore, the high glucose leads to consumption Glutathione and lack of presence which leads to the lack of activity of the GST [30], and considering that the kidney is the target organ for a large number of toxins and free radicals therefor GST trying to prevent the crash was caused by the oxidative stress, so the lack of the effectiveness of these enzymes is considered as an indicator of a serious increase oxidative stress[31], therefor is noted decrease the effectiveness of the enzymes in the (G2), but in groups treated either before or after the induction of the disease, we find that there is a marked improvement, especially in the treated group, and this because of the treatment by EEP has improved greatly in the level of these enzymes, propolis containing many important chemical elements such as (Phenyl Propanoids) in addition to the antioxidant compounds[32], as well as there are many research showed that alkaloids contribute effective in reducing the progress of renal failure in streptozotocin-induced diabetic rat [33].

The level of MDA :

This study showed a significant increase in the concentration of MDA in Group diabetic but in treatment groups by EEP showed that there is a marked improvement through lower concentration of MDA, that shown in table (2),where they can instruct the increase MDA to the lack of effectiveness of the antioxidant system and thereby increase the MAD in the case of kidney failure where MDA is considered an important indicator in the diagnosis of vascular complications [34].

These results are consistent with the results of each of the researchers(Al-Sa'aidy et al,.2012[35];Wei et al,. 2010[36]; Osama et al,.2009[37]), where their researches were on rats with diabetes .

The level of Nitric Oxide, Uric acid:

For NO Conc., found that there was a remarkable increase in its concentration in the infected group as well as for Uric acid in the blood, while treated groups recorded a significant decrease in the concentrations ($P < 0.05$) ,that shown in table (2).

Recorded numerous reports of increased NO in diabetic animal models, which stimulates the retinal complications to increase Retinopathy, as well as stimulate the early stages of kidney failure due to increased permeability and integration of renal vesicles [38, 39].

As for the increase in the Uric acid turned out reports and research that cell damage is not due to the Uric acid, but because of free radicals for Superoxide, which produces at the same time by the enzyme xanthine oxidase (XO) [40] and is considered to increase Uric acid important signal to the beginning of kidney failure associated with diabetes [41], and through the results of the study show that the dosage of EEP gave effective results in reducing concentrations of UA, NO where the researcher (Garcia-Mondiavilla et al., 2007)[42], that the dose of Propolis(30mg / ml) inhibits the production of NO by 65% by reducing the gene expression of the inducible nitric oxide synthase for NO (iNOs) where a direct impact on the inhibition of the activity of this enzyme, where the components of propolis from (quercetin, chrysin, galagin, kaempferol)play as inhibitor and a strong reductant for this enzyme. As for Uric acid has been ranked researcher (Soni et al., 2011)[43] a number of natural products such as propolis as an inhibiting factor for xanthin oxidase enzyme in experiments on laboratory animals (either mouse or rabbit), also researcher (Miguel et al., 2011)[44]show that propolis contain relatively high amounts of flavonoids that reduce the impact of the level of superoxide and thereby contribute to the inhibition of the xanthine oxidase, because the flavonoids are linked to Active site of enzyme, as well as the oxidation of xanthine to uric acid will not produce the super oxide when there is a flavonoid compounds, therefor Propolis is a natural enhancer for kidney work, there are plenty of research that proves the effect of propolis on the work of the kidney in the case of kidney failure, such as(Osama etal., 2009[37]; Wei etal., 2011[45]Orsolic etal., 2012[46]).

The level of Serum Total Protein:

As for the concentrations of serum total Protein The results of the study showed the low level of protein in group G2 ($p < o.o5$) compared to the normal group (G1, G3) found that while there is marked improvement, especially in the treatment group G5,that shown in table (2).

Attributed the reasons for decrease STP in the case of diabetes to several reasons, including because of the compounds (Advanced oxidative protein product), as well as due to free radicals, which produces compounds Protein Carbonyl Products (PCO), which is considered a sign or evidence of the crash protein also considered an indicator of a defect in the ability to nomination of vesicles due to increased permeability of the protein and thus lack the level in serum and increase in urine also possible to instruct the lack of the protein to the inhibition of the process of oxidative phosphorylation and thus lead to increase the process of protein catabolism and reducing the level of the amount of protein in the blood (Jerine et al., 2011)[47].

There are many researches horizon the results of this research, including (Yassin et al., 2007[48], Wanke and Wong., 1991[49]), as a researcher (Giurgea et al, 1989[50])the dosage of 20mg / 100 gm.b.w of standard propolis extract (SPE) to the chickens for 15 days caused an increase in the value of total protein as well as the level of globulin, this research showed thatthe purified propolisoverlap withserum protein causing increases the effectiveness of ceruloplasminealso increases the activity of the process of metabolism, especially (anabolic effect) and this in turn leads to increased immune response of the body. As well as (Ali, 1993)[51] showed that a single dose of propolis caused increasing the proportion of total protein for rabbits, and propolis can be used as a strong catalyst immune to humans or animals.

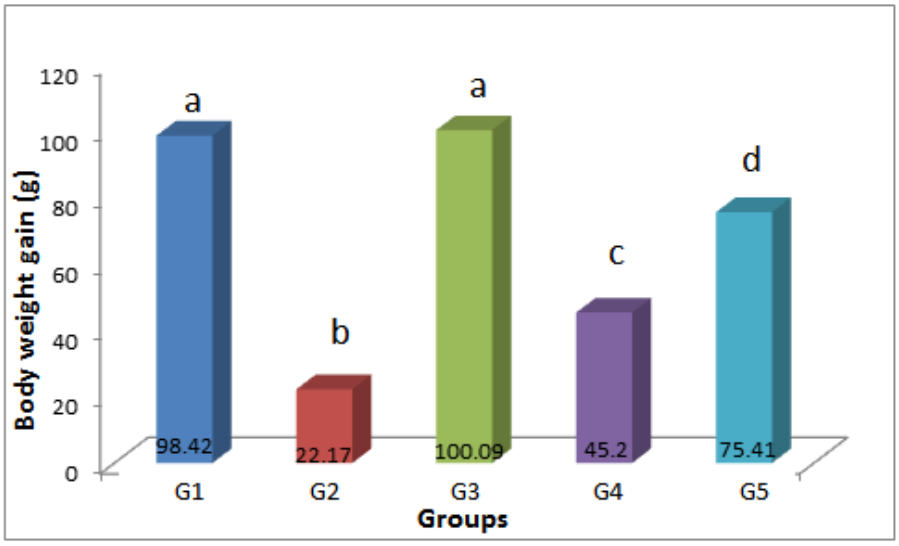

Figure (1): Effect ofEEP on body weight gain (g) after day 21 when induction of diabetic by STZ in male rats.
•G1= Control rats. •G2=Diabetic rats. •G3= Treatment with EEP.
•G4= Diabetic with Pre- treated of EEP. •G5= Diabetic with post- treated of EEP.
•Different letters represent significant difference between groups($p<0.05$).

Table (1): Effect of EEP on Blood Glucose level in STZ- induced diabetic rats.

Days	Control G1	Diabetic rats G2	Treatment with EEP. G3	Diabetic with Pre-treated of EEP. G4	Diabetic with post- treated of EEP. G5
21	93.73∓1.09 **a**	93.06∓1.66 **a**	92.66∓1.78 **a**	91∓1.78 **a**	91.26∓1.79 **a**
27	94.60∓1.29 **a**	485.13∓17.65 **b**	95.73∓1.25 **a**	289.12∓11.35 **c**	446.66∓16.17 **b**
30	96.43∓2.05 **a**	506.5∓15.58 **b**	94.2∓1.56 **a**	296 ∓ 10.01 **c**	395.61∓15.79 **d**
33	91.8 ∓ 1.55 **a**	508.14∓17.38 **b**	91.4 ∓ 1.74 **a**	301.86∓12.26 **d**	366.46∓13.89 **d**
36	92.3 ∓ 1.59 **a**	505.85∓17.12 **b**	87.94∓2.99 **a**	294.73∓10.74 **c**	354.26∓14.25 **d**
40	92.46∓1.60 **a**	512.56∓13.56 **b**	90.8 ∓ 2.01 **a**	302.2∓10.65 **d**	324.13∓14.28 **d**
43	96.1 ∓ 2.11 **a**	508.68∓14.98 **b**	91.81.681 **a**	301.21∓10.65 **d**	299.86∓12.4 **c**
46	91.7 ∓ 1.27 **a**	514.16∓16.31 **b**	92.5 ∓ 1.69 **a**	302.71∓9.79 **d**	275.53∓9.44 **c**
49	91.3 ∓ 1.79 **a**	515.81∓16.33 **b**	91.2 ∓ 2.04 **a**	301.13 ∓ 11.4 **d**	256.66∓6.24 **c**

•G1= Control rats. •G2=Diabetic rats. •G3= Treatment with EEP.
•G4= Diabetic with Pre-treated of EEP. •G5= Diabetic with post- treated of EEP.
•Different letters represent significant difference between groups($p<0.05$).
•Values represent mean∓standard error.

Table (2): Effect of EEP on serum biochemical parameters in male rats

Parameters	**Control** **G1**	**Diabetic rats** **G2**	**Treatment with EEP.** **G3**	**Diabetic with Pre-treated of EEP.** **G4**	**Diabetic with post- treated of EEP.** **G5**
SOD Activity U/L	1.98 ∓ 0.07 b	1.00 ∓ 0.02 e	2.07∓0.02 a	1.82∓0.01 d	1.91∓0.01 c
CAT Activity K/ml	0.484∓0.006 a	0.109∓0.001 d	0.489∓0.003 a	0.402∓0.002 c	0.457∓0.004 b
GST Activity U/L	11.34∓0.15 b	7.03∓0.05 e	11.96∓0.08 a	9.13∓0.16 d	10.50∓0.09 c
MDA Conc. µmole/L	1.45∓0.02 a	4.53∓0.02 d	1.32∓0.02 a	2.29∓0.09 c	1.72∓0.03 b
Nitric oxide µmole/L	5.03∓0.22 a	20.41∓0.51 d	4.65∓0.18 a	9.98∓0.14 c	8.72∓0.23 b
Uric acid conc.mg/dl	1.70∓0.05 a	8.38∓0.23 c	1.33∓0.05 a	2.97∓0.07 b	2.24∓0.09 b
Total protein g/dL	6.37∓0.06 b	3.54∓0.12 b	6.82∓0.08 a	5.45∓0.13 d	6.15∓0.08 b

•G1= Control rats. •G2=Diabetic rats. •G3=Treatment with Local EEP.
•G4= Diabetic with Pre-treated of EEP. •G5= Diabetic with post- treated of EEP. •Different letters represent significant difference between groups($p<0.05$).
•Similar letters represent insignificant difference between groups.
•Values represent mean∓ standard error

Histopathological Changes:

The results of biochemical alterations that ensured nephropathy due to diabetes in rats might be insured by microscopic examination of rats kidney, as well as the improvement in kidneys function by treatment with EEP.

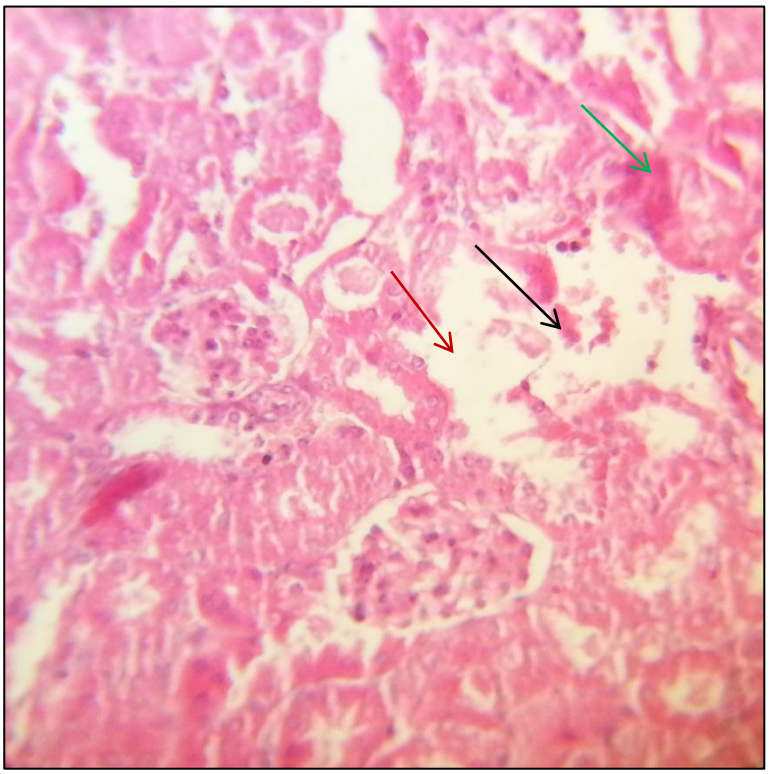

Figure (2): kidney of STZ-induced diabetic in rats after 3 weeks showing sever vascular congestion andatrophy , with vascular wall destruction(black arrow), showing areas of red blood cells extravasation into the interstitium and amidst the spaces between the tubules(green arrow),oedem changes(hydropicde generation) with inflammatory cells. with perivascular cells necrosis and mild to moderate hyaline degeneration(red arrow). 40X H&E.

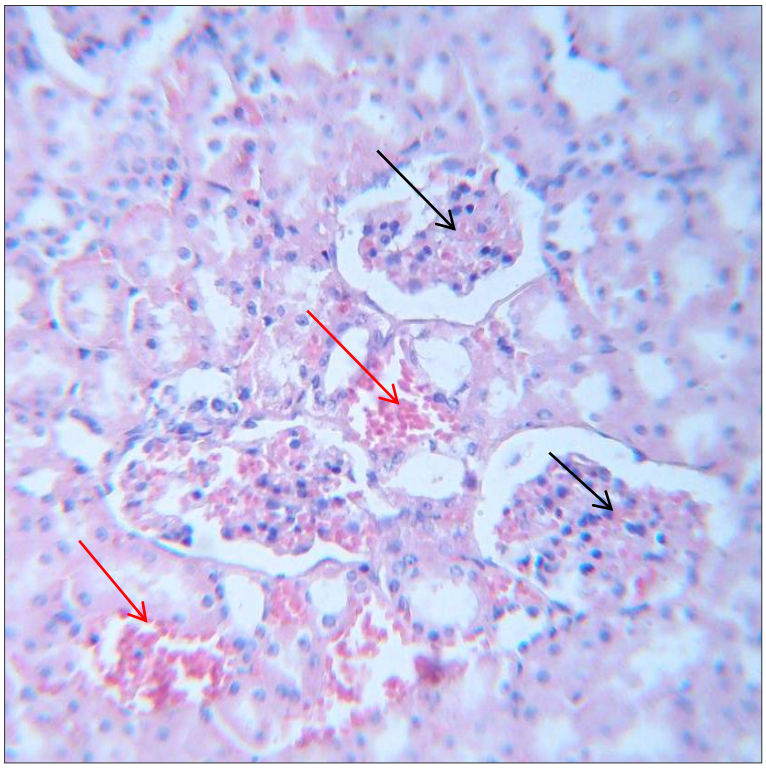

Figure (3): kidney, rats pretreated with EEP before injection of STZ-induced diabetes partially improvement of the histoarchitecture of kidney withmild to moderate vascular congestion(black arrow), with a small areas of red blood cell extravasation into the interstitium(red arrow). Bowman's capsules are preserved.40X H&E.

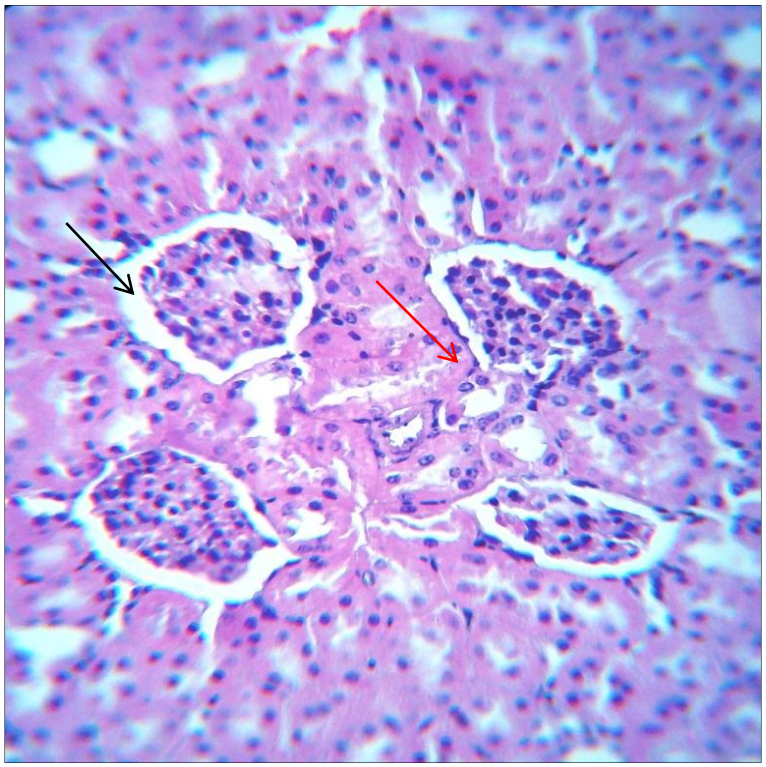

Figure (4): kidney, rats post-treated with EEP after injection of STZ-induced diabetes, showed , mild vascular congestion(red arrow), and shows mild dilation of tubules and Bowman's capsules well preserved(black arrow), showed near normal architecture of the renal tissue. 40X H&E.

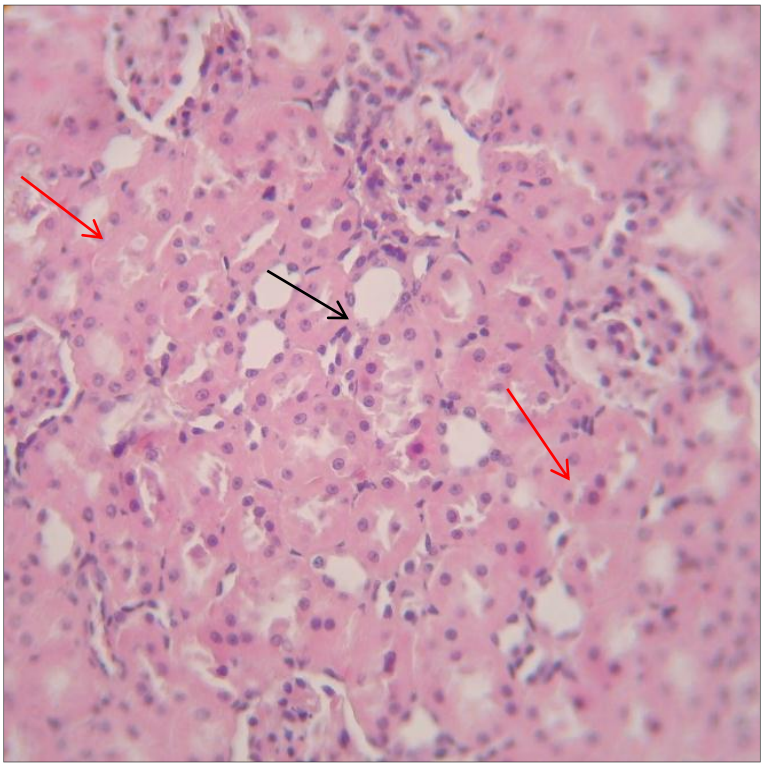

Figure (5): kidney of rat treated with local EEP, there is no pathological lesions, normal histoarchitecture of the renal tissue, shows intact tubules and glomeruli, well preserved(black arrow) normal structure of bowman capsules and un remarkable histopathology changes(red arrow). 40X H&E.

Conclusion :

Through the study shows that the local propolishas anti-oxidant activity, where it helped in the organization of metabolic processes by reducing the Oxidative stress that result from diabetes, where reducing the side effects caused by this disease such as renal failure markedly,so the study showed that propolis was a factor in the repair the kidney and reduce the level and impact of high glucose in the blood, both when used before the induction of the disease (as a preventative), or after the induction of the disease (therapeutic), but most effective when used as a natural product in the case of treatment

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