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Anti- Asprosin: A Potential Protective Role Against the Progression of Diabetic Nephropathy in Type 2 Diabetic Rats

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Abstract

Background and objective: Diabetic nephropathy (DN) is a common and serious diabetic complication. Asprosin is a protein secreted by white adipose tissue and induces hepatic glucose release. The purpose of this study was designed to evaluate the potential protective effect of asprosin blockage against progression of nephropathy in type II diabetic rats.

Methods: Type II diabetes mellitus was induced by feeding rats on high fat- diet for four weeks followed by injecting them with low dose of streptozotocin (STZ). We randomly divided thirty rats into three different groups: group I (control), group II (non-treated experimentally induced type II diabetic group) and group III (treated type II diabetic group with anti- asprosin antibody). Rats in group III were divided into three equal subgroups. Group (IIIA), (IIIB) and (IIIC): in which rats were given daily intraperitoneal injections of anti- asprosin antibody at doses of 10, 20 and 30 µ g / kg body weight respectively for 8 weeks. After the experiments were finished, serum and kidney tissue samples were gathered. In all groups, body weights (BW), thoracic and abdominal circumferences (TC and AC) were measured. Body mass index (BMI) and AC/TC ratio were calculated. After eight weeks duration, rats were euthanized. Serum levels of insulin, glucose, lipid profile, creatinine, urea, C- reactive protien (CRP) and plasminogen activator inhibitor-1 (PAI-1) were measured; homeostasis model assessment of insulin resistance (HOMA-IR) and creatinine clearance (ml/min) were calculated. Protein in urine was estimated. Moreover, the animal was connected to the Power Lab after carotid artery cannulation to record mean arterial blood pressure (MAP). In addition, the changes in the histopathological aspects of the kidney were examined.

Results: Eight weeks after induction of DM, the mean values of final BMI, final AC/TC ratio, serum levels of glucose, HOMA-IR, triglycerides (TG), total cholesterol (TC), LDL-c, urea, creatinine, CRP, PAI-1, proteinuria and MAP were significantly high ($P < 0.001$). However, the mean values of serum insulin, HDL-

c and creatinine clearance were significantly low (P<0.001) in type 2 diabetic rats (group II) when compared to controls. Furthermore, the current findings showed that, the administered anti- asprosin antibody to type 2 diabetic rats (group IIIC), significantly reduced the mean values of final BMI, final AC/TC ratio, serum levels of glucose, HOMA- IR, TG, TC, LDL-cholesterol, urea, creatinine, CRP, PAI-1, MAP and proteinuria (P<0.001, P<0.001, P <0.001, P<0.001, P<0.001, P<0.001, P<0.001, P<0.001, P<0.01, P<0.001, P<0.001, P<0.001 respectively), while, the mean values of serum levels of HDL-c and creatinine clearance were significantly high (P < 0.001) in comparison to those of group II and group IIIA. However, the mean values of serum levels of insulin showed insignificant change when compared with those of group II and group IIIA (P>0.05).

Conclusions: The current study showed that, anti- asprosin may significantly slow the pathophysiological and biochemical progression of DN in high‐ fat diet/STZ-induced type 2 diabetic rats indicating that, it may be used as a novel reno-protective agent in cases of obesity and type 2 DM.

Keywords: Diabetes, Nephropathy, Anti- asprosin, Glucose, Creatinine, Rats

1. Introduction

Diabetic nephropathy (DN) is one of the most severe vascular complications of diabetes mellitus resulting in end-stage kidney disease and mortality [1]. Chronic hyperglycemia induces non-enzymatic glycosylation reactions between protein molecules and glucose, and generates advanced glycosylation end products (AGEs) leading to glomerular hypertrophy, glomerular basement membrane thickening and glomerulosclerosis [2]. In most cases, proteinuria and decreased glomerular filtration rate occur in parallel [3].

Asprosin is a long protein of 140 amino acids that is greatly expressed in white adipose tissue. It is a C-terminal product of the fibrillin-1 protein encoded by FBN1. It is a hepatic glucose releaser. [4]. Therefore, it acutely increases with fasting and reduces with refeeding. Peripheral administration of asprosin crosses the blood-brain-barrier activating the hypothalamic feeding centers stimulating appetite and prolonging the obesity [5]. Moreover, plasma asprosin levels are significantly increased in impaired glucose regulation and newly diagnosed type 2 diabetic subjects [6].

Interestingly, the increased glucose due to high asprosin production may inhibit growth hormone leading to short stature. Asprosin may be used for the growth hormone determination [4]. However, the decreased glucose due to inadequate production of asprosin and excessive insulin release may result in the hypoglycemic attacks after meals [7].

In the present study, the potential impact of asprosin blockage on DN in type 2 diabetic rats was examined, and its effects on serum glucose, lipids, renal function parameters and kidney tissue pathology were compared. The study aimed to evaluate the potential use of asprosin blockage as a novel approach for protecting against DN in type 2 diabetic rats.

2. Materials and Methods

2.1 Experimental animals

This study was performed on a total number of 30 adult male albino rats of 12 weeks old, weighting 173-202 gm, were obtained from the animal house - Zagazig Veterinary Medicine faculty. The animals were kept in steel wire cages (6-7/cage) in the physiology animal house - Zagazig Medicine faculty under hygienic conditions. Rats were kept at comfortable temperature (20-24 ˚ C) and were maintained on a normal light/dark cycle [8]. They had free access to food and water. This study was performed from March to November 2018. All animals received care in accordance with the guide to the care and use of experimental animals of Institute of Laboratory Animal Resources [9]. Experimental protocol was approved by physiology department and institutional research board (IRB) in faculty of medicine - Zagazig University. Animals were divided in to 3 main groups, Group I: Control group (n = 10 rats): All animals were fed on diet of mixed commercial rat laboratory chow that consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat [10]. Group II: Experimentally induced type II diabetic group (n = 10 rats): In which experimental diabetes was induced as following: the

rats were fed high fat diet (60.3% fat, 21.3%, carbohydrate18.4% protein as a % of total kcal) for 5 weeks. Then HFD was replaced with standard rodent diet and the rats received intraperitoneal injection of low dose streptzotocin (35 mg/kg BW) (Sigma Chemical, St. Louis, MO, Sigma-Aldrich, U.S.A). Blood glucose was checked seven days after STZ injection. Animals with blood glucose levels of ⩾ 250 mg /dl were chosen for the study [11, 12]. This group was survived for 8 weeks after induction of diabetes mellitus. Group III: Type II diabetic treated group (n = 10 rats): After induction of diabetes, the rats in this group were intraperitoneally injected by anti- asprosin antibody (IgG isotype, Wuhan Fine Biotech Co., Catalogue No. FNab09797) in different 3 doses of 10, 20 and 30 μ g/kg/day for 8 weeks. Death rate in diabetic rats was 10%, and dead animals were replaced. Anti- asprosin antibody is used for immunologic neutralization of asprosin. It was validated by Romere et al., [4] for asprosin specificity and they performed a detailed epitope mapping for it. They found that intraperitoneal injection of a single dose of anti- asprosin antibody was sufficient to acutely drop plasma asprosin levels at 3 and 6 hour post-injection neutralizing asprosin function, with recovery to normal levels at 24 hours.

2.2 Anthropometric parameters measurement

The animals were weighed in (gm) by a digital scale; day before the experiment, twice a week and at the last day. The results were written in a record for each labeled rat. The nose to anus lengths of rats were for calculation of body mass index (BMI) (gm/cm^2) by dividing body weight (gm)/ $length^2$ (cm^2) [13,14]. Additionally, the abdominal circumference (AC) and thoracic circumference (TC) were measured for calculating AC/TC ratio [14]. Then data were plotted in records of each labeled rat.

2.3 Blood Pressure measurement

Overnight fasted anesthetized rat were placed on the surgical table. The skin on chest, right hind leg and ventral side of neck were carefully shaved and disinfected. Tracheostomy and cannulation of carotid artery were done and blood pressure was recorded. The animal was connected to the Power Lab 4/20 (data acquisition system, with MLT844 physiological pressure transducer with clip-on BP domes, AD Instruments Pty Ltd, Australia) after cannulation to record blood pressure (intravenous cannula consists of sterile polyethylene (PE) tube with an internal diameter (ID) of 0.5 mm and an outer diameter (OD) of 0.9 mm provided with a 26 G × 1/2 ″ needle). The animal was allowed to stabilize for ten min. and was monitored for bleeding. This measurement was done according to the method described by [15].

2.4 Blood sampling and biochemical analysis

Blood samples were obtained at end of the experimental period after overnight fasting and measurement of blood pressure (between 9:00-11:00 a.m.), blood samples were obtained from each rat sinus orbitus vein after inhalation of ether [16]. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes (Centrifuge Zentriguanhan, Engelsr-of DDR- 7123.Engels-drf/-eipzig/Banug/Fabr.NO.08/30 type T30, Max. 1 min: 6400 Max Fullgew Kgr. Frequency 50 Hz made in Germany). The serum was stored at -20° C.

Measuring serum glucose levels (mg/dl) according to [17] and serum insulin levels (μ IU/ml) by enzyme-linked immuno-sorbent assay (ELISA) according to [18]. Kits for serum glucose and insulin levels were purchased from Biosource Europe S.A. Belgium.

Calculating homeostasis model assessment - insulin resistance (HOMA-IR) using HOMA-IR index according to Bonora et al. [19] as follows: [HOMA-IR] = fasting serum glucose (mg/dL) × fasting serum insulin (μ IU/mL)/405.

Measuring serum total cholesterol (TC) levels according to [17], serum triglycerides (TG) levels according to [20], serum HDL levels according to [21] and serum LDL levels was calculated according to [22] as follows: LDL = TC -HDL － TG/5.

Measuring serum creatinine levels by rat kits (Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Sant esteve de bas (gi), Spain) and estimating serum urea level by rat kits (Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Sant esteve de bas (gi), Spain) according to [23, 24, 25].

Estimating serum C- reactive protien (CRP) by rat kits (MonobindInc Lake Forest, Ca 92630,

USA) by Immuno-enzymometric assay according to [26].

Estimating serum plasminogen activator inhibitor-1 (PAI-1) by rat kits for PAI-1 (Sunred Bio Shanghai 201-11-0637, CHINA) by Sandwich ELISA according to [27].

2.5 Urine collection

The rats were housed individually in metabolic cages and allowed to access water from drinking bottles. Suitable sized- funnels were arranged at the metabolic cages bottom for collecting urine and perforated plastic discs were arranged in funnels to retain fecal matter [28]. The urine sample of 24 hours was collected for each rat in a beaker at the funnel bottom. The volumes of the 24-hour urine samples were noted individually and centrifuged at 3000 rpm for 10 minutes. The supernatants were transferred into another set of clean and dry tubes and stored at -20° C until analysis.

Estimating protein in urine by using Microlab 300: Vital Scientific, Germany and estimating creatinine in urine using kits purchased from Sigma Aldrich according to the manufacturer's protocol and instruction.

Calculating creatinine clearance (Ccr): After collection of individual urine (24-hour) samples, creatinine clearance was calculated from the urinary creatinine, serum creatinine, 24-hour urine volume, and body weight, using the following equation: Ccr (mL/min/kg) = [Urine Cr (mg/dL) × urine volume (mL)/serum Cr (mg/dL)] [1000/body weight (g)] [1/1440 (min)] as previously reported by [29].

2.6 Histopathological examination

The abdominal cavities of the rats were opened to remove the kidneys. The kidneys were fixed in 10 % formalin solution and embedded in paraffin. Sections were made and stained with hematoxylin and eosin (H &E) and observed microscopically (high power—40×) as it was previously described by [30]. The pathologist was blinded to the treatment.

2.7 Statistical Analysis

The data obtained in the present study were expressed as mean SD for quantitative variables and statistically analyzed according to the methods described by [31]. The statistical analysis is done by using SPSS program (19) (SPSS Inc. Chicago, IL, USA).

ANOVA [Post hoc (LSD)] test was used to compare means among more than two groups. P value < 0.05 was considered statistically significant.

3. Results

In group II (non- treated type 2 diabetic group), while the mean values of serum levels of glucose, HOMA- IR, triglycerides, total cholesterol, LDL-cholesterol, urea, creatinine, CRP, PAI-1, final BMI, final AC/TC ratio, proteinuria and MAP were significantly high ($P < 0.001$), the mean values of serum levels of insulin, HDL-cholesterol and creatinine clearance were significantly low ($P < 0.001$) in comparison to those of group I (control group) (Table 1, Trace 1, Fig 1-8). Moreover, there were no significant changes in all parameters measured between group II and group IIIA ($P > 0.05$) (Table 1).

In group IIIB, although the mean values of serum levels of glucose, HOMA- IR, triglycerides, total cholesterol, LDL-cholesterol, urea, CRP, PAI-1, final AC/TC ratio, MAP and proteinuria were significantly low ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively), the mean values of serum levels of HDL-cholesterol and creatinine clearance were significantly high ($P < 0.001$) in comparison to those of group II (Table 1, Trace 1, Fig 1-8). However, the mean values of serum levels of insulin, creatinine and CRP, and final BMI showed non-significant change when compared with those of group II ($P > 0.05$) (Table 1).

In group IIIC, the mean values of serum levels of glucose, HOMA- IR, triglycerides, total cholesterol, LDL-cholesterol, urea, creatinine, CRP, PAI-1, final BMI, final AC/TC ratio, MAP and proteinuria were significantly low ($P < 0.001$ $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.001$, $P < 0.001$ respectively) , while, the mean values of serum levels of HDL-cholesterol and creatinine clearance were significantly high ($P < 0.001$) in comparison to those of group II and group IIIA (Table 1, Trace 1, Fig 1-8). However, the mean values of serum levels of insulin showed

insignificant change when compared with those of group II and group IIIA ($P > 0.05$) (Table 1).

Furthermore, in group IIIC the mean values of serum levels of triglycerides, total cholesterol, LDL-cholesterol, creatinine, CRP, PAI-1 , final AC/TC ratio, MAP and proteinuria were significantly low ($P < 0.001$ $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.01$ respectively) whereas, the mean values of serum levels of HDL-cholesterol and creatinine clearance were significantly high ($P < 0.01$ and $P < 0.05$ respectively) in comparison with those of group IIIB (Table 1, Trace 1, Fig 1-8). However, the mean values of serum levels of insulin, glucose, HOMA- IR, urea and final BMI showed non-significant change when compared with those of group II ($P > 0.05$) (Table 1).

Moreover, there is significant dose dependent decrease in serum levels of glucose, HOMA- IR, triglycerides, total cholesterol, LDL-cholesterol, urea, creatinine, CRP, PAI-1, final BMI, final AC/TC ratio, MAP and proteinuria. Whereas, there is significant dose dependent increase in serum levels of HDL-cholesterol and creatinine clearance (Table 2, Fig 9).

Renal histopathological examination revealed normal renal tissue formed of normal sized glomerulus surrounded by proximal and distal convoluted tubules in group I (control group) [Fig 10 A] and sever diabetic nephropathy with hyalinized small glomeruli surrounded by heavy aggregation of chronic inflammatory cells in the interstitial tissue in group II (non- treated type 2 diabetic group) [Fig 10 B]. Group IIIB (diabetic group treated with anti- asprosin antibody (20 µ g/kg/day) showed some improvement of the degree of diabetic nephropathy with dilated congested vascular space and moderate aggregation of chronic inflammatory cells in the interstitial tissue when compared with group II [Fig 10 C]. Moreover, in group IIIC (diabetic group treated with anti-asprosin antibody (20 µ g/kg/day) there was marked improvement in the degree of renal lesions with mild aggregates of chronic inflammatory cells around the glomerulus and the renal tubules in comparison to group II and group IIIB [Fig 10 D].

Table 1: Anthropometric, serum biochemical parameters, renal function tests and MAP in all studied groups

	Group I (Control)	Group II (non- treated type 2 diabetic)	Group III (treated type 2 diabetic with different doses of anti- asprosin antibody)		
			Group IIIA 10 µg / kg body weight	Group IIIB 20 µg / kg body weight	Group IIIC 30 µg / kg body weight
Final BMI (gm/ cm^2)	0.49±0.03	$0.58^{\$}$ ±0.06	0.57±0.04	0.54±0.05	$0.52^{\infty\&}$ ±0.06
AC/TC ratio	1.08±0.01	$1.15^{\$}$ ±0.02	1.14±0.02	$1.11^{\infty\&}$ ±0.02	$1.07^{\infty\&\#}$±0.01
Serum glucose (mg/dl)	84.5±6. 6	$307^{\$}$ ±29.6	302.1±41.6	$179.4^{\infty\&}$ ±12.9	$161.6^{\infty\&}$ ±14.8
Serum insulin (µIU/ml)	19.02±2.07	$12.23^{\$}$±0.65	11.37±1.2	11.91±0.95	12.24±0.74
HOMA-IR	3.96±0.55	$9.26^{\$}$ ±0.96	8.49±1.6	$5.27^{\infty\&}$ ±0.6	$4.88^{\infty\&}$ ±0.6
Serum triglycerides (TG) (mg/dl)	54.68±6.3	$151.28^{\$}$ ±9.6	144.89±10.9	$133.38^{\infty\&}$ ±10.5	$85.53^{\infty\&\#}$ ±7.9
Serum total cholesterol (TC) (mg/dl)	80.68±4.4	$213.02^{\$}$ ±12.02	199.95±10.7	$153.71^{\infty\&}$ ±25.2	$118.58^{\infty\&\#}$±15.2
Serum HDL-cholesterol (mg/dl)	43.4±4.3	$25.82^{\$}$±3.5	28.13±3.9	$33.82^{\infty\&}$ ±4.8	$39.23^{\infty\&\#}$ ±2.3

Serum LDL-cholesterol (mg/dl)	26.47±5.3	150.82$ ±8.9	143.29±8.7	110.79∞&±12.8	64.04∞&# ±16.3
Serum urea (mg/dl)	24.99±3.6	74.74$ ±7.9	71.82±8.2	37.76∞& ±4.5	37.97∞& ±4.1
Serum creatinine (mg/dl)	0.41±0.03	2.58$ ±0.29	2.53±0.32	2.48±0.26	0.85∞&# ±0.09
Creatinine clearance (ml/min)	0.77±0.06	0.39$ ±0.06	0.43±0.04	0.57∞& ±0.08	0.64∞&# ±0.08
proteinuria (mg/dl)	5.2±0.97	36.8$ ±4.7	34.3±4.3	31.6∞±5.03	13.2∞&# ±1.9
Serum CRP (µg/dl)	3.22±0.7	9.1$ ±0.7	8.87±0.6	8.72±0.6	5.76∞&# ±0.8
Serum PAI-1 (µg/ml)	9.26±1.3	17.79$±1.4	17.55±1.6	12.64∞& ±1.3	11.26∞&# ±1.6
MAP (mmHg)	85.14±6.3	124.67$ ±5.5	120.62±6.3	101.69∞&±7.8	95.06∞&#±6.4

$ = significant VS group I ∞ = significant VS group II
&= significant VS group IIIA # = Significant VS group IIIB

Table 2: Correlation between the dose of anti- asprosin antibody (10, 20, 30 µg/Kg BW) and levels of all parameters measured in group III

	Glucose	HOMA	AC/ TC ratio	TG	cholesterol	HDL-c	LDL-c	Final BMI
r	-0.852**	-0.779 **	-0.839**	-0.886**	-0.886**	0.782**	-0.930**	-0.379*
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05

	Urea	Creatinine	Creatinine clearance	Proteinuria	CRP	PAI-1	MAP
r	-0.815**	-0.844**	+0.772**	-0.853**	-0.811**	-0.840**	-0.824**
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

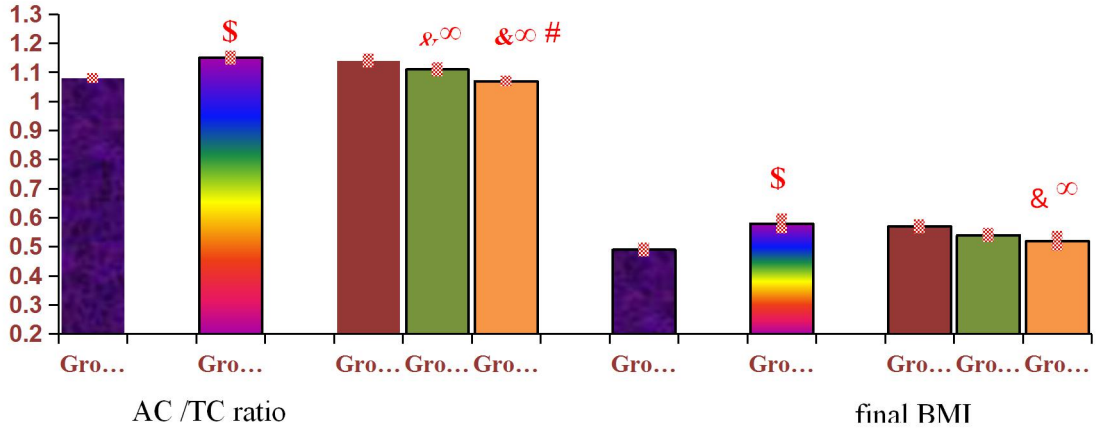

Figure 1: Histogram illustrates AC /TC ratio and final BMI in all studied groups

$= VS group I ∞ = VS group II & = VS group IIIA # = VS group IIIB

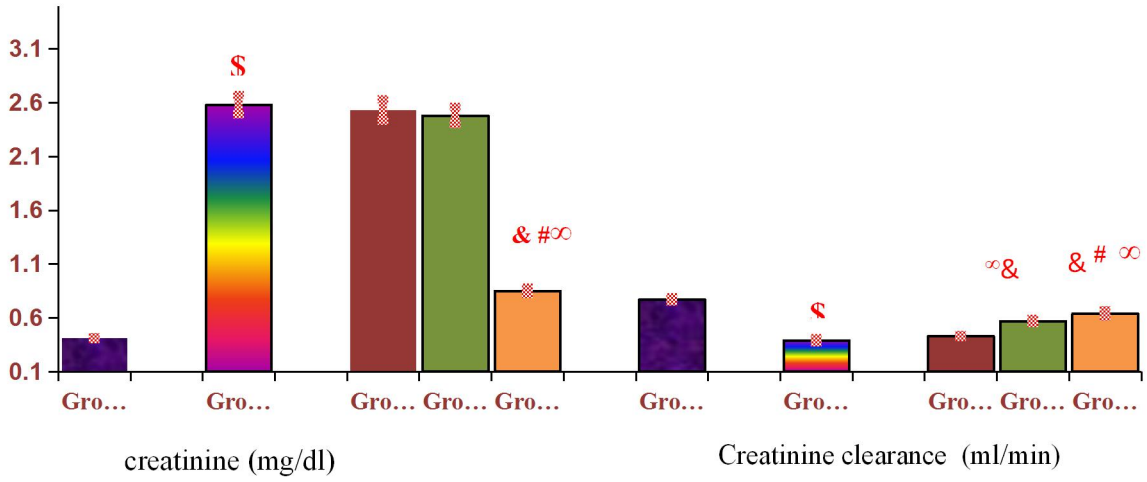

Figure 2: Histogram illustrates serum creatinine and creatinine clearance in all studied groups
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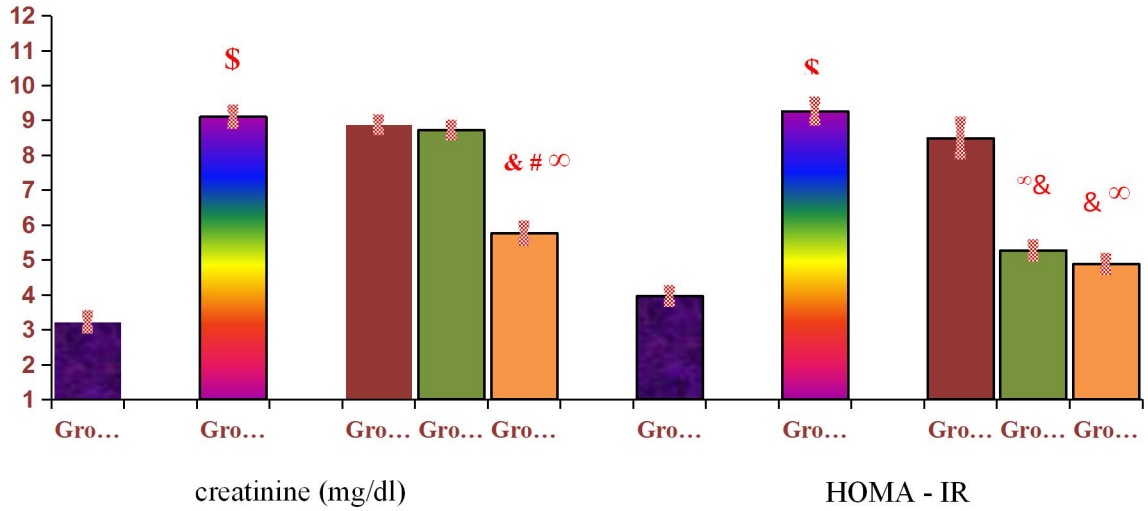

Figure 3: Histogram illustrates serum CRP and HOMA- IR in all studied groups
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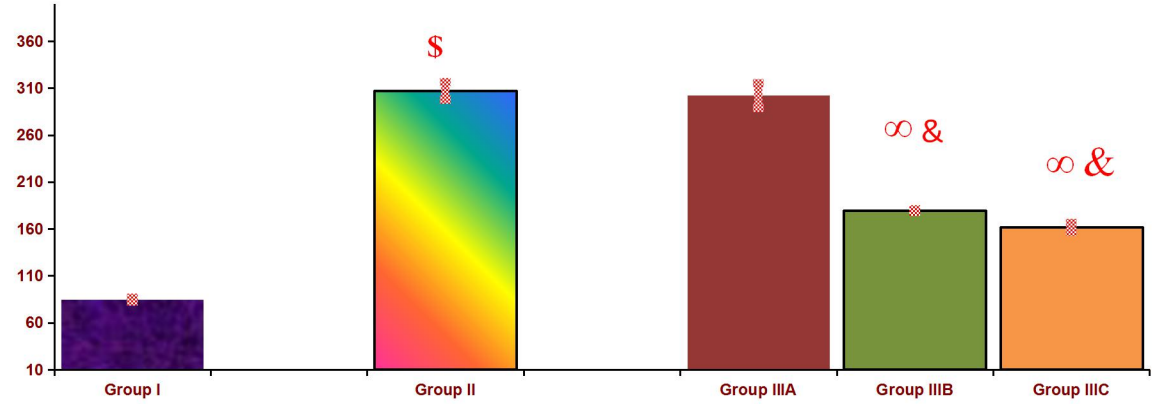

Figure 4: Histogram illustrates serum glucose (mg /dl) in all studied groups
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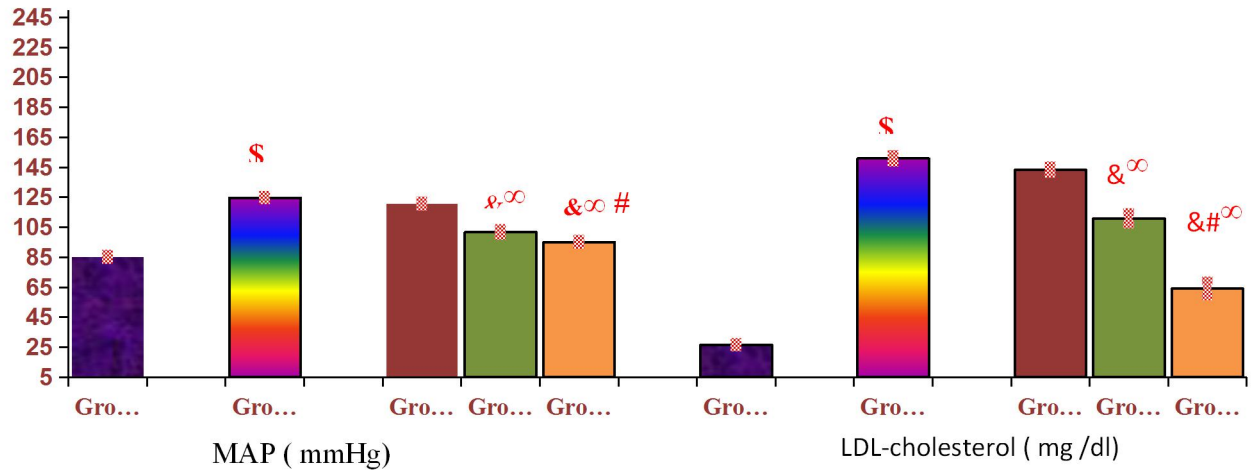

Figure 5: Histogram illustrates serum LDL-cholesterol and MAP in all studied groups
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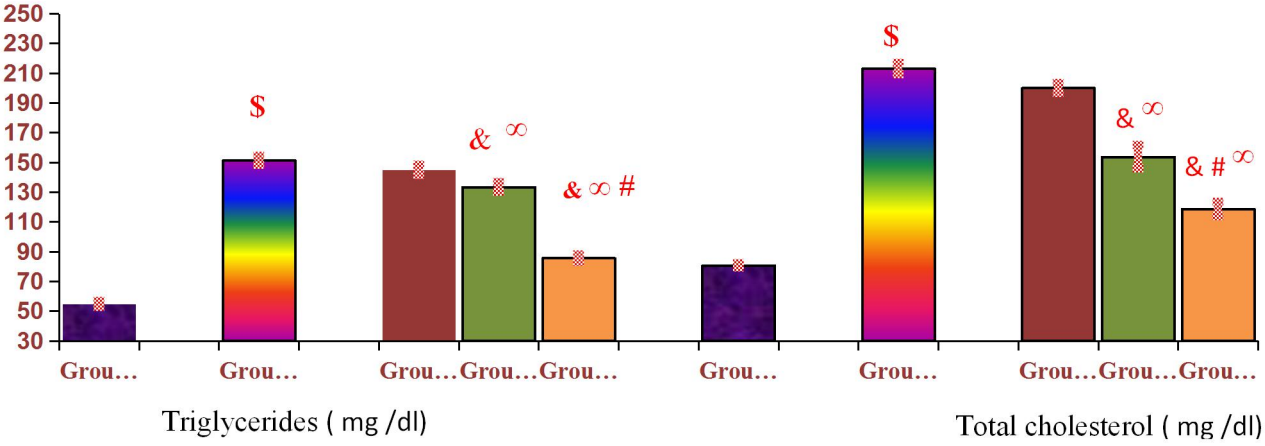

Figure 6: Histogram illustrates serum triglycerides and total cholesterol in all studied groups
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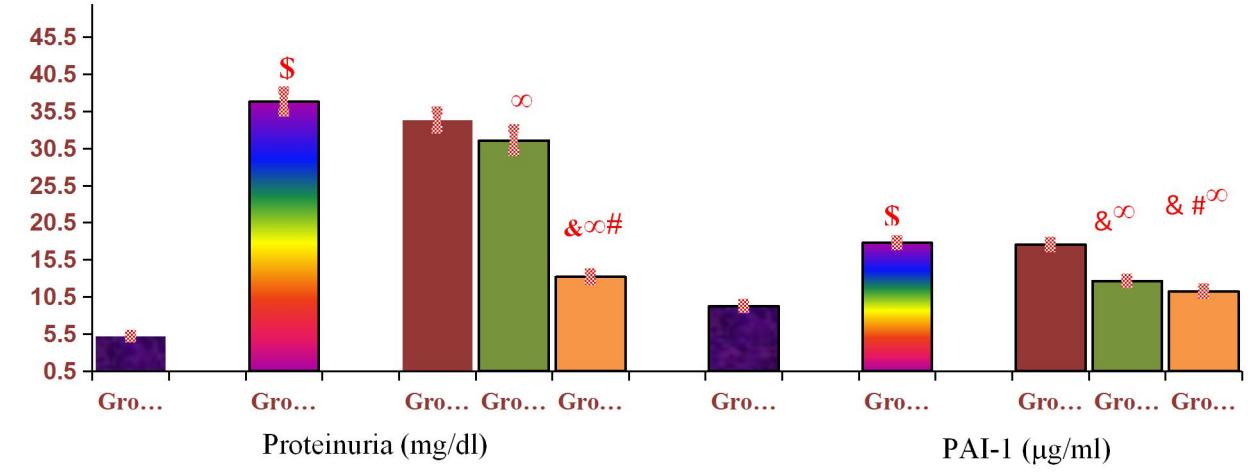

Figure 7: Histogram illustrates serum PAI- 1 and proteinuria in all studied groups
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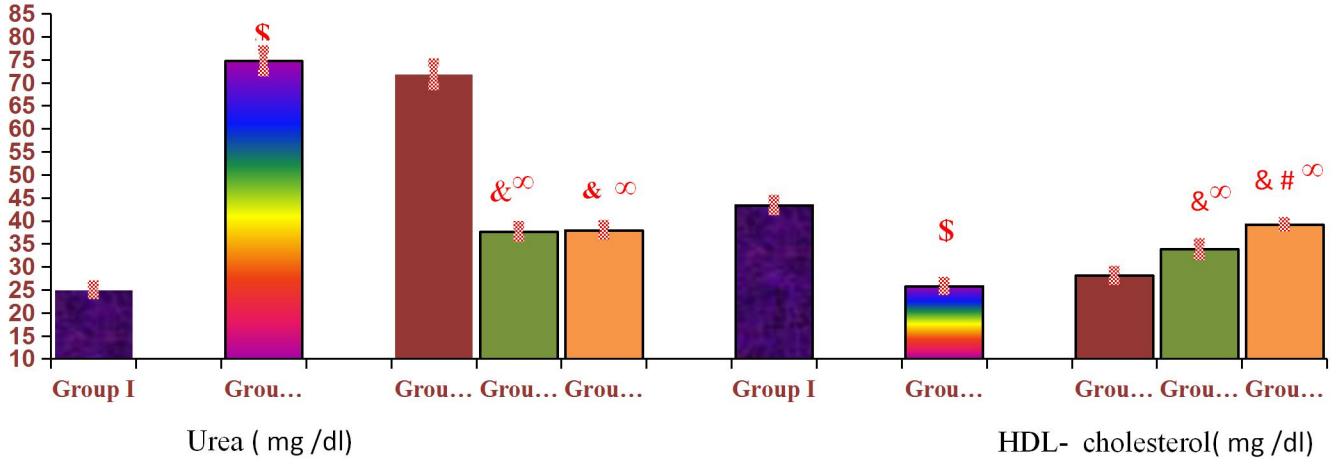

Figure 8: Histogram illustrates serum urea and HDL- cholesterol in all studied groups
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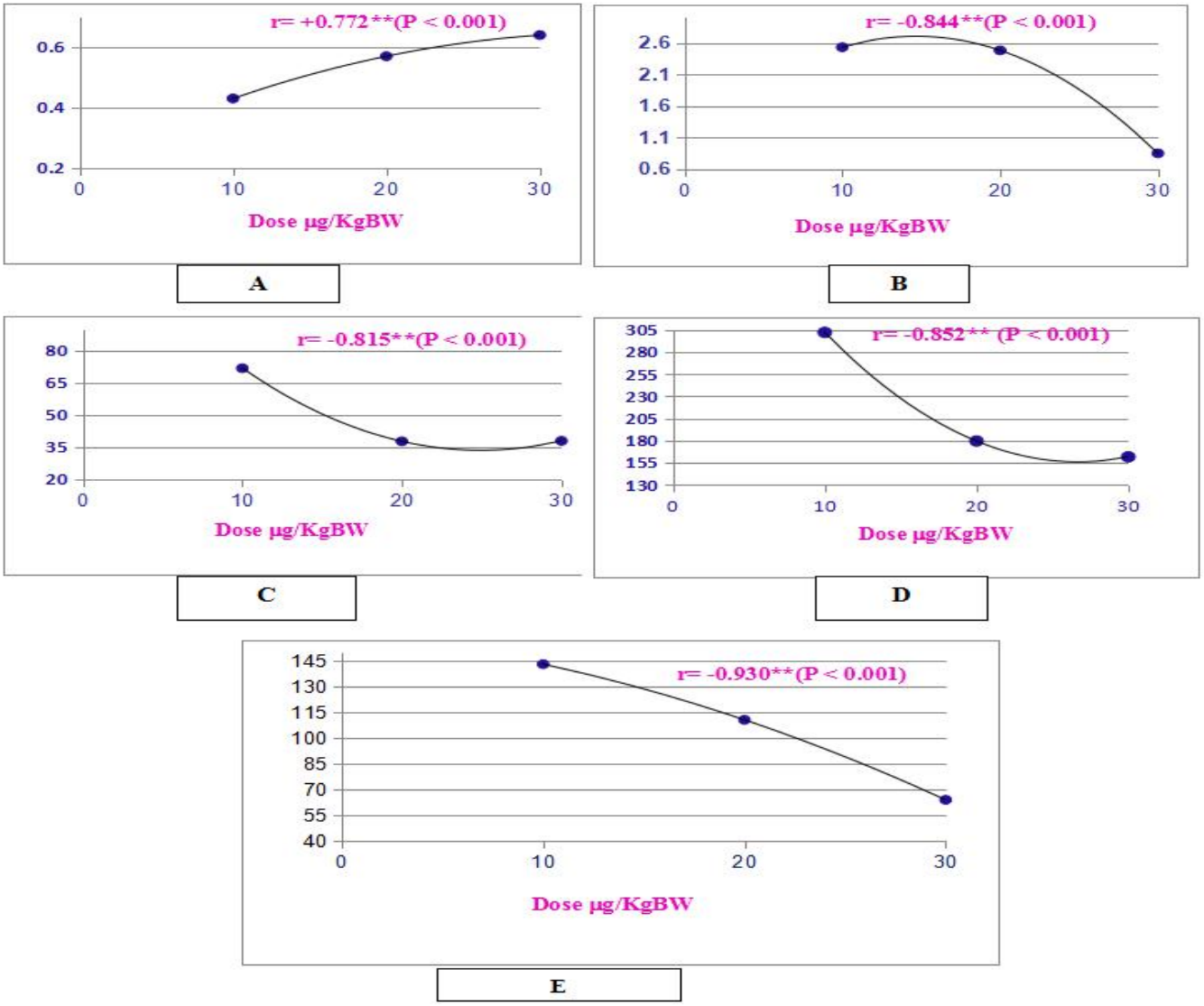
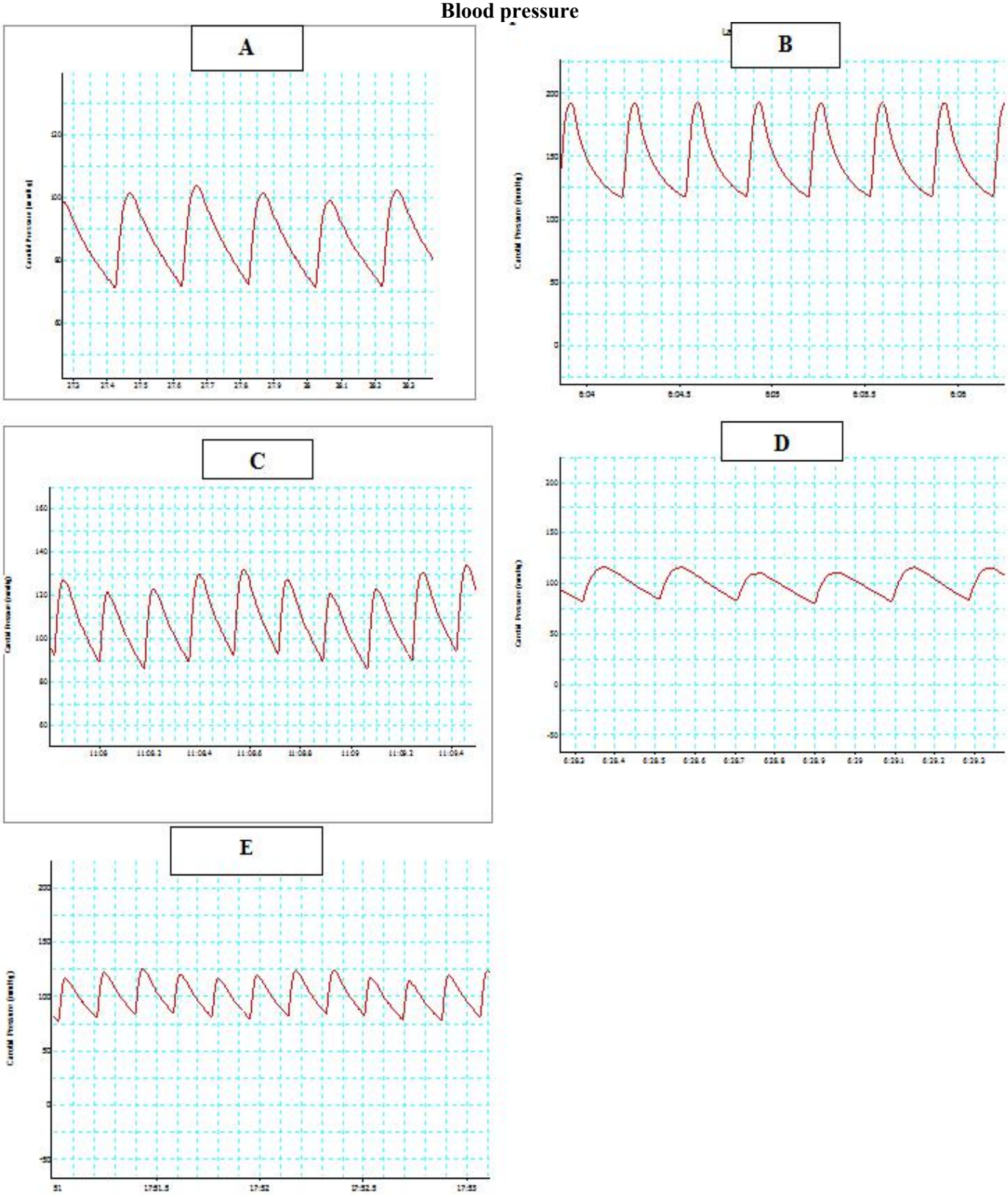


Figure 9 (A, B, C, D, E): Illustrates the dose response curve of anti-asprosin antibody (10, 20 and 30 mg/Kg BW) and the mean values of creatinine clearance (ml/min), creatinine (mg/dl), urea (mg/dl), glucose (mg/dl) & LDL-cholesterol (mg/dl) respectively in group III



Trace 1 (A, B, C, D, E): Shows an example of a record of carotid BP in subgroups I, II, IIIa, IIIb, IIIc respectively

Kidney histopathology of all studied groups

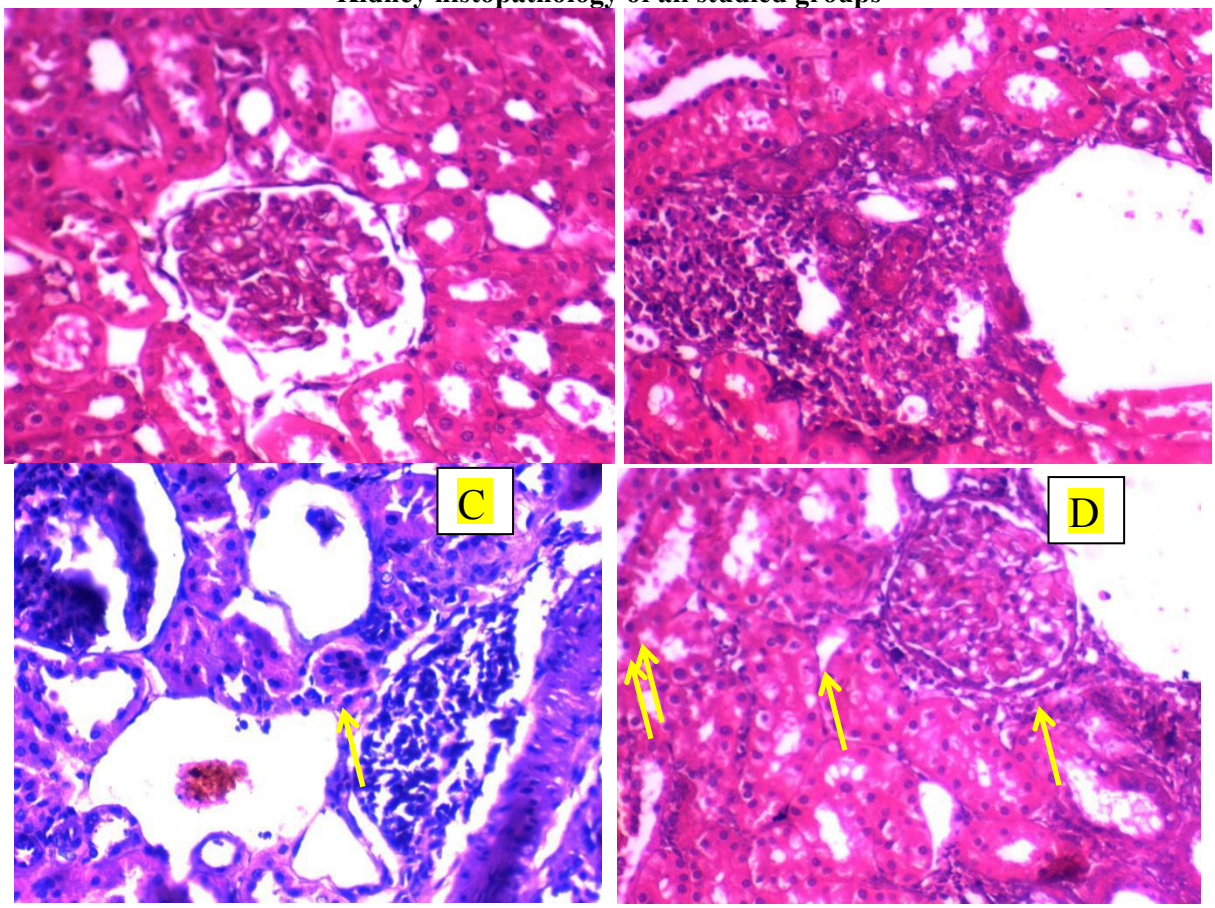


Figure 10: Photomicrograph (stained with Haematoxylin & Eosin x400)

(A): Normal renal tissue formed of normal sized glomerulus (pyramid) surrounded by proximal and distal convoluted tubules (black star). (B): of 8 weeks diabetic rat with sever diabetic nephropathy showing hyalinized small glomeruli (yellow star) surrounded by heavy aggregation of chronic inflammatory cells (arrow) in the interstitial tissue. (C): of 8 weeks diabetic rat treated with anti- asprosin antibody (20 µ g/kg/day) with moderate diabetic nephropathy showing dilated congested vascular space with moderate aggregation of chronic inflammatory cells (arrow) in the interstitial tissue. (D): of 8 weeks diabetic rat treated with anti- asprosin antibody (30 µ g/kg/day) with mild diabetic nephropathy showing mild aggregates of chronic inflammatory cells (arrow) around the glomerulus and the renal tubules.

4. Discussion

Because of the glycogenic role of asprosin in glucose production [4], it may participate in the pathogenesis of all types of diabetes and diabetic complications such as retinopathy and nephropathy [32, 33]. Therefore, in the present study, the potential impact of anti-asprosin on DN in type 2 diabetic rat models was examined. DN leads to end-stage renal disease and cardiovascular morbidity and mortality worldwide [34]. Blood pressure, glucose control and angiotensin system inactivation could delay the progression of diabetic renal injury but do not stop it [35]. Therefore, the aim of this study was to find out the possibility of using anti-asprosin as a novel approach for managing and protecting against the progression of DN in type 2 diabetic rats.

In the current study, induction of insulin resistance in rats was done via high-fat diet intake [36], followed by injection of a single low dose of STZ (35 mg/kg) to induce frank hyperglycemia. After 8 weeks duration, the type 2 diabetic rats had hyperglycemia, dyslipidemia, renal dysfunction and general inflammatory state [37]. This non-genetic model of type 2 diabetic rats has a potential advantage over the conventional type 1 diabetes

model in that kidney lesions in type 2 model appear to be more pronounced than in type 1 diabetic rats despite less proteinuria and hyperglycemia [11].

Hyperglycemia is the main pathology of DM resulting in the majority of diabetic problems. Chronic hyperglycemia leads to advanced glycation end products (AGEs) accumulation and increased risk for diabetic vascular complications [38]. Thus bad begins, and worse remains behind. Once AGEs accumulate, the oxidative stress and reactive oxygen species (ROS) - mediated pathway begin. ROS formation is elevated in proximal tubular epithelium stimulating cellular stress sensitive pathways resulting in cellular injury [39, 12]. Therefore, strict control of blood glucose level is a major management line, as it improves the oxidative stress [40, 41]. This current study showed that, anti-asprosin antibody administration significantly reduced the elevated FBG levels and HOMA-IR indicating that, the anti- asprosin may be a renal protective agent in type 2 DM via its role in glycemic control and insulin sensitivity.

The relationship between asprosin and type 2 DM is very interesting. Even one recombinant asprosin injection could increase blood glucose fast and result in hyperinsulinemia [4]. In addition, asprosin levels were increased in insulin resistance and type 2 diabetic adults suggesting that, it might be considered as a risk factor associated with type 2 diabetes pathogenesis [42, 4]. However, anti- asprosin antibody reduced plasma levels of asprosin and enhanced insulin sensitivity in insulin resistant mice [4]. Therefore, blocking asprosin activity in this current work provided a potential novel approach for protecting the kidney function against the progression of DN in type 2 diabetic rats.

Moreover, this study demonstrated the protective effect of anti- asprosin against adiposity by decreasing final BMI and final AC/TC ratio in type 2 diabetic rats. Excess adiposity is a leading cause of insulin resistance, type 2 DM and metabolic syndrome [43, 44]. Zhang et al [42] showed that, serum asprosin concentrations were positively associated with BMI, waist circumference, and Waist-hip ratio in type 2 diabetic patients. Once asprosin concentrations were increased, adiposity-related parameters were gradually increased as well.

Hyperlipidemia is a risk factor for DN as the continuously filtered lipids and lipoproteins can exacerbate the glomerular injury, urinary albumin excretion and glomerulosclerosis [45, 46, and 47]. Moreover, it increases the glomerular macrophage and oxidized LDL dependent oxidative stress [48]. The findings of the lipid profile of type 2 DN rats group (group II) were as follow: significant higher levels of TC, LDL- c and TG and lower levels of HDL- c that in comparison to controls which were supported by findings of [45,49]. In treated type 2 diabetic groups with anti- asprosin antibody (groups IIIB and IIIC), the mean values of serum TC, LDL-c and TG were significantly low. While, the mean value of serum HDL-c was significantly high in comparison to those of group II indicating that, anti-asprosin significantly attenuated these pathological alternations in blood lipid concentrations in type 2 DN rats. These findings implied that, anti- asprosin antibody may protect against the progression of diabetic nephropathy in type 2 diabetic rats by modulating lipid metabolism and dyslipidemia.

Also, our results revealed a significant progressive increase in serum CRP and PAI-1 in diabetic group supported by those of [50]. The increased PAI-1 has a role in obesity, metabolic syndrome and cardiovascular disease [51]. PAI-1 acts as a fibrogenesis stimulator in diabetic nephropathy [52]. Giannico et al [53] suggested that, excess glomerular PAI-1 results in extracellular matrix deposition leading to glomerulosclerosis. Chronic inflammation and subsequent activation of immune system are linked to type 2 DM and obesity resulting in the release of pro-inflammatory TNF- α , and IL-6 producing more serum CRP and PAI-1 [54], which finally leads to decline in insulin sensitivity. Later on, insulin resistance increases more PAI-1 and CRP accumulation [55]. The current study results demonstrated that, anti- asprosin antibody could delay the progress inflammatory processes in type 2 DN rats.

As regard to kidney function parameters in the present study, kidney dysfunction in type 2 DN rats was assessed by significant elevated serum urea, serum creatinine and proteinuria and reduced creatinine clearance levels. These findings are in consistent with that of [56] who showed that, the serum blood urea nitrogen and urinary albumin in type 2 diabetic rats were higher than those in controls. In early onset DN, kidney damage is indicated by increased urinary albumin excretion [57,

[58]. Creatinine, a breakdown product of creatine phosphate in muscle, is filtered in the glomeruli and its clearance can be used as a marker of glomerular filtration rate and an indicator for kidney function [59]. The current findings showed that, the administered anti- asprosin antibody to type 2 diabetic rats significantly reduced the serum urea, serum creatinine and proteinuria compared to controls. It also significantly elevated creatinine clearance in type 2 diabetic rats treated with anti-asprosin compared to controls. Therefore, these findings indicate that, the anti- asprosin may be used as a reno-protective agent in obese rats with type 2 DN.

In high fat diet fed rats with type 2 DM, glomerular and systemic hemodynamics develop the observed glomerular lesions. Furthermore, hypertension, hyperglycemia and dyslipidemia are main contributors of the following renal consequences [60, 3]. In the present study, diabetes induction causes progressive significant rise in MAP when compared to controls. However, MAP was significantly decreased in high‐ fat diet/STZ-induced type 2 diabetic rats treated with anti-Asprosin when compared with diabetic group.

Finally, the histopathological examination of the kidney in 8 weeks diabetic rats showed a link between the biochemical abnormalities and several structural abnormalities of DN, including glomerular basement membrane thickening, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis. However, the groups treated with anti- asprosin showed amelioration of the degree of these lesions and improvement of renal histology in a dose dependent manner. These results were in agreement with previous studies [61, 62],

5. Conclusion

The present study demonstrated the reno-protective effects of anti- asprosin against DN in high‐ fat diet/STZ-induced type 2 diabetic rats. Anti- asprosin significantly delayed the pathophysiological characteristics of type 2 DN by glycemic control protecting renal functions and morphology. However, further investigation is required to fully elucidate the underlying mechanisms.

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