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RESEARCH ARTICLE

Alleviation of Oxidative Stress and Suppression of Inflammatory Cytokines in STZ -induced Diabetes by Banana (*Musa paradisiacal)*, Kiwi (*Actinidiadeliciosa planch*) and Olive (*Oleaeuropaea*L.), by-products, Extract: Comparative Study

Habiba A. Ahmed[1], Hanan F. Aly[2*], Ahmed M. Aboul-Enein[3], Alaa A. Gaafar[1], Zeinab A. Salama[1]

1. *Plant Biochemistry Department, National Research Centre (NRC), 12622 Giza, Egypt.*
2. *Department of Therapeutic Chemistry, National Research Centre (NRC), 12622 Giza, Egypt.*
3. *Biochemistry Department, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.*

***Corresponding Author: Hanan F. Aly**

Abstract

The effect of peels (kiwi, banana and leaves (olive and banana)acetone extract 80%on streptozotocin (STZ) induced diabetic rats was investigated. Oral administration of 500 mg crude extract /kg body weight for 30 days post induction of diabetes, showed remarkable improvement in all biomarkers under investigation compared to standard drug. Olive leaves showed the highest percentage of improvement in glutathione (GSH), nitric oxide, (NO), glutathione peroxidase (GPX) glutathione –S-transferase (GST) ,catalase, lipid peroxide (MDA) , urea ,creatinine, tumor necrosis factor- α(TNF-α) interleukin 1ß(IL1-ß) (41.74,138.68, 48.77, 26.15, 27.84, 73.54, 97.92, 81.37, 52.96 and 40.24% , respectively).While, banana leaves and kiwi peels extracts showed the highest percentage of improvement in antioxidant enzyme paraoxonase (PON1) and transforming growth factor ß(TGF ß)reached 16.90 and 41.81%, respectively. From a histopathological point of view, treatment of diabetic rats with different extracts revealed regeneration and amelioration effects of extracts on the pancreas and renal tissues in diabetic rats and apparently normal. It could be concluded that the anti-hyperglycemic properties of plant extracts may offer a potential therapeutic source for the treatment of diabetes. Further work on isolation and characterization of active constituents in these wastes is needed.

Keywords: *Diabetes, Inflammatory cytokines, Antioxidant, Oxidative stress.*

Introduction

The prevalence of diabetes has dramatically increased worldwide due to the increase in the obesity rate. Diabetic nephropathy is one of the major complications of type 1 and types 2 diabetes and it is currently the leading cause of end-stage renal disease. Hyperglycemia is the driving force for the development of diabetic nephropathy. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress. While the increase in oxidative stress has been shown to contribute to the development and progression of diabetic nephropathy, the mechanisms by which this occurs are still being investigated. Historically, diabetes was not thought to be an immune disease; however, there is increasing evidence supporting a role of inflammation in type 1 and type 2 diabetes. Inflammatory cells, cytokines, and profibrotic growth factors including transforming growth factor-ß (TGF-ß), monocyte chemoattractant protein-1 (MCP-1), connective tissue growth factor (CTGF), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), and cell adhesion molecules (CAMs) have all been implicated in the pathogenesis of diabetic nephropathy *via* elevation of vascular inflammation and fibrosis [1]. The stimulus for the increase in inflammation in diabetes is still under investigation; however, reactive oxygen species are a primary candidate. Thus, targeting oxidative stress-

inflammatory cytokine signaling could improve therapeutic options for diabetic nephropathy. The current study will focus on the understanding the relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy to help in elucidation the question of which comes first in the progression of diabetic nephropathy, oxidative stress and inflammation [2]. Wu et al. [3] evaluated the *in vitro*Kiwi fruit against α-glycosidase and aldolase reductase and recorded that kiwi fruit exhibited the dose-dependent inhibitory activity against α-glycosidase and aldolase reductase with IC_{50} 42.70 and IC_{50} 18.50 mg/ml respectively. Also, Patel Et Al [4].

Described the antidiabetic potential of kiwi ethanolic, hexane and chloroform extracts as well as n-butanol soluble and n-butanol insoluble fractions of banana leaves and peels which demonstrated promising antidiabetic activity in streptozotocin (STZ) induced diabetes in an animal model. The lowering percentage of blood sugar level was for ethanolic extract of the leaves and peels (20.5 and 25.5%).

Further, the acute effect of crude extract, n-butanol and aqueous residual fractions of banana leaves on glycemia, serum insulin secretion and glycogen content showed that the crude extract, n-butanol, and aqueous residual fractions were statistically reduced glycemia and increased liver glycogen content in hyperglycemic rats as well as inhibited maltose activity and the formation of advanced glycation end-products *in vitro*[5].

Also, a significant increase in insulin secretion and muscle glycogen content in hyperglycemic rats was observed with oral administration of then-butanol fraction[5].

The phytochemical analysis demonstrated the presence of rutin in crude extract and fractions of banana leaves as the major compound, which effects on the regulation of glucose homeostasis indicated its potential antidiabetic properties[5].However, ethanolic extracts, hexane and chloroform, n-butanol soluble and n-butanol insoluble fractions of banana leaves and peels showed also promising antidiabetic activity[6]. The antidiabetic and hypoglycaemic potentials of different parts of banana plant methanolic extract revealed that the stem, fruit, leaves and flower methanolic extracts exhibited inhibition activity againstα-glucosidase andα-amylase at 100 µg/ml concentration of the plant extract. In addition, banana peelsacetone extract showed antidiabetic effects through the significant reduction in the fasting blood glucose level and the concentration of HbA1c in diabetic rats post 45 days of treatment[3,7].

On the other hand, oleuropein and hydroxytyrosol isolated from olive leaves(8 and 16 mg/kg body weight of each compound), significantly decreased the blood glucose and cholesterol levels as well as restored the antioxidant perturbations[8] . Besides, olive leaves 80% aqueous ethanol extract reduced HbA1c and fasting plasma insulin levels, through the reduction of starch digestion and absorption[8].

Hence, olive leaves extract may represent an effective adjunct therapy that normalizes glucose homeostasis in individuals with diabetes [9] . Further, Moghaddam et al. [10] studied the effect of oral feeding of olive leaves on serum glucose level and lipid profile of diabetic rats. The data showed that serum glucose level was significantly lowered at the 6th week as compared to control. Also, Husni [11] investigated the effect of ethanolic extraction of olive leaves on alloxan induced diabetic rabbits.

The author found that blood glucose level, total cholesterol, triglycerides, and LDL–cholesterol was reduced significantly in diabetic treated rabbits. So, the present study is designed to evaluate the antidiabetic effect of peels (kiwi and banana) and leaves (olive and banana)acetone extracts through measuring enzymatic and non-enzymatic antioxidant enzymes, oxidative stress biomarkers, kidney function as well as inflammatory cytokines.

Materials and Methods

Plant Materials

Peels of kiwi (*Actinidiadeliciosa*) and Peels of banana variety maghrbiy (MusaSP.) were obtained from the local market at Giza, Egypt. Leaves of banana variety maghrbiy and leaves of olive variety kalamata (*Oleaeuropea L.*) were obtained from the *Nubaria*farm (National research centre, Giza, Egypt).

Chemicals and Reagents

All solvents and kits were of analytical grade (from Sigma-Aldrich and Bio-diagnostic Company, Egypt).

Preparation of Samples

The fresh leaves (banana and olive) and fruit (kiwi and banana) were washed and peels of banana and kiwi were removed using a sharp knife. The four samples were cut into small pieces, air-dried followed by drying in an oven at 40ºC, grinding then stored in the refrigerator until extraction.

Preparation of Plant Extracts

Two grams of ground sample was weighed to which 20 ml of solvent (distilled water, 80% methanol, 80% ethanol and 80% acetone) were added, soaked and shacked for 48 h at room temperature.

The supernatant of each solvent was collected and the residue was subjected to extraction three times till it became colorless and volume was adjusted to 60 ml. Each extract obtained was filtered using Whatman No.1filter paper and evaporated to dryness using rotary evaporator. The dryness was stored in a refrigerator -20˚C.

Basal Diet

The basal diet is consisting of corn starch 65%, casein 15% and corn oil 10% (Table 1), and salt mixture 4% (Table 2), in addition to vitamins mixture 1% (Table 3) and cellulose 5% as described in AOAC[11].

Table 1: Composition of basal diet

Ingredients	Amount (g/kg)
Corn Starch	650
Casein	150
Corn oil	100
Cellulose	50
Salt mixture	40
Vitamin mixture	10

Table 2: Description of the salt mixture

Ingredients	Weight (g)
$CaCO_3$	600
K_2HPO_4	645
$CaHPO_4,7H_2O$	118
Mg $SO_4,7H_2O$	204
NaCl	334
$Fe(C_6H_5O_7)$ $6H_2O$	55
$CuSO_4.5H_2O$	0.6
KI	1.6
$ZnCl_2$	0.5
$MnSO_4$	10

Table 3: Formulation of vitamin mixture

Vitamins	Amount
Vitamin A	2000 IU
Vitamin D	200 IU
Vitamin E	10 IU
Vitamin B_{12}	2.0 mg
Vitamin K	10 mg
Vitamin E	0.5 mg
Biotin	0.04 mg
Pyriodoxin	0.4 mg
Niacin	4 mg
Inositol	10 mg
Folic Acid	0.2 mg
Choline chloride	200 mg
Pantothenic acid	4 mg
Riboflavin	1.0 mg
Paraminobenzoic Acid	10 mg

Experimental Design

One hundred and thirty-two male albino rats weighted from 200 ± 50 g were selected for this study and divided into eleven groups (twelve rats for each group)

Group 1: Normal healthy control rats.

Groups 2-5: Normal healthy rat's orally administrated with 80% acetone extracts of different tested samples (kiwi peels, banana peels, banana leaves, and olive leaves) extracted with 80% acetone (500 mg/kg body weight daily for 30 days[9, 13].

Groups 6-11: Considered as diabetic groups; where type 2 diabetes was induced by streptozotocin, each rat was injected intraperitoneally with a single dose of streptozotocin (45mg/Kg body weight), dissolved in 0.01M citrate buffer immediately before use[14,15].

After injection, animals had free access to food and water. After two hours each rat was orally administered with 2.5 ml 40% glucose solution as well as 5% glucose solution to drink overnight to counter hypoglycemic shock [16]. Hyperglycemic rats (blood glucose level ≥ 250) were used for the experiment and classified as follows:

Group 6: Diabetic group sacrificed after three days of STZ injection.

Group 7-10: Diabetic animals treated orally with 80% acetone extracts of different tested samples at dose 500 mg/kg body weight for 30 days, each rat received 55 mg/0.5 ml distilled water[17] .

Group 11: Diabetic animals treated with glibenclamide (Glucophage) 10 mg/kg body weight (each rat received 1.5 mg/0.5 ml distilled water) and considered as the reference drug[18] . All rats groups fed on the basal diet during the experimented period (30 days), water and diet gave *ad libitium*.

Sample Preparation for Analysis

Serum Sample

Each animal was weighed, blood collected by puncture the sublingual vein in a clean and dry test tube, left 10 min. to clot and centrifuged at 3000 rpm for serum separation. The separated serum was stored at -80°C until analysis.

Tissue Sample

Liver tissue was weighed and homogenized in 10 volumes of appropriate medium using a Polytron homogenizer and the supernatant stored at -80°C for further estimation as the following method. The extraction method was carried out using 1g tissue and homogenized in 9 ml physiological saline (0.9N) (to give 10% homogenate). Then the sample was centrifuged at 3000 rpm for 10 min and the supernatant was separated and used for different biochemical tissue analysis [19] (Zammit and Newsholme, 1979).

Analysis of Biochemical Parameters

Antioxidant and Oxidative Stress Biomarkers

Determination of Reduced Glutathione (GSH)

Reduced glutathione was estimated according to the method of Beutler et al. [20]. Reduced glutathione (GSH) concentration (mg/g.tissue used) in sample was calculated from the following equation: Glutathione in tissue (mmol/g.tissue) = A_{sample} X 2.22/ g.tissue.

Determination of Nitric oxide

Nitric oxide was determined in tissue liver homogenate by colorimetric assay according to the method of Montgomery and Dymock [21] **.**

Nitric oxide concentration (µmol/l) in the sample was calculated from the Nitric oxide concentration (µmol/l) = $A_{sample}/S_{standard}$ X 50

Determination of Glutathione Peroxidase (GPX)

Determination of glutathione peroxidase activity was estimated according to the method of Paglia and Valentine [22] .

Glutathione peroxidase (GPX) activity (U/g. tissue used) in the sample was calculated from the/g.tissue = A_{340}/ min. / 0.00622 X 21

Determination of Glutathione-S-transferase (G-S-T)

Determination of glutathione-s-transferase activity was estimated according to the method of Habig et al [23].

Glutathione-s-transferase (G-S-t) activity (U/g.tissue used) in sample was calculated from the equation: (U/g.tissue) = A_{sample} X

2.812/ g. tissue used.

Determination of Catalase (CAT) Activity

Catalase activity was estimated according to the method of Fossati et al. [24] .Catalase activity (U/g.tissue used) was calculated from the equation: (U/g.tissue) = ($A_{standard}$–A_{sample}/ $A_{standard}$) X (1/g. tissue used)

Determination of Lipid Peroxide (malondialdehyde) (MAD)

Lipid peroxide was determined according to the method described by Satoh [25] .

Malondialdehyde concentration was calculated from the equation: MAD = (A_{sample}/ $A_{standard}$) X (10/g. tissue used).

Determination of Renal Function

Determination of Creatinine

Creatinine level content determined according to the method described by Bartels et al. [26].

Creatinine concentration was calculated according to the following equation**:**

$$\text{mg/dl} = X\ 2\ \frac{A_2\ \text{sample} - A_1\ \text{sample}}{A_2\ \text{standard} - A_1\ \text{standard}}$$

Determination of Urea

Urea content determined according to the method described by Patton and Crouch [27].

Urea concentration was measured according to the following equation. mg/dl= A_{sample}/ $A_{standard}$ X 50

Immunosorbent Assay

Inflammatory biomarkers (TNF- α, TGF- β1 and IL-1B) levels and antioxidant enzyme (paraoxonase) were estimated in serum by ELISA; a sandwich enzyme immunoassay.

Histopathological Examination

Pancreas, and kidneys of the sacrificed rats were taken and immersed in 10 % formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Dehydrated specimens were cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin for histopathological examination according to the method described by Drury et al. [28]. The histopathological examination was done by Dr. KawkabAbd El Aziz Ahmed, Department of Pathology, Faculty of Veterinary Medicine, Cairo University.

Statistical Analysis

SPSS program combined with a *co-state* computer program was performed [29]. Where different letters are considered significant at $p \leq 0.05$.

Results and Discussion

Effect of 80% Acetone Extract of Tested Plant by-products on Antioxidant Biomarkers and Oxidative Stress

Diabetes mellitus is a metabolic disorder characterized by increased production of free radicals and oxidative stress. The oxidative stress is known to be a component of molecular and cellular tissue damage, associated with complications of diabetes. Impaired antioxidant status is known to be an indicator of oxidative stress[30,31].

Reduced glutathione (GSH) is a major intracellular nonprotein sulfhydryl compound and intracellular hydrophilic important considered as the most antioxidant[32] .GSH acts as a co-substrate for glutathione peroxidase (GPX) and as a co-factor for many enzymes. Stress resistance of many cells is associated with low intracellular levels of GSH. Decreased GSH content may predispose the cells to lower defense against the condition of oxidative stress during several degenerative diseases including diabetes [33].

Nitric oxide (NO) has been identified as a mediator of protein-induced hyperfiltration. While the mechanisms whereby which diabetes mellitus may argument NO production are still unclear. Excessive NO biosynthesis could result from either the increased availability of its precursor, L-arginine, or the enhanced activity of NOS.

Diabetic rats usually intake much more food, which may contain L- arginine [34]. The direct toxicity of NO is enhanced by its reaction with superoxide radical to give secondary toxic oxidizing species; peroxynitrite which is capable of oxidizing cellular structure and causes lipid peroxidation[35].

Table (4) shows the GSH and NO levels in the liver of different experimental groups. Reduction in GSH content (2.2 mmol/g) and elevation of NO levels (33.82 µmol/L) in diabetic rats, were observed compared to normal control rats (5.37 mmol/g and 13.95 µmol/L, respectively). There were no significant differences between normal control and treated normal rats groups, where administration of the tested extracts tends to bring the NO and GSH to reach the normal level.

The percentage improvements of GSH and NO in diabetic rats after treatments by the four extracts ranged from 35.40 to 41.74% for GSH compared to the drug (Glucophage) which showed 43.02% and 115.09 to 138.68% for NO compared to drug being 128.30%. The highest percentage of improvement in GSH and NO was observed after treatment with olive leaves extract (Table 4). These results are in a good agreement with that of Kaimal et al. [36], Shehata and Soltan [37], Weinstein et al [9].Navghare and Dha wale [38], for kiwi, banana and olive leave extracts, respectively.

They reported that GSH contents decreased drastically in the liver and pancreas of diabetic rats while the reduction in NO levels of diabetic-treated rats with polyphenol compounds of plant extracts. On the other hand, glucose is known to induce lipid peroxidation through activation of lipoxygenase enzymes and contributed to the significant reduction in GSH content [39].

Also, rutin has antioxidant and anti-inflammatory effects that lead to a reduction of blood glucose level in the STZ-induced diabetic rats. Besides, it functionally and formatively protects pancreas, heart, liver, kidneys, and retina tissues that attributed to diabetic complications [40].

Thus, the protective effect of acetone extract of the tested by-products may be related to the presence of flavonoid compounds that lead to reducing the oxidative stress consequently, normalize hepatic and pancreatic tissues structure and functions[41].

Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) played detoxification important roles in cell cycle defense [42] . Previous studies on animal model suggested that, as a consequence of diabetes, an increase in hepatic GPX and GST activities was noticed, which may be a compensatory response to oxidative stress, and play a crucial role in the protection of DNA from oxidative damage[43,44].

Table 4: Effect of 80% acetone extracts of tested plant by-products on GSH and NO levels

GSH level			
Groups	**Concentration (mmol/g)**	**Changes relative to normal control %**	**Improvement %**
Nor. Con	$5.37^{a} \pm 0.50$	100.00	----
Dia. Con	$2.21^{b} \pm 0.07$	41.20	----
Nor. KPE	$5.03^{a} \pm 0.71$	93.75	----
Nor. BPE	$4.95^{a} \pm 0.68$	92.18	----
Nor. BLE	$4.84^{a} \pm 0.73$	90.13	----
Nor. OLE	$5.18^{a} \pm 1.29$	96.54	----
Dia. KPE	$4.35^{a} \pm 1.19$	81.05	39.85
Dia. BPE	$4.11^{a} \pm 1.02$	76.60	35.40
Dia. BLE	$4.27^{a} \pm 0.57$	79.47	38.27
Dia. OLE	$4.45^{a} \pm 1.29$	82.94	41.74
Dia. Drug	$4.52^{a} \pm 0.74$	84.23	43.02
Nitric oxide level	**(µmole/L)**		
Nor. Con	$13.95^{b} \pm 2.25$	100.00	----
Dia. Con	$33.82^{a} \pm 2.16$	242.45	----
Nor. KPE	$12.89^{b} \pm 1.58$	92.45	----
Nor. BPE	$14.34^{b} \pm 2.99$	102.83	----
Nor. BLE	$14.87^{b} \pm 3.53$	106.60	----
Nor. OLE	$13.55^{b} \pm 1.78$	97.17	----

Dia. KPE	$16.05^{b} \pm 3.28$	115.09	127.36
Dia. BPE	$17.11^{b} \pm 4.03$	122.64	119.81
Dia. BLE	$17.76^{b} \pm 2.51$	127.36	115.09
Dia. OLE	$14.47^{b} \pm 5.18$	103.77	138.68
Dia. Drug	$15.92^{b} \pm 2.61$	114.15	128.30

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis is carried out using the SPSS computer program combined with a co-state computer program, where different letters are considered significant at $p \leq 0..05$.

Data in Table (5) shows the activities of GPX and GST in the liver of rats. The activity of GPX and GST in the liver was significantly increased in diabetic rats (249.00 and 81.04 U/g, respectively), compared to control rats (151.74 and 62.26 U/g, respectively), also the results exhibited insignificant change between normal control and normal rats treated with the four extracts.

The results demonstrated that the kiwi peels had the highest improvement for GPX activity (51.28%) compared to the drug (38.46%), while olive leaves improved GST (26.15%) compared to the drug (21.41%). Also, Table(6) shows the catalase (CAT) activity and the level of MDA as oxidative stress biomarker in the liver of different experimental groups.

It is known that the hyperglycemia induced by STZ leads to increased concentration of H_2O_2, which eventually causes the induction of CAT activity as an antioxidant enzyme[45] . Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems[46]. While, Rajeswari et al. [47], declared that, high blood glucose levels are known to induce lipid peroxidation through activation of lipoxygenase enzymes.

Also, the increase of MAD due to excessive oxidative stress causes the exceeding free radicals product especially reactive oxygen species (ROS). ROS has been known to produce cellular and tissue injuries through covalent binding, DNA strand breaking, lipid peroxidation (LPO) and augment fibrosis [48].

Also, Kaimal et al. [49] , observed that the level of lipid peroxides increased significantly while GSH content decreased drastically in the liver and pancreas of diabetic rats.

The activity of CAT and MAD levels in the liver was significantly increased in diabetic rats (897.35 U/g and 608.82 nmole/g, respectively) compared to normal control rats (686.73 U/g and 348.97 nmole/g, respectively). This parameter came towards the normal level gradually after administration of acetone extract of the four by-products into diabetic rats.

The activity of CAT and MAD levels in liver showed no significant changes between treated normal rats and treated diabetic groups which ranged from 690.30 to 720.35 U/g for cat activity and from 343.97 to 376.62 mole/g for MAD level. The percentage of improvements in CAT activity and MAD level were ranged from 25.77 to 29.38% and from 66.54 to 73.54% compared to drug-treated animals (which were 27.06 and 72.99%, respectively).

In the present study, the amelioration may be related to the treatments with extracts of the four byproducts which are rich in polyphenol compounds. These compounds enhance the secretion of antioxidant enzyme (GST, GPX, and catalase), and protect cells from damage by scavenging free radicals of oxygen.

In this concerns, Al-Attar, and Alsalmi[50] and Ben et al. [51] , confirmed the antidiabetic, antioxidant and protective effects of olive leaves extract and attributed these activities to its antioxidant effect of the active constituent.

Also, carotenoids can significantly decrease MDA levels. This finding is in agreement with the results of Dixon et al. [52] and sun et al. [53] studies , who reported that the scavenging of H_2O_2, increased antioxidant levels, lower inflammatory markers iL-1 and iL-6 and these may be attributed to their phenolics, content which can donate H^+ to H_2O_2, thus neutralizing it to water.

Table 5: Effect of 80% acetone extracts of tested plant by-products on GPX and GST activities

GPX activity			
Groups	**Activity (U/g)**	**Changes relative to normal control %**	**Improvement %**
Nor. Con	$151.74^{b} \pm 16.28$	100.00	----
Dia. Con	$249.00^{a} \pm 39.87$	164.10	----
Nor. KPE	$163.41^{b} \pm 22.18$	107.69	----
Nor. BPE	$182.86^{b} \pm 10.66$	120.51	----
Nor. BLE	$175.08^{b} \pm 23.83$	115.38	----
Nor. OLE	$167.30^{b} \pm 32.55$	110.26	----
Dia. KPE	$171.19^{b} \pm 31.97$	112.82	51.28
Dia. BPE	$186.75^{b} \pm 10.66$	123.08	41.03
Dia. BLE	$182.86^{b} \pm 17.40$	120.51	43.59
Dia. OLE	$175.08^{b} \pm 36.39$	115.38	48.72
Dia. Drug	$190.64^{b} \pm 25.36$	125.64	38.46
GST activity			
Nor. Con	$62.26^{b} \pm 7.39$	100.00	----
Dia. Con	$81.04^{a} \pm 1.66$	130.17	----
Nor. KPE	$63.78^{b} \pm 5.50$	102.44	----
Nor. BPE	$65.69^{b} \pm 7.39$	105.51	----
Nor. BLE	$64.39^{b} \pm 6.18$	103.43	----
Nor. OLE	$63.94^{b} \pm 3.50$	102.71	----
Dia. KPE	$65.82^{b} \pm 6.51$	105.72	24.45
Dia. BPE	$66.99^{b} \pm 3.34$	107.60	22.57
Dia. BLE	$68.01^{b} \pm 1.22$	109.24	20.93
Dia. OLE	$64.76^{b} \pm 4.43$	104.02	26.15
Dia. Drug	$67.71^{b} \pm 3.37$	108.76	21.41

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis is carried out using the SPSS computer program combined with a co-state computer program, where different letters are considered significant at $p \leq 0..05$.

Table 6: Effect of 80% acetone extracts of tested plant by-products on catalase activity and lipid peroxide level.

Catalase activity			
Groups	**Activity(U/g)**	**Changes relative to normal control %**	**Improvement %**
Nor. Con	$686.73^{b} \pm 28.40$	100.00	----
Dia. Con	$897.35^{a} \pm 21.31$	130.67	----
Nor. KPE	$690.30^{b} \pm 25.03$	100.52	----
Nor. BPE	$693.81^{b} \pm 41.79$	101.03	----
Nor. BLE	$702.65^{b} \pm 44.51$	102.32	----
Nor. OLE	$692.04^{b} \pm 47.82$	100.77	----
Dia. KPE	$695.57^{b} \pm 49.51$	101.29	29.38
Dia. BPE	$720.35^{b} \pm 67.28$	104.90	25.77
Dia. BLE	$715.04^{b} \pm 48.23$	104.12	26.55
Dia. OLE	$706.19^{b} \pm 69.23$	102.84	27.84
Dia. Drug	$711.50^{b} \pm 29.75$	103.61	27.06
Lipid peroxide level	**(ηmole/g)**		
Nor. Con	$348.97^{b} \pm 9.64$	100.00	----
Dia. Rats	$608.82^{a} \pm 22.56$	174.46	----
Nor. KPE	$343.97^{b} \pm 20.49$	98.57	----
Nor. BPE	$350.74^{b} \pm 13.80$	100.51	----
Nor. BLE	$356.32^{b} \pm 12.23$	102.11	----
Nor. OLE	$347.65^{b} \pm 9.91$	99.62	----
Dia. KPE	$362.94^{b} \pm 20.64$	104.00	70.46

Dia. BPE	$367.94^{b} \pm 19.58$	105.44	69.03
Dia. BLE	$376.62^{b} \pm 12.58$	107.92	66.54
Dia. OLE	$352.21^{b} \pm 47.23$	100.93	73.54
Dia. Drug	$354.12^{b} \pm 12.67$	101.47	72.99

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis was carried out using SPSS program combined with a *co-state* computer program where different letters are considered significant at $p \leq 0.05$.

Effect of 80% Acetone Extracts of the Tested Plant byproducts on Renal Functions

Measurement of the urea and creatinine is widely regarded as a test of renal functions [54].Diabetes nephropathy is the kidneys disease that occurs as a result of diabetes. After many years of diabetes, the delicate filtering system in the kidneys becomes destroyed, initially becoming leaky to larger blood proteins such as albumin which are then lost in the urine. This is more likely to occur if the blood sugar is poorly controlled [55]. The mean serum urea and creatinine levels of different experimental groups are given in Table (7). Their levels were significantly increased in the diabetic rats (0.47 and 59.56 mg/dl, respectively) compared to control rats (0.24 for creatinine and 30.78 mg/dl for urea). Administration of acetone extracts of the four tested samples caused a significant reduction in serum.

Table 7: Effect of 80% acetone extracts of the tested plant by-products creatinine and urea levels

Creatinine level			
Groups	**Levels (mg/dl)**	**Changes relative to normal control %**	**Improvement %**
Nor. Con	$0.24^{b} \pm 0.06$	100.00	----
Dia. Con	$0.47^{a} \pm 0.07$	195.83	----
Nor. KPE	$0.22^{b} \pm 0.09$	89.58	----
Nor. BPE	$0.25^{b} \pm 0.06$	102.08	----
Nor. BLE	$0.26^{b} \pm 0.07$	106.25	----
Nor. OLE	$0.21^{b} \pm 0.03$	87.50	----
Dia. KPE	$0.29^{b} \pm 0.05$	118.75	77.08
Dia. BPE	$0.30^{b} \pm 0.06$	122.92	72.92
Dia. BLE	$0.33^{b} \pm 0.03$	137.50	58.33
Dia. OLE	$0.24^{b} \pm 0.05$	97.92	97.92
Dia. Drug	$0.25^{b} \pm 0.08$	104.17	91.67
Urea level			
Nor. Con	$30.78^{b} \pm 2.52$	100.00	----
Dia. Con	$59.56^{a} \pm 6.06$	193.50	----
Nor. KPE	$30.02^{b} \pm 4.01$	104.04	----
Nor. BPE	$29.47^{b} \pm 2.75$	95.74	----
Nor. BLE	$31.44^{b} \pm 1.56$	102.17	----
Nor. OLE	$30.93^{b} \pm 3.75$	100.51	----
Dia. KPE	$33.07^{b} \pm 3.02$	107.44	86.07
Dia. BPE	$35.84^{b} \pm 8.40$	116.46	77.04
Dia. BLE	$38.07^{b} \pm 3.92$	123.68	69.82
Dia. OLE	$34.51^{b} \pm 6.10$	112.13	81.37
Dia. Drug	$33.38^{b} \pm 1.71$	108.45	85.05

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis was carried out using SPSS program combined with a *co-state* computer program where different letters are considered significant at $p \leq 0.05$.

Creatinine levels from 0.47 to 0.24 mg/dl and urea from 59.56 to 33.07 mg/dl in the diabetic treated rats. However, serum total urea levels did not statistically differ between the normal control group and normal- treated rats. Olive leaves exhibited the highest percentage of improvement for creatinine (97.92%), and kiwi peels for urea (86.07%). On the other hand, banana leaves extract showed the lowest percentage of

improvement for creatinine and urea (58.33 and 69.82%, respectively) compared to diabetic rats treated with the standard drug (91.67 and 85.05%, respectively). The treatment with acetone extracts of by-products can increase antioxidant enzymes, which have the ability to decrease the oxidative stress, and protects the hepatic and renal tissues of diabetic rats [56].

Effect of 80% Acetone Extracts of Tested Plant by-products on TNF- α, TGF-β1 and IL-1B levels and Paraoxonase Antioxidant Enzyme

Inflammation is a protective mechanism of the body; however, in chronic diseases like diabetes mellitus, hypertension, asthma, etc. this protective mechanism becomes an important mechanism for the progression of the disease. Interleukin (IL)-1ß is a pro-inflammatory cytokine involved in the autoimmune process of Type 1 diabetes.

In Type 2 diabetes mellitus the decreasing ß cell mass is also associated with glucose toxicity mediated through IL-1ß induced apoptosis. There are many studies emphasizing the presence and importance of inflammatory component in the pathogenesis of diabetes mellitus. A very important role is played by adipose tissue, which releases various pro-inflammatory cytokines, such as, tumor necrosis factor- alpha (TNF-α), interleukin-1 (IL-1) [57, 58]. Tables 8,9 show the effects of different acetone extracts on the inflammatory markers and paraoxonase as an anti-inflammatory and antioxidant enzyme. The diabetic rats had the highest levels of TNF-α and IL-1B (385.23 Pg/ml and 2.91 ηg/ml, respectively) compared to control rats (110.49 Pg/ml and 1.95 ηg/ml, respectively).

However, TNF-α and IL-1B markers level showed no significant difference between control rats and normal rats treated with the four acetone extracts (Table 8). Post one month of the experimental treatment using extracts and standard drug, the percentage of improvement was ranged from 33.39 to 63.10% compared to the drug (89.58%) for TNF-α and ranged from 38.01 to 41.78% compared to the drug (33.22%) for IL-1B.

The results declared that acetone extract of kiwi peels have the highest percentage of improvementforTNF-α (63.10%), while banana leaves, kiwi peels, and olive leaves showed values of 41.78, 40.41 and 40.24% for IL-1B, respectively. Transforming growth factor-beta (TGF-ß) is a cytokine has a vital role in the regulation of many biological processes, including cellular proliferation and differentiation, modulation of the immune response, extracellular matrix deposition, angiogenesis and tissue repair [59].

Table 8: Effect of 80% acetone extracts of tested plant by-products on TNF- α and IL-1B levels

TNF-α level			
Groups	**(Pg/ml)**	**Change relative to normal control %**	**Improvement%**
Nor. Con	$110.49^{f} \pm 6.10$	100.00	----
Dia. Con	$385.23^{a} \pm 8.67$	348.67	----
Nor. KPE	$117.27^{f} \pm 4.15$	106.14	----
Nor. BPE	$118.54^{f} \pm 8.65$	107.29	----
Nor. BLE	$124.01^{f} \pm 6.82$	112.24	----
Nor. OLE	$107.39^{f} \pm 5.35$	97.20	----
Dia. KPE	$315.52^{d} \pm 3.94$	285.57	63.10
Dia. BPE	$348.34^{b} \pm 4.82$	315.27	33.39
Dia. BLE	$337.26^{bc} \pm 6.57$	305.25	43.41
Dia. OLE	$326.71^{cd} \pm 5.49$	295.70	52.96
Dia. Drug	$286.25^{e} \pm 4.32$	259.08	89.58
IL1-B level	**ηg/ml**		
Nor. Con	$1.95^{de} \pm 0.05$	100.00	----
Dia. Con	$2.91^{a} \pm 0.07$	149.66	----
Nor. KPE	$1.93^{de} \pm 0.08$	99.14	----
Nor. BPE	$1.98^{cde} \pm 0.06$	101.54	----
Nor. BLE	$1.97^{cde} \pm 0.52$	101.37	----
Nor. OLE	$1.86^{e} \pm 0.06$	95.72	----
Dia. KPE	$2.13^{bcd} \pm 0.03$	109.25	40.41
Dia. BPE	$2.17^{bc} \pm 0.07$	111.64	38.01
Dia. BLE	$2.10^{bcd} \pm 0.18$	107.88	41.78

Dia. OLE	$2.13^{bcd} \pm 0.04$	109.42	40.24
Dia. Drug	$2.27^{b} \pm 0.07$	116.44	33.22

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis was carried out using SPSS program combined with a *co-state* computer program where different letters are considered significant at $p \leq 0.05$.

Data in Table (9) shows the level of TGF-*β* and paraoxonase activity in normal and different therapeutic groups. The level of TGF-*β* was the highest (477.62 pg/ml) in the diabetic group, while paraoxonase activity showed the lowest in the diabetic group (6.14 ηg/ml) compared to the normal control group (249.05 pg/ml and 10.10 ηg/ml, respectively).

In addition, TGF-ß level and paraoxonase enzyme activity showed no significant changes between the normal control group and normal rat groups under the present treatments. Treatments for diabetic rats using acetone extracts of tested samples and drug showed an improvement ranged from 24.73 to 41.81% for TGF-ß compared to the drug (48.27%) and ranged from 10.20 to 16.90% for paraoxonase activity compared to the drug (13.77%). It is known that the derived proinflammatory cytokines and other cytokines which are mainly derived from macrophages (such as IL-1, TNF-α, TGF, and IL-6) may have prominent proinflammatory functions acting downstream of Th_1/Th_{17} cell-mediated autoimmunity[60]. The present results demonstrated that the acetone extracts of kiwi and banana peels, as well as banana and olive, leaves reduced the proinflammatory cytokines and anti-inflammatory enzyme.

Also, possessed potential efficacy as an anti-inflammatory effect *in vivo* and suggested that the samples (peels and leaves) might be a good therapeutic agent for the treatment of inflammatory diseases. The anti-inflammatory properties of samples may be due to polyphenol compounds in samples. It is well known that these compounds possess antioxidant properties protecting against inflammation by scavenging free radicals [61].

Table 9: Effect of 80% acetone extracts of tested plant by-products on TGF-β level and paraoxonase activity

Paraoxonase activity			
Groups	**(ηg/ml)**	**Changes relative to normal control %**	**Improvement %**
Nor. Con	$10.10^{a} \pm 0.51$	100.00	----
Dia. Con	$6.14^{e} \pm 0.16$	60.78	----
Nor. KPE	$10.21^{a} \pm 1.04$	101.09	----
Nor. BPE	$8.77^{abc} \pm 0.55$	86.83	----
Nor. BLE	$9.05^{ab} \pm 0.83$	89.63	----
Nor. OLE	$9.41^{a} \pm 0.49$	93.17	----
Dia. KPE	$7.53^{cde} \pm 0.35$	74.55	13.77
Dia. BPE	$7.39^{cde} \pm 0.08$	73.16	12.38
Dia. BLE	$7.84^{bcd} \pm 0.10$	77.68	16.90
Dia. OLE	$7.17^{de} \pm 0.16$	70.98	10.20
Dia. Drug	$7.53^{cde} \pm 0.35$	74.55	13.77
TGF-β level	**pg/ml**		
Nor. Con	$249.05^{d} \pm 24.25$	100.00	----
Dia. Con	$477.62^{a} \pm 17.36$	191.78	----
Nor. KPE	$252.41^{d} \pm 18.78$	101.35	----
Nor. BPE	$284.17^{d} \pm 12.84$	114.10	----
Nor. BLE	$278.40^{d} \pm 19.07$	111.78	----
Nor. OLE	$251.97^{d} \pm 22.06$	101.17	----

Dia. KPE	$373.50^{bc} \pm 5.89$	149.97	41.81
Dia. BPE	$416.02^{b} \pm 12.10$	167.04	24.73
Dia. BLE	$414.23^{b} \pm 9.30$	166.32	25.45
Dia. OLE	$405.61^{b} \pm 5.05$	162.86	28.91
Dia. Drug	$357.41^{c} \pm 4.45$	143.51	48.27

All values represented as mean ± S.D.
Means with different letters are significantly different ($p<0.05$).
(Nor.): normal, (Dia.): diabetic, (Con.) control, (KPE): kiwi peels extract, (BPE): banana peels extract, (BLE): banana leaves extract and (OLE): olive leaves extract. Statistical analysis was carried out using SPSS program combined with a *co-state* computer program where different letters are considered significant at $p \leq 0.05$.

Histopathological Examination

Pancreas

The majority of islet cells are formed by ß cells which are responsible for producing insulin. Depletion of ß cells will, therefore, result in insulin deficiency leading to carbohydrate, protein and fat metabolism disorders with resultant hyperglycemia [62]. The histopathological examinations of the pancreas from different experimental groups are demonstrated in photomicrographs (1-11).

Photomicrographs (1-5) show the pancreas of a normal control group and normal control rats treated with kiwi and banana peels, banana leaves and olive leaves respectively which showed healthy pancreatic lobules normal pancreatic acini. While, photomicrograph (6) demonstrated diabetic rats with the focal hemorrhagic area, interlobular edema with a thick-walled dilated blood vessel, hemorrhage, and vacuolations of some ß cells.

The histopathological examination of the pancreas in diabetic rats may be due to STZ, which selectively destroy ß cells of the islet induced type 2 diabetes mellitus. It is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells [63,64]. Pancreas section of rats from diabetic rats treated with acetone extracts are demonstrated in Photomicrographs (7-11).

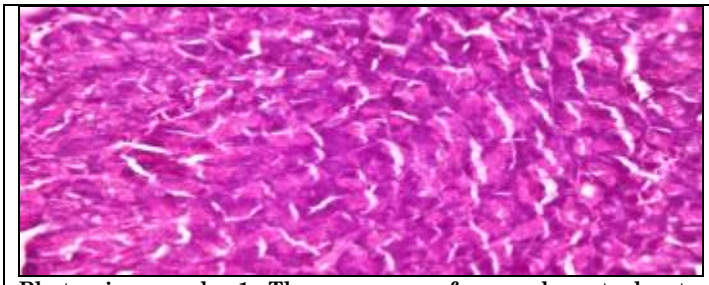
The diabetic rats treated with kiwi peels extract showed healthy pancreatic with slightly hyperplastic pancreatic islets, and this result is completely identical to normal rats treated with kiwi peels extract (photomicrograph7), while pancreas of diabetic rats treated with banana peels extract showed slightly dilated blood vessel (photomicrograph8). On the other hand, the diabetic rats treated with banana leaves extract showed apparently healthy pancreatic lobules with normal pancreatic acini like normal control group (photomicrograph 9).

While the diabetic rats treated with olive leaves extract and the standard drug showed slight interlobular edema with a thick-walled dilated blood vessel (photomicrographs10 and11). Islet cells of diabetic rats treated with 500 mg/kg/day of acetone extracts showed regenerated considerably suggesting the presence of stable cells in the islets with the ability to regenerate[62].

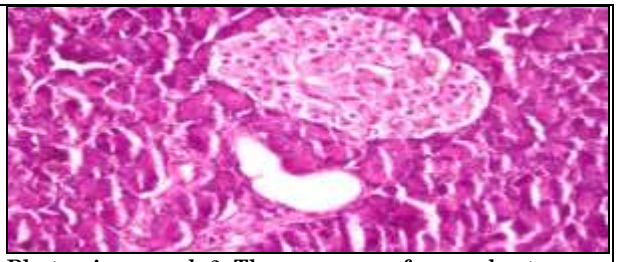
This suggested that the plant extract at this dose had the ability to induce the quiescent cells to proliferate to replace the lost cells. The exact mechanism is not known but it has been documented that the flavonoid fraction of this samples extract decreased blood glucose and increased the number of ß cells [65].

On the other hand, the presented results may be explained on the basis of the samples contain a phenolic compound, which may have stopped further destruction of the remaining ß cells in the islet by mopping up the circulating reactive oxygen species generated by the STZ.

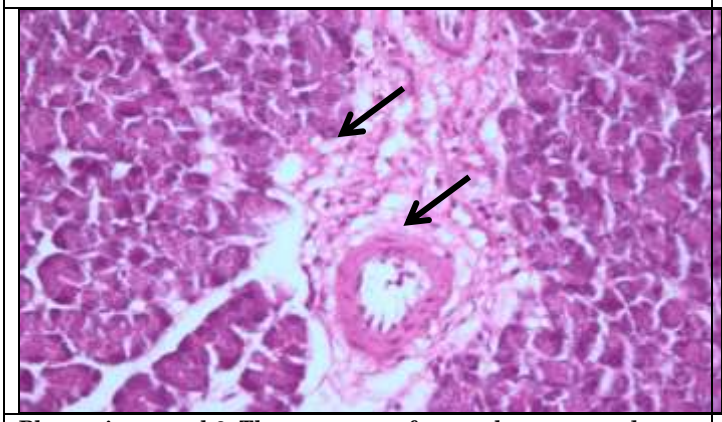
This drug destroys the ß cells and then allowing other phytochemicals of the plants to induce regenerative activities [66].



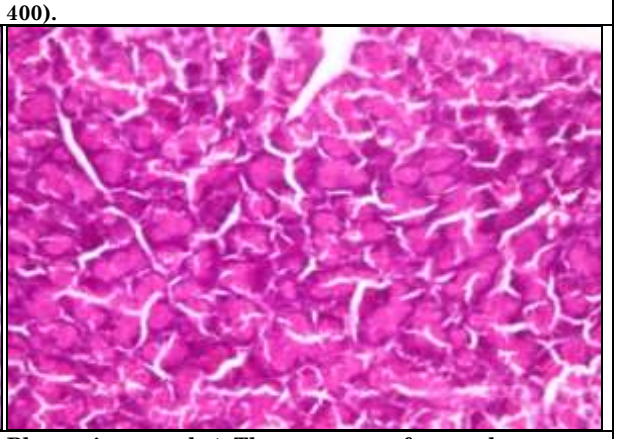
Photomicrographs 1: The pancreas of normal control rats showing the apparently healthy pancreatic lobules with normal pancreatic acini (H&E X 400).



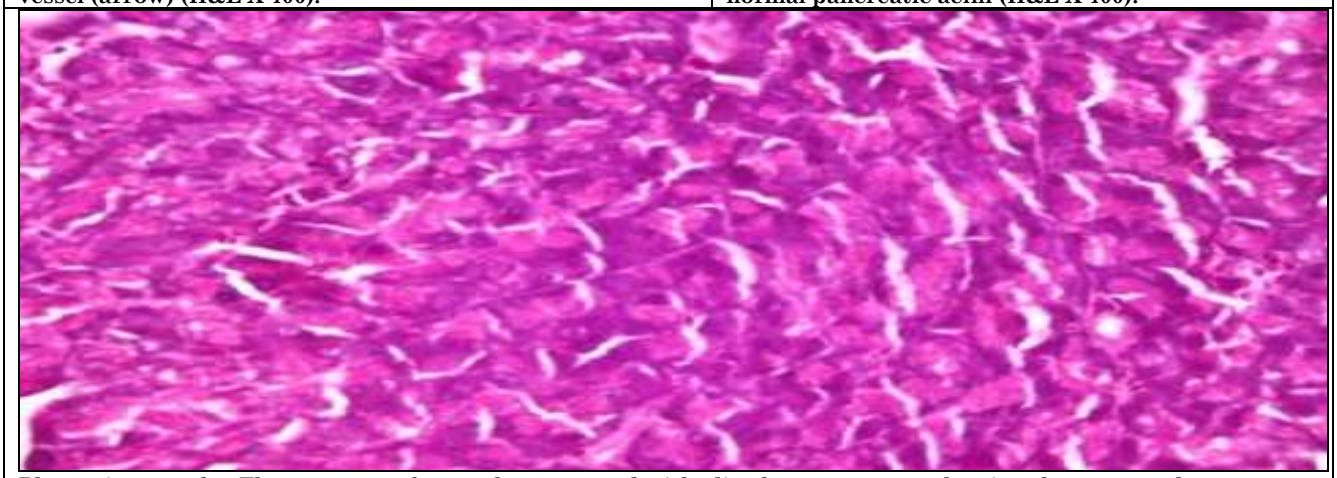
Photomicrograph 2. The pancreas of normal rats treated with kiwi peels extract showing the apparently healthy pancreatic with slightly hyperplastic pancreatic islets (arrow) (H&E X 400).



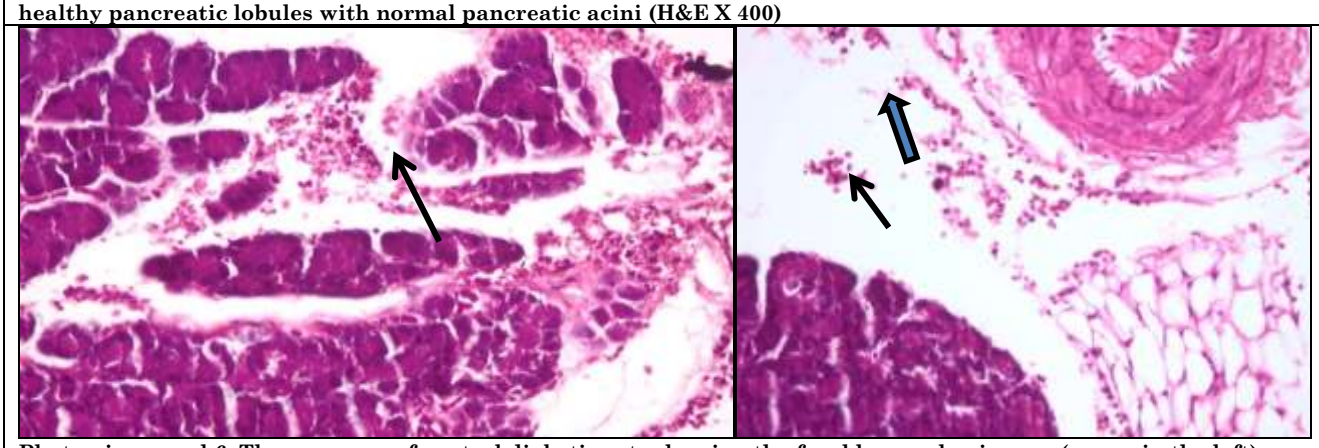
Photomicrograph3: The pancreas of normal rats treated with banana peels extract showing the healthy pancreatic with slightly dilated interlobular blood vessel (arrow) (H&E X 400).



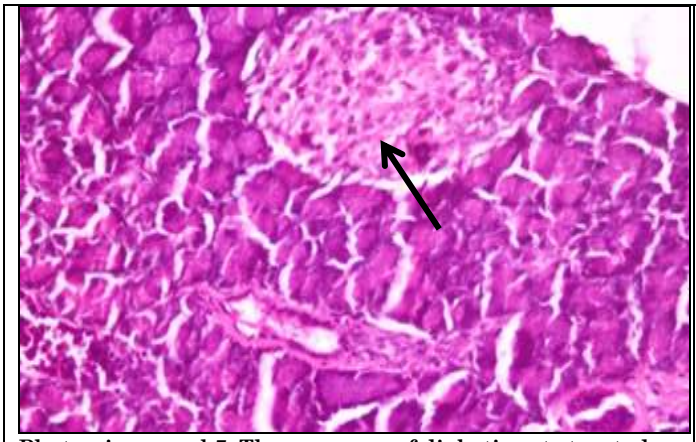
Photomicrograph 4: The pancreas of normal rats treated with banana leaves extract showing the apparently healthy pancreatic lobules with normal pancreatic acini (H&E X 400).



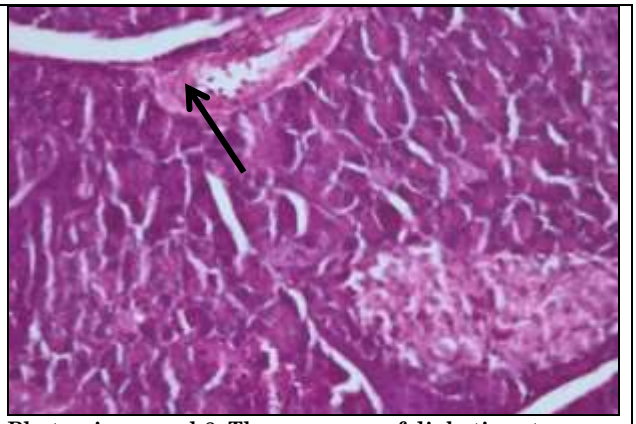
Photomicrograph5: The pancreas of normal rats treated with olive leaves extracts showing the apparently healthy pancreatic lobules with normal pancreatic acini (H&E X 400)



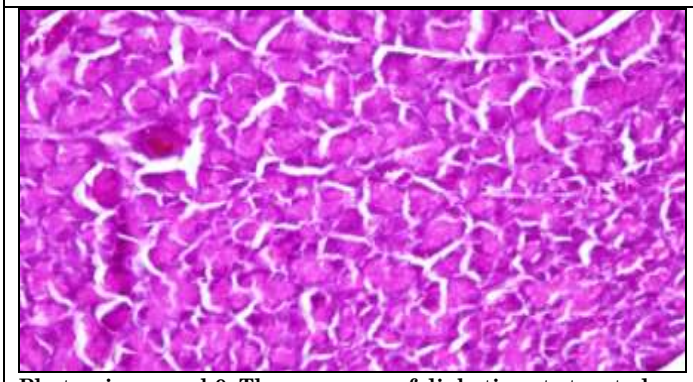
Photomicrograph6: The pancreas of control diabetic rats showing the focal hemorrhagic area (arrow in the left), interlobular edema with a thick-walled dilated blood vessel (arrow head in the right) and hemorrhage (arrow in the right) (H&E X 400)



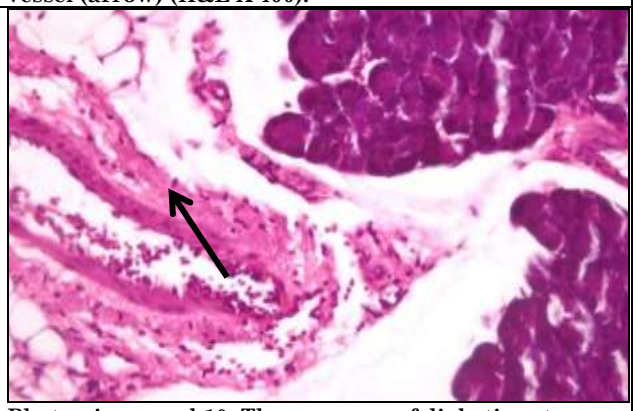
Photomicrograph7: The pancreas of diabetic rats treated with kiwi peels extracts showing the slightly hyperplastic pancreatic islets (arrow) (H&E X 400).



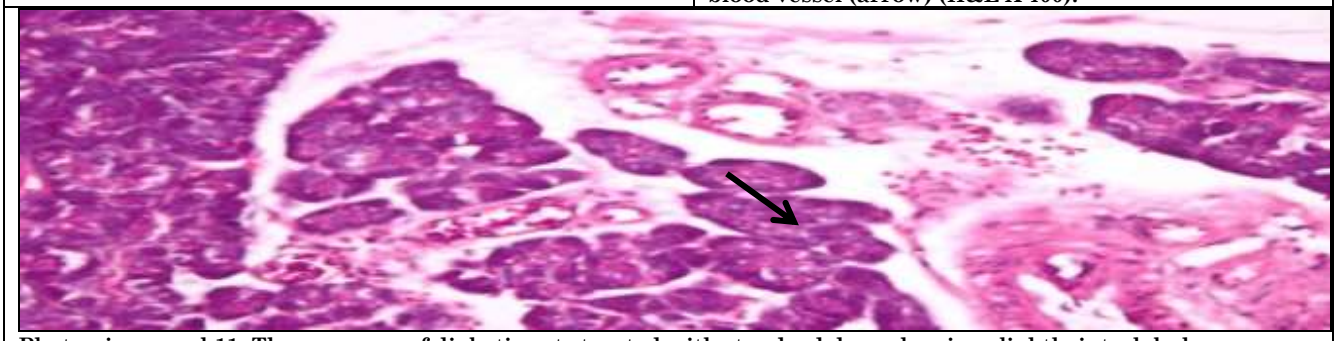
Photomicrograph8: The pancreas of diabetic rats treated with banana peels extracts showing the healthy pancreatic with a slightly dilated blood vessel (arrow) (H&E X 400).



Photomicrograph9: The pancreas of diabetic rats treated with banana leaves extract showing the apparently healthy pancreatic lobules with normal pancreatic acini (H&E X 400)



Photomicrograph10: The pancreas of diabetic rats treated with olive leaves extract showing slightly interlobular edema with thick walled dilated blood vessel (arrow) (H&E X 400).



Photomicrograph11: The pancreas of diabetic rats treated with standard drug showing slightly interlobular edema with a thick-walled dilated blood vessel (arrow) (H&E X 400).

Kidneys

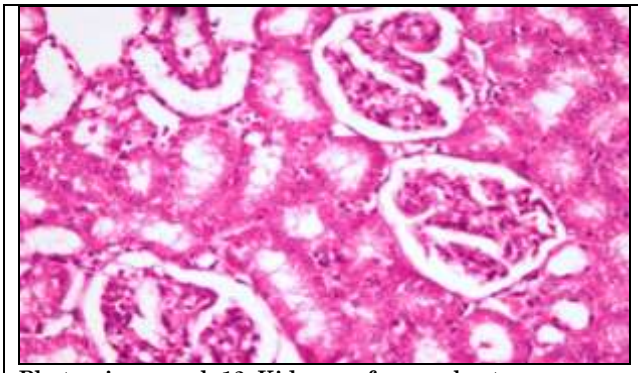
The histopathological examination of kidney sections of rats from control and control treated rats with different extracts (12-16), demonstrated normal histopathological structures. While diabetic rats (photomicrograph 17)showed vacuolated renal tubular epithelium, congestion of portal triad with mild inflammation and remarkable fibrosis near the central vein in the liver, tubular inflammation and glomeruli shrinkage in the kidneys compared to the normal control group (photomicrograph 12). These are provoked by the increased production of highly reactive oxygen species, which are normally detoxified by an endogenous antioxidant enzyme in the excessive presentation.

The depletion of the exogenous antioxidant store can permit the reactive intermediate to react with and destroy the hepatic and renal cells such pathological changes can be in the diabetic rats. The treatment can increase antioxidant enzyme, which has the ability to ameliorate oxidative stress and protects the hepatic and renal tissues in diabetic rats [56].

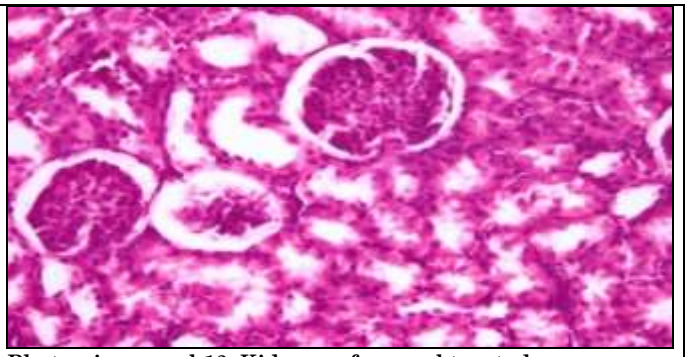
On the other hand, diabetic rats treated with the same extracts (Photomicrographs 18-22) showed normal histopathological observation except for diffuse interstitial infiltration with

leucocytic cells with diabetic group treatment with olive leaves extract (photomicrograph 21).

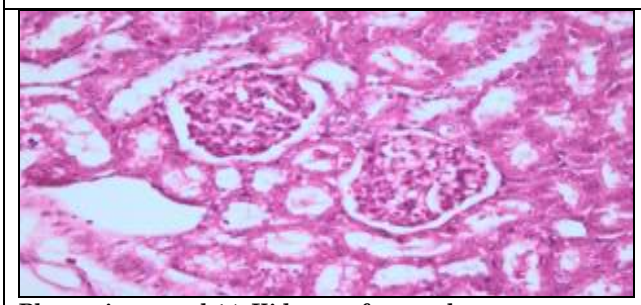
Also, data showed slightly congested blood capillaries with the diabetic group treated with kiwi peels extract (Photomicrograph 18).



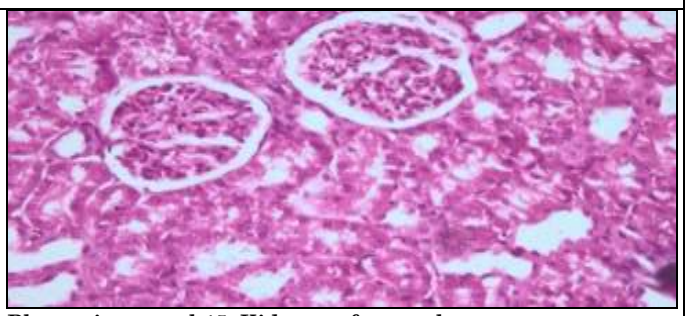
Photomicrograph 12. Kidneys of normal rats showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



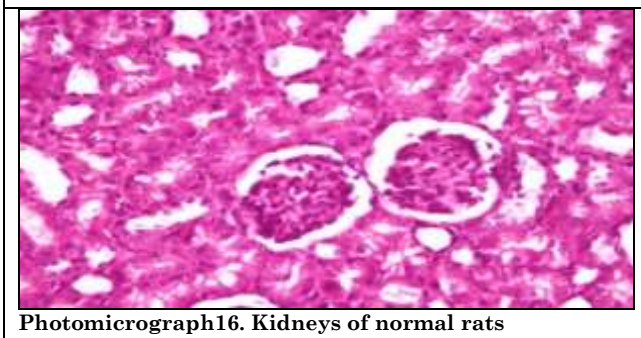
Photomicrograph13. Kidneys of normal treated with kiwi, peels extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



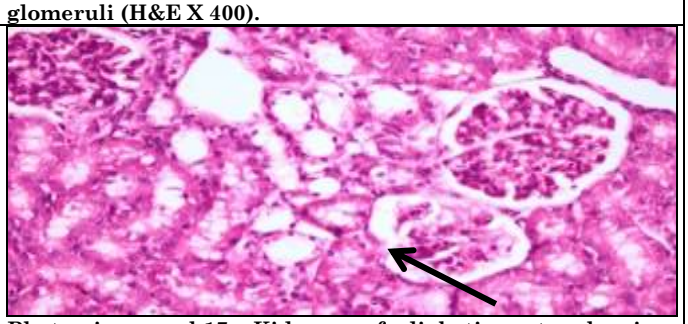
Photomicrograph14. Kidneys of normal rats treated with banana peels extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



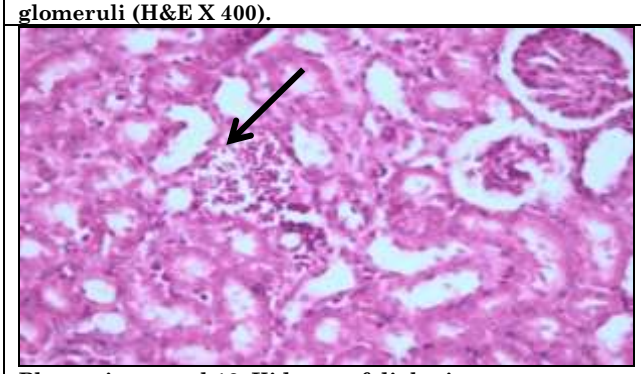
Photomicrograph15. Kidneys of normal rats treated with banana leaves extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



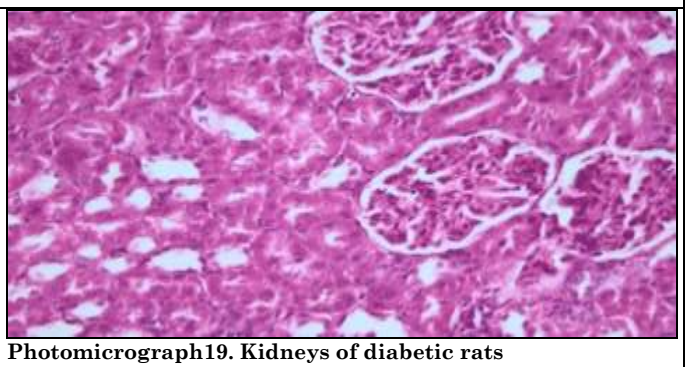
Photomicrograph16. Kidneys of normal rats treated with olive leaves extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



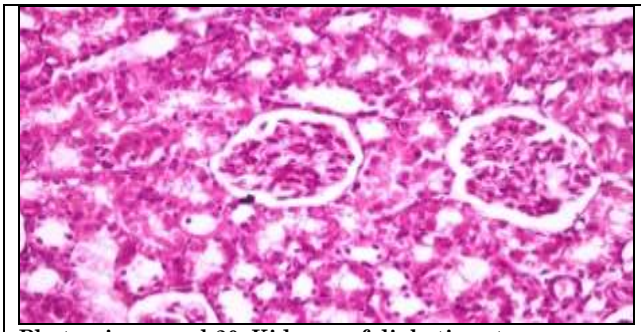
Photomicrograph17. Kidneys of diabetic rats showing vacuolated renal tubular epithelium (H&E X 400).



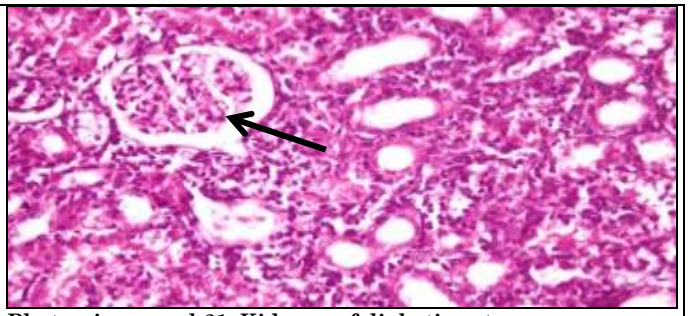
Photomicrograph18. Kidneys of diabetic rats treated with kiwi peels extract showing slightly congested blood capillaries (arrow) (H&E X 400).



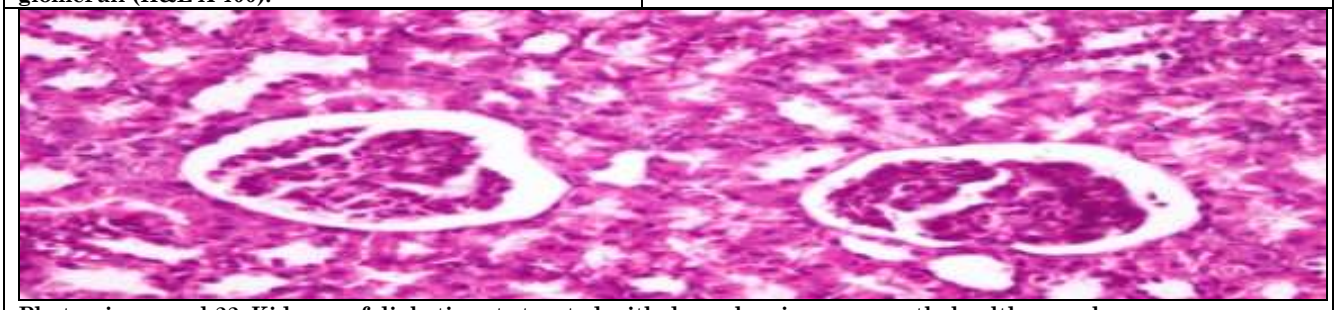
Photomicrograph19. Kidneys of diabetic rats treated with banana peels extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



Photomicrograph20. Kidneys of diabetic rats treated with banana leaves extract showing apparently healthy renal tubules and renal glomeruli (H&E X 400).



Photomicrograph21. Kidneys of diabetic rats treated with olive leaves extract showing diffuse interstitial infiltration with leucocytic cells (arrows) (H&E X 400).



Photomicrograph22. Kidneys of diabetic rats treated with drug showing apparently healthy renal tubules and renal glomeruli (H&E X 400)

Conclusion

All the treated groups recorded changes in pancreatic function, renal function, antioxidant biomarkers, oxidative stress, inflammatory markers, and anti-inflammatory enzyme after a single dose of streptozotocin (45 mg/kg body weight). These parameters came towards around the normal control level gradually after daily administration of 80% acetone extract of all tested samples (500 mg/kg body weight). The histopathological study revealed the ameliorative effect of acetone extracts on pancreatic ß-cells and renal tissues of diabetic rats and apparently normal. Hence, in conclusion, the present study clearly refers to the possibility of using by-products from kiwi peels, banana peels, banana leaves, and olive leaves to develop new formula as antioxidants and hypoglycaemic agents.

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