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HYPOGLYCEMIC EFFECTS OF WHITE CABBAGE AND RED CABBAGE (BRASSICA OLERACEA) IN STZ INDUCED TYPE-2 DIABETES IN RATS

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ABSTRACT

The present study aims to investigate the comparative potential and ameliorative effect of both white and red cabbages (*Brassica oleracea*) in the management of STZ induces diabetes in rats. Diabetes was induced in male Westar rats using streptozotocin; STZ, 60 mg/kg body weight. Diabetic rats showed significant elevation in serum total cholesterol (TC) , low density lipoprotein-Cholesterol (LDL-C) and triacylglycerol (TG) , while, significant decrease in serum high density lipoprotein –cholesterol (HDL-C) level. Oral supplementation of white and red cabbage extracts to diabetic rats resulted in insignificant change in serum total lipids level, TC, TG and HDL .LDL showed significant increase with white cabbagetreatment ,while, red cabbage demonstrated significant decrease as compared to normal control rats.Diabetic rats exhibited significant increase in liver function enzymes; alanine and aspartate aminotransferase (AST and ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels. Treatment of diabetic rats with white cabbage demonstrated significant increase in AST enzyme activity, while, insignificant change in AST was noticed with red cabbage extract. However, ALT enzyme activity recorded insignificant change with white and red cabbage extracts. White cabbage treated - diabetic rats demonstrated significant increase in ALP enzyme activity, although, diabetic rats treated with red cabbage declared insignificant change. Whereas TB level, showed insignificant change with both treatments. The present

results reveal also, significant lower levels of total protein (TP) and albumin (ALB) in diabetic rat's .Treatment of diabetic rats with white and red cabbage extracts, showed insignificant change in TP and ALB levels. Blood glucose level of diabetic rats showed significantly increase. Treatment of diabetic rats with white and red cabbage extracts restored blood glucose level to normal value .In addition , diabetic rats recorded significant reduction in glutathione (GSH) content while, malondialdehyde (MDA) level showed significant elevation. However, diabetic -rats treated with white and red cabbage extracts exhibited insignificant change in GSH ,while significant increase in MDA levels .Significant increase in pro-inflammatory cytokines; tumor necrosis factor- α(TNF-α) and C- reactive protein (CRP), was detected also in diabetic rats .Treatment of diabetic-rats with both white, red cabbage extracts showed significant increase in TNF-α levels ,while, insignificant change was observed in CRP level. Moreover, diabetic status caused significantly elevated levels of serum total urea and creatinine. White and red cabbage extracts treated- diabetic rats showed, insignificant change in total urea and creatinine levels as compared to normal control rats. Histopathological investigation of diabetic rat's liver showed hydropic degeneration of hepatocytes and oval cells proliferation. Histopathological examination of diabetic kidney, demonstrated interstitial nephritis.However, pancreas of diabetic rats revealed necrosis of islets of Langerhan's and inflammatory cells infiltration. Treatments of diabetic rats with white and red cabbage extracts declared amelioration in hepatic, renal as well as pancreas architectures and apparent normal .So, it could be concluded that, the antioxidant, anti-inflammatory and anti-hyperglycemic properties of *Brassica oleracea* extracts may offer a potential therapeutic source for the treatment of diabetes.

Keywords: White cabbage, Red cabbage, Hypoglycemic Effects, antidiabetic.

INTRODUCTION

Micro vascular complications of diabetes share a common pathophysiology that may be explained as a direct or indirect consequence of hyperglycemia-mediated overproduction of reactive oxygen species (ROS). Microvascular deterioration is preventable either by the inhibition of superoxide accumulation or by modulating the blood glucose levels, and among several micro vascular disorders; nephropathy[1] can be improved by antioxidants.

The antioxidant protection of natural plants is a promising therapeutic remedy for free radical pathologies [2]. Among myriad natural plants, red cabbage (RC) (*Brassica oleracea*var*capitata*) and other *Brassica* vegetables, vegetables endemic to the Mediterranean

region, have been found to have antioxidant, antihyperglycemic, anticancer and hypocholesterolemic properties. RC extract has also prevented oxidative stress induced in livers and brains of animals exposed to paraquate and *N*-methyl-d-aspartate[3].The principle constituents of RC are isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins[4]. Anthocyanins, a group of phenolic natural pigments present in RC, were found to have the strongest antioxidizing power of 150 flavonoids[5].

Experimental and clinical as well as population studies confirmed the benefits of diet rich in fruits and vegetables in prevention of cardiovascular diseases, cancer, hypertension, diabetes and obesity. In particular, several epidemiological studies report an inverse correlation between consumption of *Brassicaceae* and risk of cancer[6].

Cruciferous vegetables such as white cabbages *(Brassica oleraceavarcapitataf. alba*) are among the most important dietary vegetables consumed in Europe owing to their availability in local markets, cheapness and consumer preference. The mechanism of chemo-preventive action of cruciferous vegetables is still not fully clarified, however animal and human intervention studies suggested that the substances present in these plants, especially glucosinolates (GLS) and products of their decomposition are able to modulate activity of phase I and II enzymes. GLS degradation products are believed to act as anti-carcinogens by decreasing carcinogen activation through the inhibition of phase I enzymes, while increasing detoxification by induction of the phase II enzymes that affect xenobiotic transformations. These compounds were also shown to inhibit tumor cell growth and to stimulate apoptosis[3], [4], [7].Though less extensively studied; the protective effects against chronic diseases could also depend on the antioxidant activity of compounds present in cruciferous vegetables. Although some studies have been conducted to determine the antioxidant activity, the content of polyphenols and flavonoids as well as other antioxidants in white cabbage [8], [9]is still not well known and their identification not sufficient. It seems, however, that such data will be of great value, as current knowledge indicates that the occurrence of at least some of mentioned above diseases as well as aging processes may result from oxidative stress leading to a variety of alterations within the human organism caused by reactive oxygen species (ROS). Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from an environmental or behavioral stressor. If not controlled properly, the excess of ROS

can lead to damage of cellular lipids, proteins or DNA, impairing their normal function. The organism defends itself against ROS by engaging several enzymatic systems and endogenous antioxidants. This natural defense is enhanced by antioxidants delivered with diet. Considering the chemical diversity of the antioxidant compounds present in foods and the interaction occurring among these different molecules, the evaluation of the total antioxidant capacity of foods seems to be a more useful marker than the evaluation of individual compounds. However, no single method to test the total antioxidant capacity of foods fully considers, at the same time, the activity of all the antioxidants[8].In addition, *Brassica rapa* ssp. Campestris (*Brassicaceae*),phytochemical and pharmacological studies revealed that the biological active compounds isolated from the roots of *Brassicarapa* ssp. Campestris such as a novel phenanthrene derivative, 6-methoxy-1-[10-methoxy-7-(3-methylbut-2-enyl)phenanthren-3-yl]undecane-2,4-dione, named *Brassica*phenanthrene A (3) along with two known diarylheptanoid compounds, 6-paradol (1) and trans-6-shogaol (2) were determined [10].These compounds also, were shown to exhibit high inhibitory activity against the growth of human cancer lines, HCT-116, MCF-7, and HeLa, with IC_{50} values ranging from 15.0 to 35.0 μM and against LDL-oxidation with IC_{50} values ranging from 2.9 to 7.1 Mm[10].Thus the present results aim to investigate the comparative potential roles of both white and red cabbages in the management of STZ induce type 2 diabetes in rats.

MARERIALS AND METHODS

Chemicals

All chemicals in the present study are of analytical grade, products of Sigma, Merck and Aldrich. All kits were the products of Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic Company (Cairo, Egypt).

Preparation of plant extract

The leaves were extracted. Briefly, 10 g of the dried powder from red and white cabbages were soaked with 100 ml of distilled water and shaking at room temperature for 48 h. The extracts were filtered and the extraction was repeated twice. The resulting aqueous extract was used for the determination of different biochemical examination.

Animals and the experimental design

Experimental animals

70 Male Wister rats (200±250), were used for the evaluation of anti-diabetic effects of white and red cabbages crude extracts, were provided by the Animal house of the National

Research Center (NRC) and housed in a temperature-controlled environment (26-29°C) with a fixed light/dark cycle for 2 weeks as an adaptation period to acclimatize under normal combination with free access to water and food *ad libitum*. The present study is approved by the Ethical Committee of the National Research Center (NRC), Egypt, provided that the animals will not suffer at any stage of the experiment.

Experimental design

Seventy male albino rats were selected for this study and divided into seven groups (ten rats in each group) as follows: **Group 1:** normal healthy control rats, **Groups 2, 3**: normal rats treated orally with 1g/kg body weight white and red cabbages crude extracts[3],for 45 days .**Group 4:** considered as diabetic groups; where type 2 diabetes was induced by STZ. Each rat was injected intraperitoneally with a single dose of STZ (60 mg/kg body weight dissolved in 0.01 M citrate buffer immediately before use[11].After injection, animals had free access for food and water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. Animals were checked daily for the presence of glycosuria[12]. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and fasting blood sugar was determined (>300 mg/dl). Hyperglycemic rats were used for the experiment and classified as follows: **Group 5,6:** diabetic rats oral administered 1 g/kg body weight white and red cabbages crude extracts for 45 Day, **Group 7:** diabetic rats administered orally antidiabeticglibenclamide reference drug 5 mg /kg body weight daily for 45 days[13].

Sample preparations

After 45 days of treatments, rats were fasted overnight (12-14 hours), anesthetized by diethyl ether and blood collected by puncture of the sublingual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was used for biochemical analysis of liver function enzymes, blood glucose level, lipid profile, albumin level , total protein content and inflammatory markers [tumour necrosis factor - α (TNF-α) and C-reactive protein (CRP)] . After blood collection, rats of each group were sacrificed, the liver, kidney and pancreas were removed immediately (a part was fixed in 10% formalin for histopathological examination). Liver in each exponential group weighed and homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 4000 rpm for 15 min, the supernatants(10%) were collected and placed in

Eppendorff tubes and stored at –80°C and used for determination of oxidative stress marker (malondialdehyde,MDA) and non-enzymatic antioxidant (GSH) .

Biochemical examination

Lipid Profile

Serum cholesterol concentration was estimated according to the method of [14], serum triacylglycerol (TGs) concentration was determined according to the method of [15], serum HDL-C concentration was measured according to the method of [16], and serum LDL-C concentration was determined according to the equation of [17]. All lipid profile was estimated using colorimetric kits. ALT and AST enzyme activities were assayed according to the method of [18] using colorimetric kits. ALP enzyme activity was determined according to the method described by [19] using colorimetric kits. Total bilirubin was assayed according to the method of [20] using colorimetric kits. Total protein was assayed in serum according to the method of [21]. Albumin was measured according to the method of[22]using colorimetric kits. Glucose was determined in blood serum according to the method of [23] using colorimetric kits. The level of hepatic glutathione was assayed in liver homogenate according to the method of [24]. Liver MDA was estimated according to the method of [25]. Urea concentration was estimated according to the method of [26] using colorimetric kits and serum creatinine concentration was measured according to the method of [27] using colorimetric kits.

Estimation of serum inflammatory markers TNF-α and CRP

Quantitative measurements of TNF-α and CRP, was performed by ELISA; a sandwich enzyme Immunoassay.

Histopathological analysis

Liver , kidney and pancreas slices were fixed instantaneously in buffer neutral formalin (10%) for 24 hours for fixation then processed in automatic processors, embedded in paraffin wax (melting point 55-60 ^{o} C) and paraffin blocks were obtained. Sections of 6 µm thicknesses were prepared and stained with Haematoxylin and Eosin (H&E) stain [28]. The cytoplasm stained shades of pink and red and the nuclei gave blue color. The slides were examined and photographed under a light microscope at a magnification power of x400.

Statistical Analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) SPSS computer program and Co-state computer program, where significance is considered at $p \leq 0.05$. Unshared letter is significant at $p \leq 0.05$

RESULTS

The current study is design to examine the anti-diabetic, antioxidative and anti-inflammatory activities of white and red cabbages extracts.

Lipid profile

As compared to negative control rats, an insignificant change was detected in lipid profile in healthy negative control rats treated with white and red cabbage extracts as compared to normal control rats. Diabetic rats showed significant elevation in serum total cholesterol (87.43%), LDL-C (201.73%) and triacylglycerol (391.22%), while significant decrease in serum HDL-C level (76.76%), as compared to negative control rats (Table: 1). Oral supplementation of white and red cabbage crude extracts to diabetic rats resulted in an insignificant change in the levels of serum total lipids (4.21 and 2.71% respectively), TC (13.11 and 9.77%, respectively), TG (13.11 and9.77%, respectively) and HDL (10.08 and 14.33 %, respectively) .While, LDL showed significant increase with treatment of white cabbage (84.17), however, red cabbage demonstrated significant decrease (15.25) as compared to normal control rats. On the other hand, antidiabeticglibenclamide standard drug –treated diabetic rats showed insignificant change (10.07% and 4.55%, respectively), for serum total lipids and HDL, while TG and LDL significant increase was recorded reached to 77.77 and 33.6% , respectively as compared to normal control rats (Table 1), the percentages of improvement post crude extracts treatment to diabetic rats is elucidated in Table (1) .

Table (1): Comparative Effects of Red and White Cabbage extracts supplementations on lipid profile TC, TG, LDL and HDL in STZ- Induced diabetic rats and in different therapeutic groups.

Groups	Parameters	TC (µg/dl)	TG (µg/dl)	LDL (µg/dl)	HDL (mg/dl)
Negative control	Mean ± S.D.	54.60 ± 11.20 [a]	22.50 ± 6.74 [c]	17.44 ± 7.00 [f]	29.65 ± 1.90 [i]
Negative	Mean ± S.D.	52.10 ± 3.23 [a]	22.10 ± 3.23 [c]	20.00 ± 0.56 [f]	25.03 ± 2.23 [i]

White cabbage extract	% Change to control	4.57	1.77	-14.67	15.58
Negative Red cabbage extract	Mean ± S.D.	54.22 ± 8.28 [a]	22.42 ± 2.04 [c]	23.00 ± 2.09 [f]	25.30 ± 2.14 [i]
	% Change to control	0.69	0.35	-31.88	14.67
Diabetic Rats	Mean ±S.D	102.34 ± 10.04 [b]	67.89 ± 5.56 [d]	85.67±6.55 [g]	6.89±0.98 [j]
	% Change to control	87.43	201.73	391.22	76.76
Diabetic – White cabbage extract	Mean ± S.D.	56.9 ± 5.10 [a]	25.45 ± 2.00 [c]	32.12 ± 4.03 [h]	26.66 ± 1.97 [k]
	% Change to control	4.21	13.11	84.17	10.08
	% of improvement	77.73	188.62	307.05	66.67
Diabetic – Red cabbage extract	Mean ± S.D.	53.12 ± 2.22 [a]	20.30 ± 1.40 [c]	14.78 ± 2.20 [f]	33.90 ± 3.20l [k]
	% Change to control	2.71	9.77	15.25	14.33
	% of improvement	90.15	211.51	406.48	91.09
Diabetic -- anti diabetic drug	Mean ± S.D.	60.10 ± 4.50 [a]	40.00 ± 5.40 [e]	23.30 ± 2.30 [h]	31.00 ± 2.45 [k]
	% Change to control	10.07	77.77	33.6	4.55
	% of improvement	77.36	123.95	357.63	81.32

(TG): Triglycerides and (TC): Total cholesterol, (LDL-C): low density lipoprotein cholesterol; (HDL-C): high-density lipoprotein cholesterol. Data presented as mean ± SD, n=8.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at $P \leq 0.05$.

Liver enzymes activity and TB

White and red cabbage crude extracts administered to healthy rats showed an insignificant change in serum AST, ALT, ALP enzyme activities and TB levels. As compared to normal control healthy rats, diabetic rats exhibited significant increase in liver function enzymes; AST, ALT, ALP and TB levels by 70.86, 42.79, 114.71 and52.30%, respectively. Treatment of diabetic rats with white cabbage and antidiabetic drug demonstrated significant increase in AST enzyme activity by 26.88 and 35.44%, respectively, while, insignificant change in AST activity was noticed with red cabbage extract. However, ALT enzyme activity recorded insignificant change with white and red cabbage crude extracts, however antidiabetic drug treated rats exhibited significant increase in ALT enzyme activity with percentage reached 29.73 as compared to normal control rats. With respect to ALP, white cabbage and antidiaetic

drug - treated rats demonstrated significant increase amounting to 18.59 and 21.64% respectively. Although, diabetic rats treated with red cabbage demonstrated insignificant change (5.57%). Whereas TB level, showed insignificant change with varies treatment as compared to normal control rats (Table 2). The percentage of improvement post crude extracts treatment to diabetic rats is elucidated in Table (2).

Table (2): Comparative Effects of Red and White Cabbage extracts supplementations on serum AST, ALT, ALP enzyme activities, and TB in STZ-Induced diabeticrats and in different therapeutic groups.

Groups	Parameters	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	TB (mg/dl)
Negative control	Mean ± S.D.	39.50 ± 2.66 [a]	64.56 ± 2.78 [a]	43.57 ± 3.06 [a]	0.65 ± 0.05 [a]
Negative White cabbage extract	Mean ± S.D.	37.88 ± 2.19 [a]	63.65 ±3.87 [a]	44.53 ± 1.87 [a]	0.69 ± 0.04 [a]
	% Change to control	4.10	1.4	2.20	6.15
Negative Red cabbage extract	Mean ± S.D.	36.23 ± 2.65 [a]	65.00 ± 3.20 [a]	45.50 ± 4.10 [a]	0.70 ± 0.03 [a]
	% Change to control	8.27	0.68	25.08	7.69
Diabetic Rats	Mean ±S.D	67.49 ± 5.00 [b]	92.19 ± 6.78 [b]	93.55 ± 8.00 [b]	0.99 ± 0.12 [b]
	% Change to control	70.86	42.79	114.71	52.30
Diabetic – White cabbage extract	Mean ± S.D.	50.12 ± 2.29 [c]	67.00 ± 3.00 [a]	51.67 ± 4.40 [c]	0.64 ± 0.02 [a]
	% Change to control	26.88	3.77	18.59	1.53
	% of improvement	43.97	39.02	96.12	53.85
Diabetic – Red cabbage extract	Mean ± S.D.	40.50 ± 2.10 [a]	63.89 ± 3.90 [a]	46.00 ± 3.74 [a]	0.68 ± 0.03 [a]
	% Change to control	2.53	1.037	5.57	4.62
	% of improvement	68.33	43.84	109.13	47.69
Diabetic -- anti diabetic drug	Mean ± S.D.	53.60 ± 3.45 [c]	83.76 ± 5.26 [b]	53.00 ± 2.07 [c]	0.70 ± 0.04 [a]
	% Change to control	35.44	29.73	21.64	7.69
	% of improvement	35.16	13.05	93.06	44.62

(ALT): Alanine aminotransferase; (AST): Aspartate aminotransferase; (ALP): Alkaline phosphatase; (TB): Total bilirubin. Data presented as mean ± SD, n=10.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at $P \leq 0.05$.

Total protein (TP) content and albumin (ALB) level

Insignificant change was detected in TP content and ALB level in healthy negative control rats treated with both cabbage extracts as compared to normal untreated -control rats. The present study reveals significant lower levels of TP and ALB in diabetic rats as compared to normal control.Diabetic rats showed significant reduction in TP content and ALB level by 29.64 and 38.59 %, respectively as compared to normal control. Treatment of diabetic rats with crude extracts of white and red cabbages, as well as, antidiabetic drug showed insignificant change in TP and ALB levels as compared to normal control. The percentages of amelioration are seen in Table (3).

Blood glucose level

Insignificant change was detected in glucose level in healthy negative control rats treated with white and red cabbage extracts as compared to normal control rats. Blood glucose level of diabetic rats showed significantly ($P \leq 0.05$) increase (300.17%), as compared to normal control rats. Treatment of diabetic rats with white and cold extracts and reference drug restored blood glucose level to normal level ,where insignificant change was recorded(Table. 3).

Table (3): Comparative Effects of Red and White Cabbage extracts supplementations on serum TP content, ALB and glucose levels in STZ induced diabetic rats and in different therapeutic groups.

Groups	Parameters	TP (mg)	Albumin (mg/dl)	Glucose (mg/dl)
Negative control	Mean ± S.D.	39.80 ± 1.23 [a]	6.40 ± 0.62 [a]	89.60 ± 3.56 [a]
Negative White cabbage extract	Mean ± S.D.	39.08±2.34 [a]	6.89 ± 0.57 [a]	88.00±3.90 [a]
	% Change to control	1.80	7.65	1.78
Negative Red cabbage extract	Mean ± S.D.	38.79 ± 1.98 [a]	6.70 ± 1.10 [a]	87.87±3.29 [a]
	% Change to control	2.53	4.68	1.93
Diabetic Rats	Mean ±S.D	28.00 ± 2.20 [b]	3.93 ± 0.45 [b]	358.56 ± 6.78 [b]
	% Change to control	29.64	38.59	300.17

Diabetic – White cabbage extract	Mean ± S.D.	36.00 ± 0.89 [a]	5.60 ± 0.26 [a]	86.89 ± 6.78 [a]
	% Change to control	9.54	12.50	3.02
	% of improvement	20.10	26.09	303.20
Diabetic – Red cabbage extract	Mean ± S.D.	37.34 ± 1.44 [a]	5.70 ± 0.56 [a]	81.16 ± 3.89 [a]
	% Change to control	6.18	10.93	9.42
	% of improvement	23.47	27.66	309.59
Diabetic -- anti diabetic drug	Mean ± S.D.	37. 56 ± 3.03 [a]	6.00 ± 0.33 [a]	89.00 ± 2.99 [a]
	% Change to control	5.62	6.25	0.66
	% of improvement	24.02	32.34	300.85

(TP): Total proteins, Data presented as mean ± SD, n=10.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P≤0.05.

Non-enzymatic antioxidant

Insignificant change was detected in GSH and MDA levels in healthy negative control rats treated with both cabagge extracts as compared to normal control rats. Diabetic rats recorded significant reduction in GSH concentration B(62.71%). While, MDA level showed significant elevation (142.66%).However, diabetic rats treated with white and red cabbage extracts and glibenclamide showed insignificant change in the concentration of GSH as compared to normal control rats (15.25, 6.77 and 3.38 %, respectively). On the contrary, MDA level recorded significant increase with percentages amounting to 43.16, 33.16 and 33.00%, for white , red cabbage extracts and antidiabetic drug , respectively as compared to normal control rats with percentages of amelioration amounting to 99.50, 109.50 and 1099.66%, respectively (Table 4).

Table (4): Comparative Effects of Red and White Cabbage extracts supplementation on serum MDA and GSH levels in liver tissue of STZ induced diabetic rats and different therapeutic groups.

Groups	Parameters	GSH mg/g tissue	LPO (MDA) µmol/g tissue
Negative control	Mean ± S.D.	0.59 ± 0.03 [a]	6.00 ± 0.45 [a]
Negative White cabbage extract	Mean ± S.D.	0.63 ± 0.05 [a]	6.20 ± 0.43 [a]
	% Change to control	6.77	3.33
Negative Red cabbage extract	Mean ± S.D.	0.64 ± 0.03 [a]	5.58 ± 0.70 [a]
	% Change to control	8.47	7
Diabetic Rats	Mean ±S.D	0.22 ± 0.03 [b]	14.56 ± 1.27 [b]
	% Change to control	62.71	142.66
Diabetic – White cabbage extract	Mean ± S.D.	0.50 ± 0.04 [a]	8.59 ± 0.56 [c]
	% Change to control	15.25	43.16
	% of improvement	47.45	99.50
Diabetic – Red cabbage extract	Mean ± S.D.	0.55 ± 0.03 [a]	7.99 ± 0.50 [c]
	% Change to control	6.77	33.16
	% of improvement	55.93	109.50
Diabetic -- anti diabetic drug	Mean ± S.D.	0.57 ± 0.02 [a]	7.98 ± 0.67 [c]
	% Change to control	3.38	33
	% of improvement	59.32	109.66

(LPO): Lipid peroxide, (GSH): Glutathione reduced, Data presented as mean ± SD, n=10.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P≤0.05.

Inflammatory cytokines CRP and TNF-α level

From the manipulated data (Table: 5), it can be demonstrated, insignificant changes in CRP and TNF-α levels in healthy negative control rats treated with both extracts as compared to normal control rats. Diabetic rats showed significant increase in pro-inflammatory cytokines; TNF-α (73.91%) and CRP (114.75%), as compared to normal control rats. Treatment of diabetic-rats with both white, red cabbage extracts as well as antidiabetic drug, showed significant increase in TNF-α levels by 27.53, 18.25 and 37.65%, respectively as compared to normal control rats with percentages of amelioration reached to 46.36, 55.66 and 36.25 %, respectively. While, insignificant change was detected in CRP levels as compared to normal control rats. Thus, significant positive correlations between diabetic status and TNF-α and CRP was noticed (Table 5).

Table (5): Comparative effects of Red and White Cabbage extracts supplementations on the anti-inflammatory TNF-α and CRP levels in STZ induced diabetic rats and different therapeutic groups.

Groups	Parameters	TNF-α (ρg/ml)	CRP (ηg/ml)
Negative control	Mean ± S.D.	109.77 ± 10.20 [a]	5.90 ± 0.19 [a]
Negative White cabbage extract	Mean ± S.D.	110.00 ± 11.78 [a]	5.44 ± 0.20 [a]
	% Change to control	0.21	7.79
Negative Red cabbage extract	Mean ± S.D.	117.20 ± 8.23 [a]	5.30 ± 0.34 [a]
	% Change to control	6.76	10.16
Diabetic Rats	Mean ±S.D	190.90 ± 12.90 [b]	12.67 ± 1.88 [b]
	% Change to control	73.91	114.75
Diabetic – White cabbage extract	Mean ± S.D.	140.00 ± 0.27 [c]	6.53 ± 0.04 [a]
	% Change to control	27.53	10.67
	% of improvement	46.36	104.06
Diabetic – Red cabbage extract	Mean ± S.D.	129.80 ± 9.16 [c]	6.00 ± 0.29 [a]
	% Change to control	18.25	1.69
	% of improvement	55.66	113.05
Diabetic -- anti diabetic drug	Mean ± S.D.	151.10 ± 10.15 [c]	6.00 ± 0.56 [a]
	% Change to control	37.65	1.69
	% of improvement	36.25	113.05

Data presented as mean ± SD, n=10.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P≤0.05.

Total urea and creatinine levels

Insignificant decrease was detected in serum total urea and creatinine levels in healthy control rats treated with both cabbage extracts as compared to normal untreated rats. Diabetic status caused significantly elevation in serum total urea and creatinine levels reached to 206.83 and 44.23%, respectively as compared to normal rats (Table: 6).White and red cabbage extracts as well as antidiabetic drug treated- diabetic rats showed, insignificant change in total urea (14.41, , 11.63 and 14.65%, respectively) and creatinine levels (5.77, 9.62 and 8.65%, respectively), as compared to the normal control (Table 6).

Histopathological observation

Histopathological investigation of normal control rats liver revealed, normal histological structure of hepatic liver (Fig.1). While, liver of diabetic rat showing hydropic degeneration of hepatocytes and oval cells proliferation (Fig.2).Diabetic rats treated with white and red cabbage extracts showed hydropic degeneration of hepatocytes (Fig.3) and congestion of central vein (Fig.4) respectively. While, liver of antidiabetic drug- treated rats declared

nohistopathological changes (Fig.5).With respect to, histopathological examination of kidney, normal control rats showed the normal histological structure of renal parenchyma (Fig.6).Although, kidney of diabetic rats demonstrated interstitial nephritis (Fig.7).On the other hand, diabetic rats- treated with white cabbage extracts showed vacuolation of epithelial lining renal tubules and perivascular oedema(Fig.8). While, kidney of diabetic rats treated with red cabbage showing vacuolation of epithelial lining renal tubules (Fig.9). Also, kidney of antidiabetic drug treated rats showing slight congestion of glomerular tuft (Fig.10). Pancreas of control negative rat demonstrates no histopathological changes (Fig.11). However, pancreas of diabetic rats revealed necrosis of islets of Langerhans's and inflammatory cells infiltration (Fig.12). Treatments of diabetic rats with white and red cabbage extracts declared focal hemorrhage (Fig.13) and apparent normal pancreas respectively (Fig.14).

Table (6) Comparative effects of Red and White Cabbage extracts supplementations on kidney-function tests (total urea and creatinine), in STZ induced diabetic rats and in different therapeutic groups.

Groups	Parameters	Urea (mg/dl)	Creatinine (mg/dl)
Negative control	Mean ± S.D.	32.34 ±2.50[a]	1.04±0.02[a]
Negative White cabbage extract	Mean ± S.D.	31.07±1.56[a]	1.00±0.04[a]
	% Change to control	3.93	3.85
Negative Red cabbage extract	Mean ± S.D.	33.89 ± 1.29 [a]	1.09 ± 0.02 [a]
	% Change to control	4.79	4.81
Diabetic Rats	Mean ±S.D	99.23 ± 6.78 [b]	1.50 ± 0.12 [b]
	% Change to control	206.83	44.23
Diabetic – White cabbage extract	Mean ± S.D.	37.00 ± 2.34 [a]	0.98 ± 0.04 [a]
	% Change to control	14.41	5.77
	% of improvement	192.42	50
Diabetic – Red cabbage extract	Mean ± S.D.	36.10 ± 1.22 [a]	0.94 ± 0.02 [a]
	% Change to control	11.63	9.62
	% of improvement	195.20	53.84
Diabetic -- anti diabetic drug	Mean ± S.D.	37.08 ± 3.00 [a]	0.95 ± 0.03 [a]
	% Change to control	14.65	8.65
	% of improvement	192.17	52.88

Data presented as mean ± SD, n=10.Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P≤0.05.

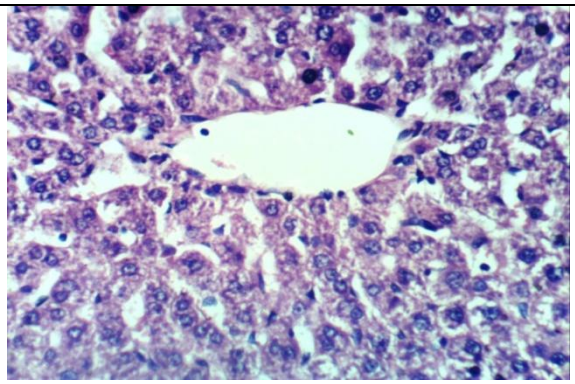

Fig. (1): Liver of normalcontrol rat showing the normal histological structure of hepatic lobule (H & E X 400).

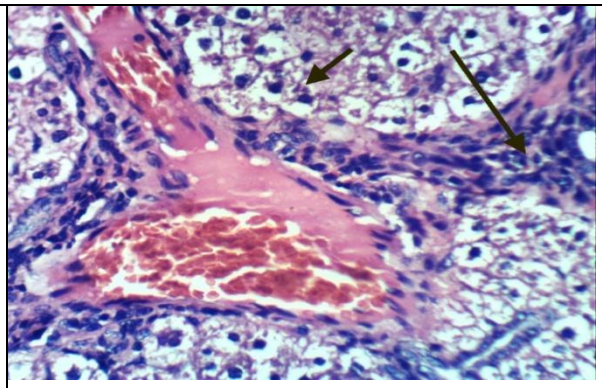

Fig. (2):Liver of diabetic rat showing hydropic degeneration of hepatocytes and oval cells proliferation (H & E X 400).

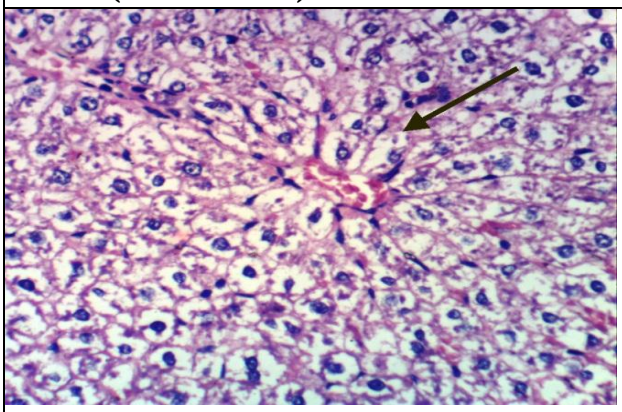

Fig. (3):Liver of rat from diabetic rats treated with red cabbage extract showing hydropic degeneration of hepatocytes (H & E X 400).

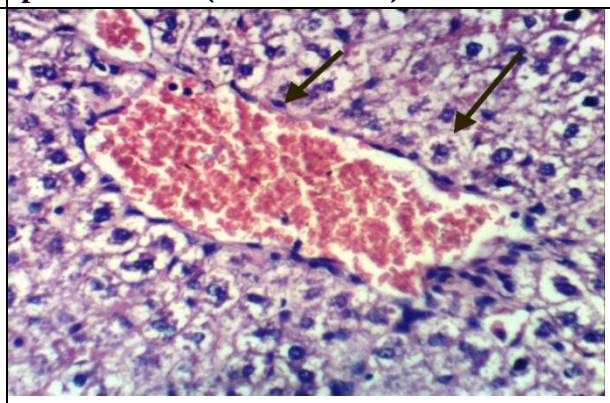

Fig. (4):Liver of rat from diabetic rats treated with white cabbage extract showing congestion of central vein and hydropic degeneration of hepatocytes (H & E X 400).

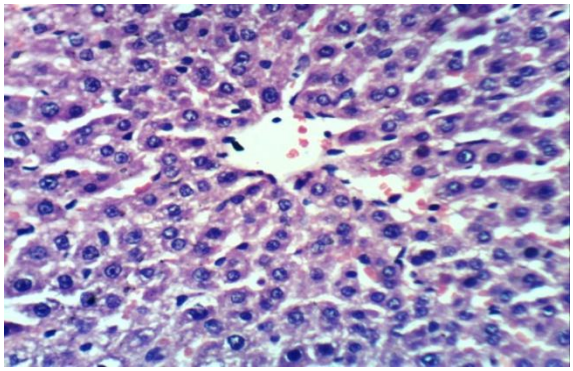

Fig. (5):Liver of rat from antidiabetic drug treated rats showing no histopathological changes (H & E X 400).

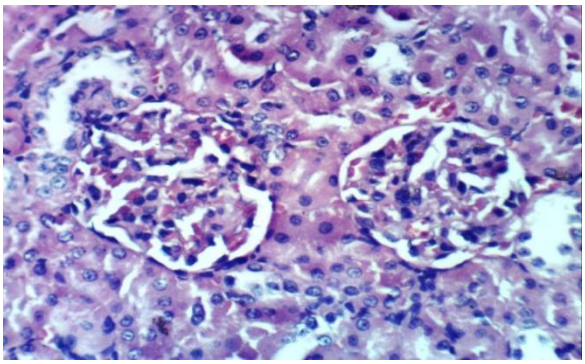

Fig.(6):Kidney of normal control rat showing The normal histological structure of renal parenchyma (H & E X 400).

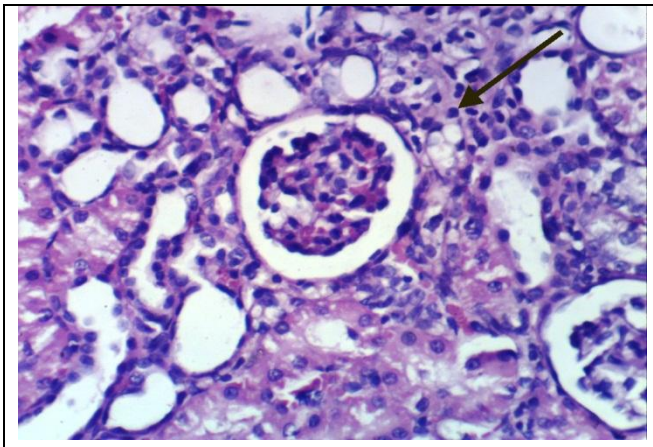

Fig.(7):Kidney of diabetic rat showing interstitial nephritis (H & E X 400).

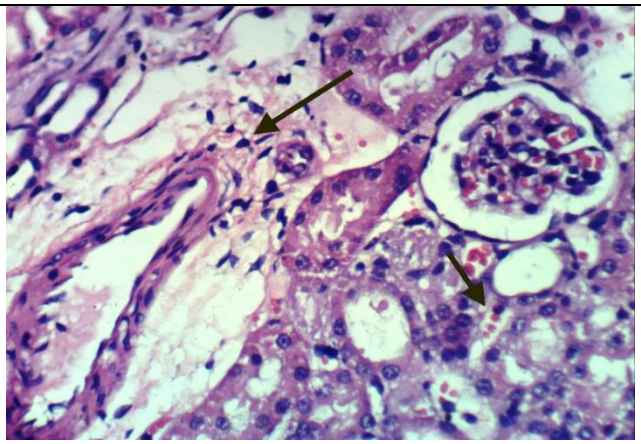

Fig. (8):Kidney of rat from diabetic rats treated with white cabbage extracts showing vacuolation of epithelial lining renal tubules and perivascular oedema (H & E X 400).

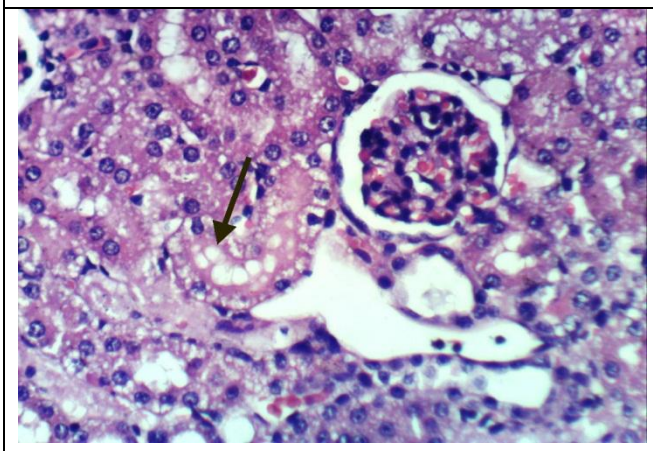

Fig. (9): Kidney of rat from diabetic rats treated with red cabbage showing vacuolation of epithelial lining renal tubules (H & E X 400).

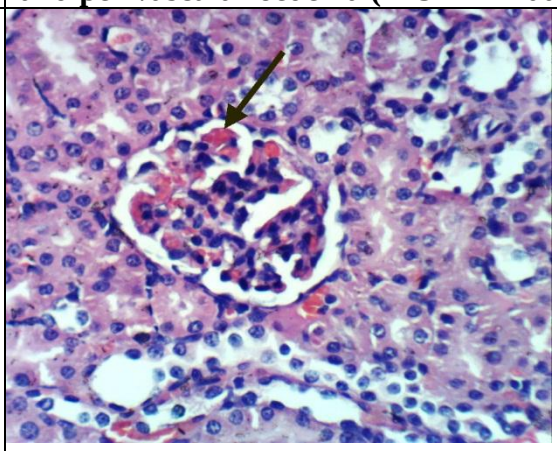

Fig. (10): Kidney of rat from antidiabetic drug treated rats showing slight congestion of glomerular tuft (H & E X 400).

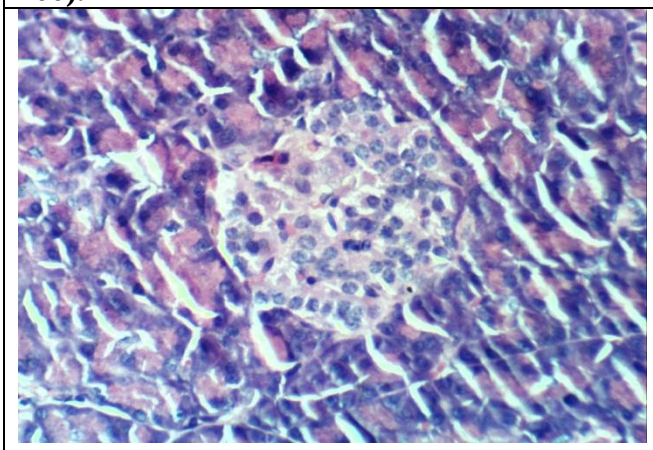

Fig. (11): Pancreas of control negative rat showing no histopathologicalchanges (H & E X 400).

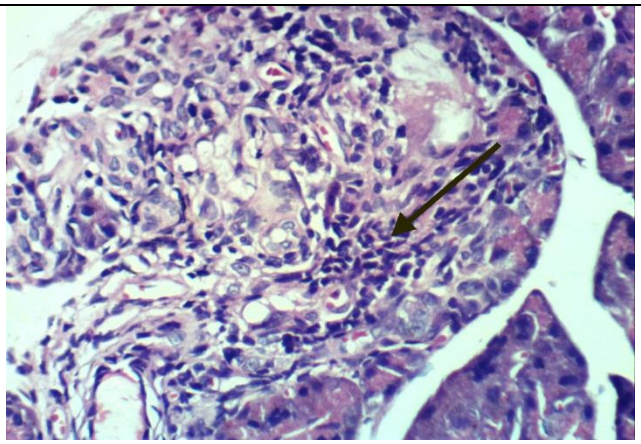

Fig. (12): Pancreas of control positive rat showing necrosis of islets of Langerhan's and inflammatory cells infiltration (H & E X 400).

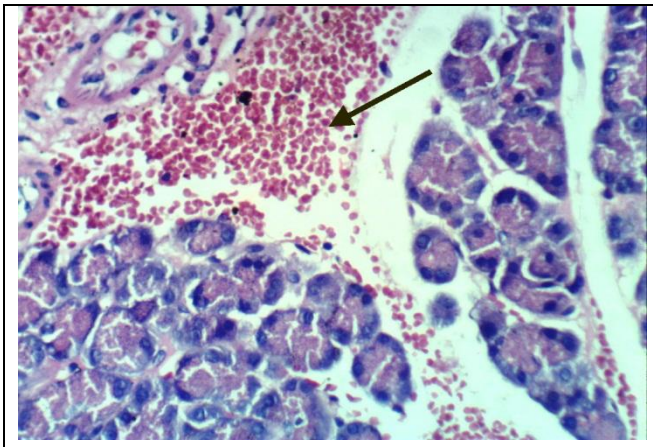

Fig. (13): Pancreas of diabetic rats treated with white cabbage showing focal haemorrhage (H & E X 400).

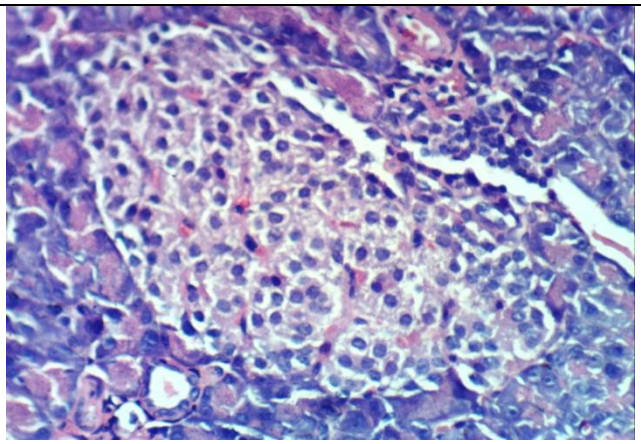

Fig. (14): Pancreas of diabetic rats treated with red cabbage showing apparent normal pancreas (H & E X 400).

DISCUSSION

Oxidative stress has been found to play an important role in the pathogenesis of diabetes. In turn, the generation of ROS, has been shown to play an integral and possibly a causative part in the pathogenesis of diabetic retinopathy[29]. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose autoxidation, polyol pathway, protein glycation), are initiated and augmented under oxidative stress. Furthermore, exposure of endothelial cells to high glucose (as indicated in the results), leads to augmented production of superoxide anions, which may quench nitric oxide, a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature. In further support of the consequential injurious role of oxidative stress, is the finding that many of the adverse effects of high glucose on endothelial functions are reversed by antioxidants [13]. Moreover, antioxidant therapy may be a suitable approach for halting the intrinsic changes within liver and retinal capillary bed that lead to the development of diabetic liver fibrosis and retinopathy[29].

The present histological examination at the cellular level reveal foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes in hepatocytes of rats indicating establishment of diabetic state (Fig. 2). Although, kidney of diabetic rats showing interstitial nephritis (Fig.7). However, pancreas of diabetic rats reveals necrosis of islets of Langerhans's and inflammatory cells infiltration (Fig.12).[30]Stated that, as a result of streptozotocin action, beta cells undergo destruction by necrosis. STZ is widely used for inducing type 2 diabetes in a variety of animals. It selectively induces degenerative alterations and necrosis of pancreatic beta-cells resulting in, insulin deficiency

and impairment in glucose oxidation. In accordance to the present study, [31] earlier reported that, the diabetic liver showed degeneration and congestion after injection of STZ. Hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in insulin levels. [32]confirmed the destruction of islet cells in pancreatic biopsy of diabetic rats due to the effect of streptozotocin and added that 60 mg/kg dose of STZ ensured induction of diabetes in rats and hyperglycemia.

The present study reveals, abnormal glucose metabolism, diabetes often involves abnormal lipid metabolism which is considered as additional metabolic disorder, in diabetic complications. The same results were achieved by [33], who found significant elevation in lipid profile in serum of diabetic rats. In a good agreement with the present data, [34] revealed that hyperglycemia produced marked increase in the serum level of triglycerides, total–cholesterol,LDL- cholesterol,while HDL- cholesterol showed reduced concentration in diabetic rats. It was reported that, hepatic fat accumulation is a well – recognized complication of DM. The most common clinical presentation in DM is hepatomegaly. This hyperlipidemia associated with DM may be attributed to insulin deficiency and elevated cortisol level, which has an important role in the process of fat accumulation.[35] Under normal circumstances insulin activates lipoprotein lipase which hydrolyzes triglycerides. Insulin deficiency results in failure to activate the enzyme, thereby causing hyper-triglyceridemia.[3]On the other hand, in insulin deficiency, the plasma free fatty acids concentration is elevated as a result of increased free fatty acids outflow from fat depots, where the balance of the free fatty acids esterification, triglycerides lipolysis is displaced in favours of lipolysis [36]. Also elevated cortisol promotes the liberation of free fatty acids from adipose tissue into blood stream by inducing and maintaining the synthesis of the hormone sensitive lipase, thus increasing free fatty acids level which contributes to cardio-vascular risk [37]. The reduction in cardioprotective HDL-C means decrease of cholesterol afflux from the tissues, the first step in reverse cholesterol transport from the peripheral tissues to the liver. The antioxidant and anti-atherogenic activities of HDL-C are enhanced when its circulating level is increased. LDL- C particles become small and dense which undergo oxidative modification, thus leading to a diabetic complication [38]. The presence of high level of fatty acids in the serum, promotes their conversion into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins [39]. In addition, the presence of high concentration of total lipid in serum of diabetic rabbits may be attributed

mainly to increase mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase [39].

In addition, the present results demonstrate significant elevation in liver function markers associated with significant reduction in total protein content and albumin level as compared to normal control group. The high serum levels of these enzymes post STZ treatment are associated with inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury and apoptosis. In parallel with the present work, previous reports revealed significant increased activities of serum enzymes relative to their normal levels[40]. Supporting our findings, it has been found that hyperglycemia resulted in hepatolysis reflected by increased blood serum aminotransferases as one of the consequences of diabetic complication. The increment of such serum markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of hepatomegaly (fatty liver) [40]. The significant reduction in total protein content and albumin level in diabetic rats is in concomitant with the results of [41], who found significant reduction in serum total protein concentrations in diabetic rats and this may be due to reduction in the three major phases in protein secretion, intracellular transport and discharge. The reduction in total protein was due to significant increase in protein excretion[42], [43].Non-enzymaticglycation of albumin was the potential to alter its biological structure and function[44]. It is mainly due to the formation of a Schiff base between amino-group of lysine (and sometimes arginine) residues and excess glucose molecules in blood to form glycoalbumin. Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis[45].

The present results indicate also, significant elevation in oxidative stress marker, lipid peroxidation products; MDA in liver of diabetic rats. These elevated levels may be due to oxidative stress which is considered as one of the causative factors that link diabetes with the pathogenic complications of disease [46]. Lipid peroxidation can damage protein, lipid, carbohydrates and nucleic acids, and is one of the risk factor of protein glycation. Oxygen free radicals are implicated as mediators of tissue injury in cardiovascular pathology. Cytotoxic effect of ROS is related to lipid peroxidation and subsequent membrane destruction [47]. Oxygen free radicals, in addition to the myocardial damaging effect, may also be responsible for the release of lysosomal or hydrolytic enzymes such as elastase[48]. On other hand, the study of [49], and[43]reported elevated rates of liver lipid peroxidation accompanied with the deterioration in glucose tolerance in GSH-depleted rats. It has been

suggested that, in free radicals initiating systems, the deterioration in glucose tolerance is attributed to impaired insulin action [50]. Initiating lipid peroxidation by free radicals, in the lipid moiety of the cell membrane was supposed to result in distortion of the structural and functional integrity of the cell membrane or internal cellular components. This would interfere with the ability of insulin to initiate and propagate its normal sequence of actions which may account, at least in part, for STZ-induced hyperglycemia[51]. Moreover, the current data show also that, STZ caused a reduction in GSH level in the liver of diabetic rats. The significant depletion of GSH in liver of diabetic rats indicates damage to the second line of antioxidant defence. This probably further exacerbates oxidative damage by adversely affecting critical GSH related processes such as free-radical scavenging, detoxification of electrophilic compounds, mod modulation of cellular redox status and thiol-disulphide status of proteins and regulation of cell signalling and repair pathways [52].

It was found that, during renal dysfunction or renal damage, the concentration of the metabolites increased in blood that may be due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels[53]. Urea is the major nitrogen-containing metabolic product of protein catabolism and creatinine is another product of protein metabolism. The increase in serum urea and creatinine levels in STZ diabetic group indicates impairment in the normal kidney function of the animal, as the mechanism of removing there from the blood might have been affected. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney, it is well known that, many biochemical and histopathological findings confirmed renal damage in diabetic conditions[43].

The current study demonstrates also, significant increase in the pro-inflammatory cytokines; TNF- α and CRP, in diabetic rats as compared to normal control group. It was found that, diabetic condition is frequently associated with increased soluble markers of systemic inflammation and trigger release of inflammatory mediator such as CRP[4]

Tight control of blood glucose can reduce clinical complications in diabetic patients; however, alternative treatment strategies are needed to prevent oxidative stress complications and to optimize recovery[54]. It is well documented that modulation of oxidative stress through treatment with antioxidants can effectively reduce diabetic snag. Oxidative stress induced by hyperglycemia in diabetics is a major cause for development and progression of diabetic microvascular complications such as nephropathy [54].

We investigated the therapeutic value of white and red cabbages against diabetic status. These plants, members of the Cruciferae and genus Brassica such as cabbage, broccoli, cauliflower, kale and Brussel sprouts, contain anthocyanin pigments that are described as free-radical scavenging and antioxidant agents. Anthocyanin, which is a phenolic natural pigment present in red cabbage, was demonstrated to have the autoxidizing power of 150 flavonoids[5]. The significant antihyperglycemic activity of red cabbage polar extract may, at least in part, modulate the oxidative stress caused by hyperglycemia-induced generation free radicals. Natural health products of vegetable origin have shown promise for the prevention of chronic diseases[3]. Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoid provide protection against myriad physiological failures such as lipid peroxidation, decreasing capillary permeability, fragility and membrane strengthening. Red cabbage extract contains vitamins A, B and, all of which have protective roles against oxidative damage [3]. In addition,[55] revealed that, selenium(Se) from Se-enriched cabbage is highly bioavailable and can potentially be beneficial in enhancing Se status and GPX activity.STZ-diabetic rats showed symptoms of renal nephropathy such as kidney hypertrophy that were attributed to an increase in blood glucose and polyuria. In addition, STZ-diabetic rats showed elevated serum urea and creatinine, as in previous report of[56]. RC polar extract prevented renal enlargement and attenuated polyuria. Meanwhile, elevated serum urea and creatinine in STZ-diabetic rats were also normalized to control values after 60 days with ingestion of red cabbage polar extract. This was consistent with other reports using chemical antioxidants, and diet of natural antioxidant plants [57] to normalize nephropathy in STZ-diabetic rats.

Diabetic rats had elevated levels of MDA, while reduced concentrations of non-enzymic antioxidant GSH. Red cabbage extract ameliorated these changes. The elevations in GSH and low MDA levels in diabetic treated rats may be compensatory mechanisms for the chronic overproduction of free radicals and oxidative stress. The down-regulation of GSH in diabetic rats is consistent with reports of [58],[59]. The protective qualities of both white and red cabbages are quite consistent with their abilities to protect against oxidative damage induced by toxic agents in other tissues of animals [60], [61].

Other investigations have shown that acylatedanthocyanins isolated from red cabbage protect against paraquat-induced oxidative stress in rats [60]. Also, acylatedanthocyanins were effective antioxidants against hepatic lipid peroxidation elevation and GSH depletion induced by paraquat in rats. Several vegetable aqueous extracts including Brassica vegetables such as

white cabbage, red cabbage, green mustard leaf, red mustard leaf, white turnip and red turnip were examined for their neuroprotective action against oxidative stress induced by *N*-methyl-d-aspartate in brains of mice [61]. Only red cabbage showed a markedly restored glutathione levels and prevented lipid peroxide induced by *N*-methyl-d-aspartate in the brains of mice at a dose of 1 gm/kg body weight. RC polar extract was more protective than white cabbage against amyloid protein induced neuronal damage [3]. These results indicated that the neuronal cell protective capacities of red cabbage might be due to high total phenolic contents and antioxidative activity. Among the three different cultivated forms of cabbage, red cabbage has higher vitamin C (24.38 mg/100g), dl-α-tocopherol (0.261mg/100g) and phenolic content (101.30 mg/100 g) as compared with white and savoy cabbage, all of which protect against oxidative damage [3].

On the other hand, [62] contribute the potential health benefits of white cabbage to a significant source of phenolic antioxidants, with quercetin derivatives being the most abundant. The ameliorative signs of white cabbage may be also related to the high level of antioxidant, the major contributors to the antioxidant activity were polyphenol and high level of flavonoids. Generally very good correlation between antioxidative activity and abundance of polyphenolic compounds in the cabbage. Another group of bioactive compounds is the high level of total GLS which is paralleled with antioxidative potential [63]. Moreover, [64] attributed the down regulation in inflammatory cytokines TNF-α and CRP to the white cabbage antioxidants and anti-inflammatory compounds caused lowering in oxidative stress and inflammation.

Thus it could be concluded that, treatment of diabetic rats with both white and red cabbages ameliorate hyperglycemia, oxidative stress and inflammatory markers with more potent effects for red cabbage. In addition, histopathological amelioration was noticed in the hepatic, renal and pancreas architectures.

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