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Original Article

Effect of high fructose administration on histopathology of kidney, heart and aorta of rats

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ABSTRACT

Objective: This study was conducted to assess the hazardous effects of high fructose administration on kidney, heart and aorta in rats.
Materials and methods: Twenty adult healthy male albino rats weighing about 200-220 gm each were used in this study. The rats were divided into 2 duplicate groups; control group and fructose group. Fructose was administered to rats in fresh drinking water daily for 8 weeks (the whole experimental period). Serum urea, creatinine and sodium concentration were determined by using ready-made kits. Spectrophotometric and colorimetric methods were also used for the detection of other serum components. Histopathological examination of the tissues was done by staining with H&E, PAS and Masson trichrome stains.
Results: Nephropathy was achieved in fructose group after one month as indicated by biochemical assay. Pathological observation showed that high fructose administration decreased size of cardio-myocytes, increased cardiac interstitial fibrosis score and aortic wall thickness. In kidneys, high fructose administration decreased glomerular tuft area and corpuscular area, increased percentage in the rats affected with interstitial renal fibrosis score 1 and percentage of rats had glomerular sclerosis score 2.
Conclusion: High fructose in diet should be avoided because it can damage kidney, heart and aorta in rats.

KEYWORDS

Aorta; Fructose; Heart; Histopathology; Kidney

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INTRODUCTION

Fructose is used extensively in our modern diet in carbonated beverages, dairy products, canned fruits and baked goods (Hanover and White, 1993). Excessive consumption of a high-fructose diet may lead to the epidemic of chronic renal disease (Nakagawa et al., 2006) and metabolic syndrome characterized by visceral adiposity, dyslipidemia and insulin resistance (Stanhope and Havel, 2008; Tappy et al., 2010). Insulin resistance is not only associated with diabetes mellitus but also with enhanced oxidative stress, endothelial dysfunction and cardiovascular disease (Oudot et al., 2009). Thus intake of fructose- rich foods was an important cause of heart disease manifested by ventricular dilatation and hypertrophy, decreased ventricular contractile function, and inflammation (Chang et al., 2007; Patel et al., 2009). It was found that high-fructose diet increases uric acid levels (Cirillo et al., 2009) that contribute to diabetic nephropathy along with microvascular disease (Jalal et al., 2011). The aim of this work was to estimate the histopathological effects of fructose rich diet on kidney, heart and aorta in rat.

MATERIALS AND METHODS

Experimental animals: In this study, twenty adult healthy male albino rats with average weight 200-220 gm were used. Rats were purchased from the animal house in Helwan and left for one week to acclimatize animal house in department of animal Physiology, Faculty of Veterinary Medicine and Mansoura University. Rats were kept under controlled environment, maintained under a 12 h light:dark cycle, 24°C (±3°C) and 50-70% humidity.

Ethical approval: All animal procedures followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Experimental design: Rats were randomly divided into 2 duplicate groups (five rats in each cage). The first group served as a control where rats were provided with standard diet and water ad-libitum. Rats in fructose group were subjected to fructose 20% in fresh drinking water daily for 8 weeks; the duration of experiment according to Hu et al. (2012).

Sample collection: Blood samples were collected in plain test tubes via retro-orbital bleeding after 12 h of fasting at 4 and 8 weeks of the experiment. Blood samples were left at room temperature for 1 h then centrifuged for 10 min at 3000 g to obtain the serum. Serum samples were stored at −80°C for further analysis. Five rats were killed each sacrifice by decapitation for collection of kidneys, aorta and heart. Each kidney was cut into two halves and right ventricles were obtained. Levels of malondialdehyde (MDA) and nitric oxide (NO) were estimated in kidney and heart tissue homogenates after been washed three times in ice cold saline and blotted individually on ash-free filter paper. The crude tissue homogenate was centrifuged at 10,000xg for 15 min in cold centrifuge, and the resultant supernatant was separated. Aortas, other halves of kidneys and left ventricles were fixed in neutral buffered formalin until be routinely processed for histopathological examination.

Biochemical serum analysis: Determination of serum urea, creatinine and sodium concentration was performed by using ready-made kits from Diamond Diagnostics Company (Egypt) (Tietz, 1995). Glutamic oxaloacetic transaminase (GOT); glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH) activity were measured by a kinetic method using commercial kits (Egyptian company for biotechnology) (Young, 1990). Serum lipid profile including cholesterol, triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) was estimated using commercial kits Vitro Scient Diagnostic Company (Egypt) (Lalouschek et al., 2003). Malonyldialdehyde (MDA) was determined spectrophotometrically according to (Ohkawa et al., 1979). Nitric oxide (NOB) was assayed in the serum by a colorimetric method using the diazotization procedure according to Bartholomew (1984), while NOH and NOK were estimated in the heart and kidney homogenates, respectively according to Montgomery and Dymock (1961).

Histopathological examinations: Paraffin sections from kidneys, heart and aorta (3 µm thickness) were cut and evaluated using standard staining protocol for H&E. Selected slides from each group were also stained with PAS and Masson trichrome as previously described (Shishido et al., 2003). A total of 50 corpuscular and 15 interstitial images were captured from prepared kidney sections at each sacrifice and analyzed. Corpuscular area and capillary tuft area were assessed using an electronic image analysis (image J = http://imagej.en.softonic.com). The parameters measured in Masson trichrome stained kidney slides were glomerular sclerosis and interstitial fibrosis. Grades of glomerular sclerosis were scored in PAS stained slides as the following: 0: no lesions, 1: 1-25%, 2: 25-50%, 3: 50-75% and 4: 75-100%. All scores obtained by each rat were subjected for statistical analysis according to Li et al. (2012). Renal interstitial fibrosis was evaluated in Masson trichrome stained slides by counting the percentage of areas with interstitial fibrosis per field of cortex in 3 fields from each section as mentioned by Oruc et al. (2010). The given scores were

from 0 to 5: 0: normal interstitium, 1: <10% of areas injured, 2: 11-25% of areas injured, 3: 26-50% of areas injured, 4: 51-75% of areas injured and 5: >76% of areas injured. The fibrosis fraction was quantitatively analyzed in at least 20 fields from each Masson trichrome stained heart slide through calculating the ratio of blue stained connective tissue area to total red stained myocardial area using image analysis software. Cross-sectional area of 100 transversely cut cardiomyocytes was measured in H&E stained slides using the electronic image analysis and means of measurements were calculated from each section according to Nozaki et al. (2004). In addition, the thickness of aortic wall was measured according to Elbe et al. (2014) in 5 randomly selected points in aorta examined with 40X objective magnification.

Statistical analysis: Statistical analysis was done using the software SPSS 19 (SPSS Inc, Chicago, Illinois). Morphometric parameters were tested using two-way ANOVA and Duncan's multiple comparisons of the means to compare data obtained. Data were expressed as means standard errors. Semi-quantitative analysis of glomerular sclerosis scores and interstitial fibrosis scores in the kidneys were compared between both groups and two time points (4&8 weeks) using Chi-square tests. *P-values* in the rows showed significance between both groups at each sacrifice ($P<0.05$), meanwhile, *P-values* in the columns showed significance inside each group ($P<0.05$).

RESULTS

Biochemical assay: Nephropathy was noted in fructose group after 4 weeks of administration as assessed by significant increase in levels of serum urea and creatinine, GPT, GOT, LDH, Na, NOH, NOB, NOK, cholesterol, TG, LDL, MDAH**,** MDAB and MDAK with significant decrease of HDL levels (**Table 1).**

Renal histopathology: Kidneys in the control group showed normal glomeruli and tubules. Meanwhile, kidneys of fructose group showed cloudy swelling in renal tubules, tubular hyaline casts, early coagulative necrosis of some renal tubules with signs of regeneration in some other renal tubules. Multiple foci of mononuclear cells aggregation were seen in interstitial tissue (**Figure 1 A-F).** Glomerular sclerosis was examined in H&E, PAS and Masson trichrome stained kidney slides from fructose group (**Figure 2 A-F)**. Statistical analysis of corpuscular area and glomerular tuft area measurements and interstitial fibrosis scores showed highly significant difference between control and fructose groups at two time points without significant changes inside each group between 4 and 8 weeks' time points. Corpuscular area and glomerular tuft area were statistically lower in fructose group than in control group (**Table 2**). After 4 weeks, the percentage of rats in fructose group had glomerular sclerosis score 1 was 54.9% which increased to be 55.5% after 8 weeks (**Table 3**). Meanwhile, the percentage of rats in fructose group affected with interstitial renal fibrosis score 1 was 66.7% after 4 weeks and 70% after 8 weeks (**Table 3**). Interstitial renal fibrosis observed in fructose group was demonstrated in (**Figure 2F)**.

Cardiac histopathology: Heart in the control group showed normal histological picture. Meanwhile, heart of fructose group after 4 weeks showed mild hyaline degeneration perivascular edema with few MNCs infiltration. After 8 weeks, vacuolation of cardiomyocytes and multifocal areas of MNCs aggregation with variable areas of degeneration and necrosis were seen **(Figure 3 A-D**).

Table 1: Biochemical measurements after 4 weeks

Measurements	Control	Fructose	*P value*
Creatinine (mg/dL)	0.6700±0.03786[a]	6.400±0.1872[b]	< 0.0001
GPT (u/L)	15.33±0.8819[a]	39.67±2.028[bc]	< 0.0001
GOT (u/L)	39.00±3.215[a]	80.33±0.8819[b]	< 0.0001
LDH (uL)	259.0±8.386[a]	435.0±24.91[b]	< 0.0001
Na (mmol/L)	134.7±1.519[a]	159.5±0.5207[b]	< 0.0001
NOH (umol/gm)	24.30±0.4041[a]	65.83±0.1202[c]	< 0.0001
NOB (umol/dL)	19.20±0.4359[a]	49.30±0.3786[c]	< 0.0001
NOK (umol/gm)	16.40±0.6245[a]	45.50±0.3606[c]	< 0.0001
Cholesterol (mg/dL)	110.0±5.033[a]	240.3±26.52[b]	0.0013
TG (mg/dL)	77.33±5.548[a]	142.3±9.667[b]	0.0151
HDL (mg/dL)	49.00±5.568[a]	20.00±0.5774[b]	0.0003
LDL (mg/dL)	45.33±6.009[a]	192.3±27.41[b]	0.0009
MDAB (mmol/L)	24.83±0.3528[a]	52.93±6.359[b]	0.0004
MDAH (mmol/gm)	31.90±0.5033[a]	62.97±7.308[b]	0.0146
MDAK (mmol/gm)	24.37±2.534[a]	48.70±6.787[b]	0.0035

Means±SE. Different superscript small letters in the same row indicate significant difference between groups when (P≤0.05)

Table 2: Histomorphometric measurements in control and experimental groups after 4 and 8 weeks

Measurements	Time interval	Groups		*P*-value
		Control	Fructose	
		Kidneys		
Corpuscular area (μ)	4 weeks	634.28±87.82	577±81.36	0.0045
	8 weeks	684.12±89.049	540.6±102.89	0.0001
	P-value	0.9231	0.1036	
Glomerular tuft area (μ)	4 weeks	620.13±121.54	573.28±85.55	0.0001
	8 weeks	597.22±124.8	591.4±130.8	0.0001
	***P*-value**	**0.804**	**0.0007**	
		Heart		
Size of cardiomyocytes (μ)	4 weeks	513.52±101.51	468.35±76.62	0.0001
	8 weeks	650.77±142.37	581.23±136.42	0.0001
	***P*-value**	**0.0009**	**0.0001**	
Cardiac interstitial fibrosis expressed as a ratio of blue stained area to total red stained myocardial area	4 weeks	1.0255±0.132	1.1295±0.06	0.0001
	8 weeks	1.029±0.118	1.1285±0.05	0.0001
	***P*-value**	**0.6497**	**0.8556**	
		Aorta		
Aorta wall thickness	4 weeks	295.11±71.88	348.7±79.52	0.0002
	8 weeks	322.75±56.61	403.52±109.9	0.001
	***P*-value**	**0.3186**	**0.1790**	

Means±SE; P-values in rows shows significant difference between groups. P-values in columns show significant difference inside groups between 4 and 8 weeks' time points.

Table 3: Semi-quantitative analysis of scores in control and experimental groups after 4 and 8 weeks

Measurements	Time interval	Group		Chi-Square value, X^2	Degree of freedom	*P*-value
		Control	Fructose			
The highest % of fields affected with glomerular sclerosis inside each group expressed as a score (0-+4)	4 weeks	0:86% 1:14% 2:0% 3:0% 4:0%	0:14.5% 1:54.9% 2:29.8% 3:0.7% 4:0%	1466.225[b]	12	0.000
	8 weeks	0:86.2% 1:13.8% 2:0% 3:0% 4:0%	0:14% 1:55.5% 2:29.8% 3:0.7% 4:0%	1494.937[c]	12	0.000
	Chi-Square value, X2	.008c	.072f			
	Degree of freedom	1	3			
	***P*-value**	**.931**	**.995**			
The highest % of fields affected with renal interstitial fibrosis inside each group expressed as a score (0-+5)	4 weeks	0:100% 1:0% 2:0% 3:0% 4:0% 5:0%	0:16.7 1:66.7% 2:16.7 3:0% 4:0% 5:0%	77.859[b]	12	0.000
	8 weeks	0: 100% 1:0% 2:0% 3:0% 4:0% 5:0%	0:13.3% 1:70% 2:16.7% 3:0% 4:0% 5:0%	86.256[b]	12	0.000
	Chi-Square value, X^2	.[c]	.136[e]			
	Degree of freedom		2			
	***P*-value**		**0.934**			

P-value in rows shows significant difference between groups. P-value in columns show significant difference inside groups between 4 and 8 weeks' time points

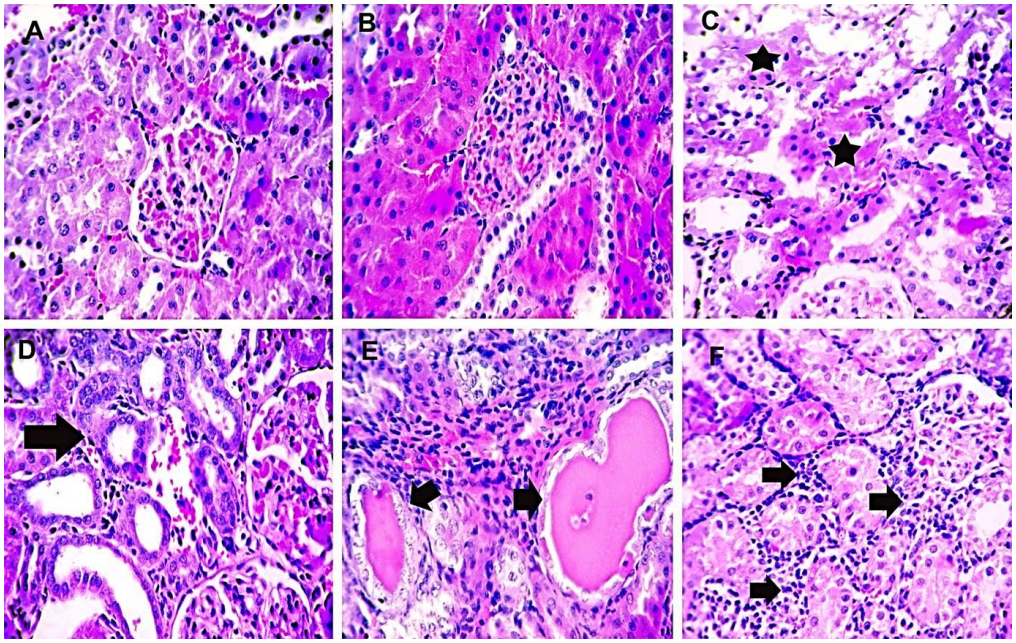

Figure 1: A: Kidneys of control group show normal histological picture**. (B-F)**: Kidneys of fructose group show **B:** cloudy swelling in renal tubules, **C** early coagulative necrosis of renal tubules (asterisks), **D:** newly formed renal tubules surrounded by mononuclear cells (arrow), **E:** dilated renal tubules with hyaline casts (arrows) and **F:** multiple foci of mononuclear cells aggregation in interstitial tissue (arrows) (H&E, X 200).

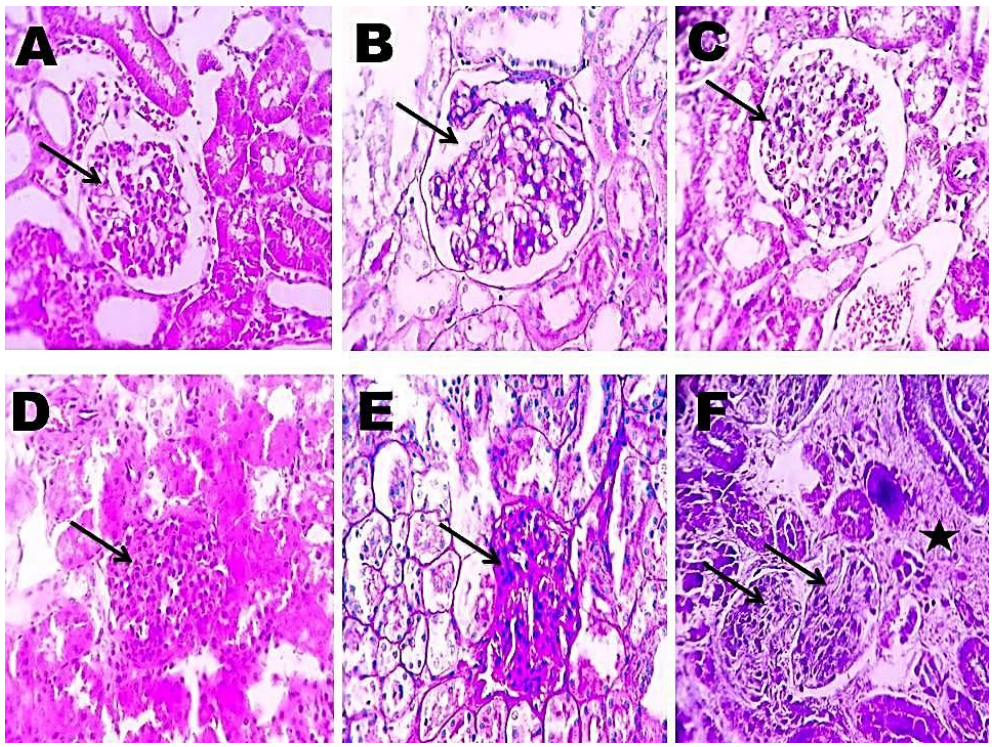

Figure 2: (A-C): Kidneys of control group showing normal glomerulus (arrow) with H&E (**A**), PAS (**B**) and Masson trichrome (**C**). **(D-F):** Kidneys of fructose group showing sclerotic glomerulus (arrow) with H&E (**D**), PAS (**E**) and Masson trichrome (**F**). Note increased interstitial renal fibrosis (asterisks) in fructose group (**F**) when compared with control group (**C**) (X 200).

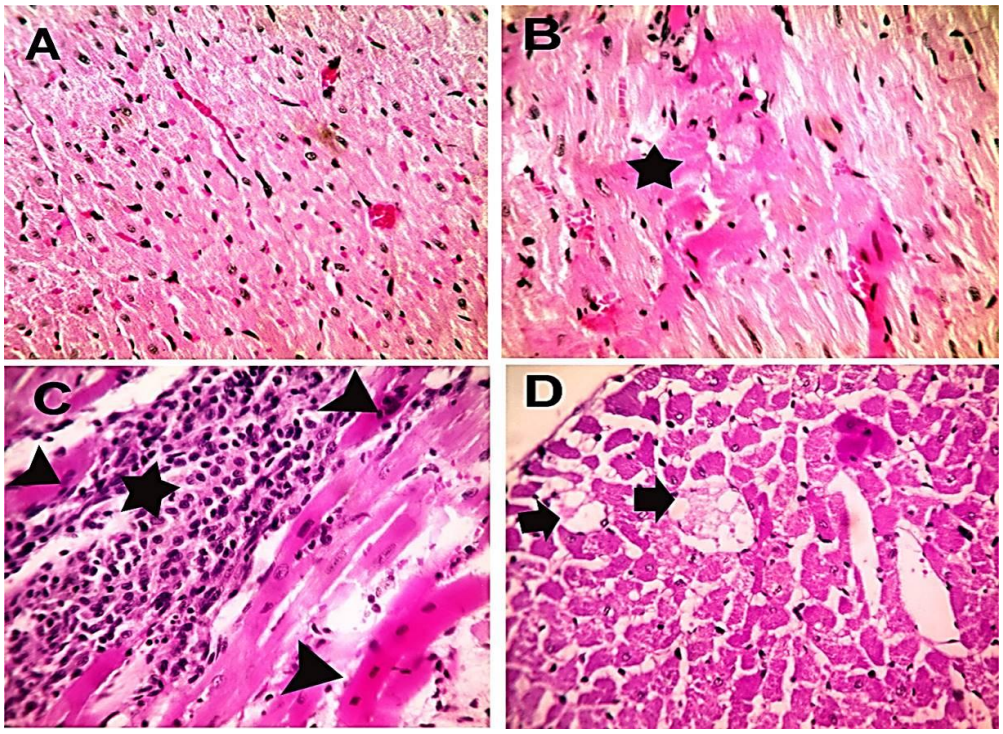

Figure 3: A. Heart of control group shows normal histological picture. **(B-D)**: Heart of fructose group shows **B.** mild hyaline degeneration (asterisk), **C.** necrosis (arrowheads) and multifocal areas of mononuclear cells aggregation (asterisk) and **D.** vacuolation of cardiomyocytes (arrows) (H&E, X 200).

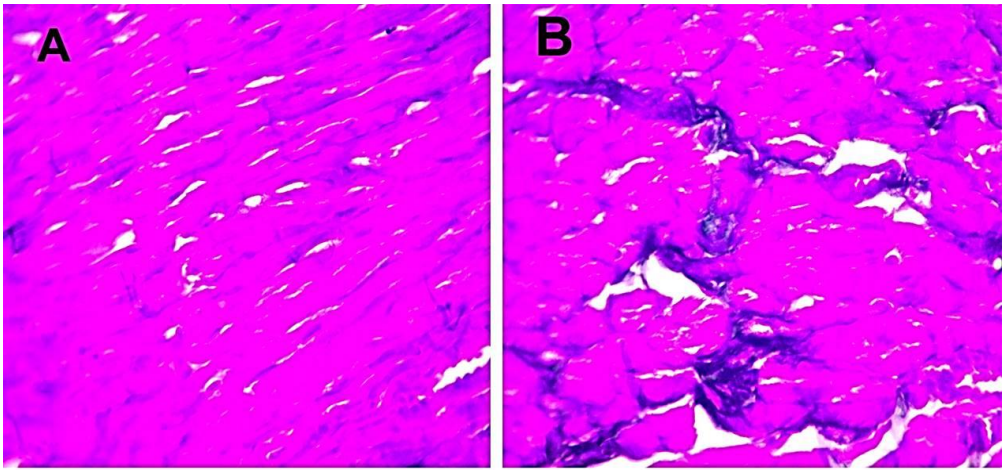

Figure 4 (A&B): Masson trichrome stained heart slides in **A** (control rats) and **B** (fructose group) (H&E, X 200).

Statistical analysis of cardio-myocytes sizes and interstitial fibrosis scores showed highly significant difference between both groups at two time points (**Table 2**). Significant increases in size of cardio-myocytes were recorded inside both groups after 2nd sacrifice (at 8 weeks) when compared with its results in 1st sacrifice (at 4 weeks) (**Table 2**). However, smaller cardiomyocytes were recorded in fructose group than in control group. In Masson trichrome stained heart slides, the fibrosis fraction increased in fructose group when compared with control group **(Figure 4**).

Aorta histopathology: Statistical analysis of the measured aortal wall thickness showed significant difference between both groups without significant changes observed at 2 sacrifices. Aortic wall thickness was significantly higher in fructose group at 2 time points than in control group (**Table 2**). The increased wall thickness was due to increased density of tunica media smooth muscle nuclei and number of elastin bands **(Figure 5**).

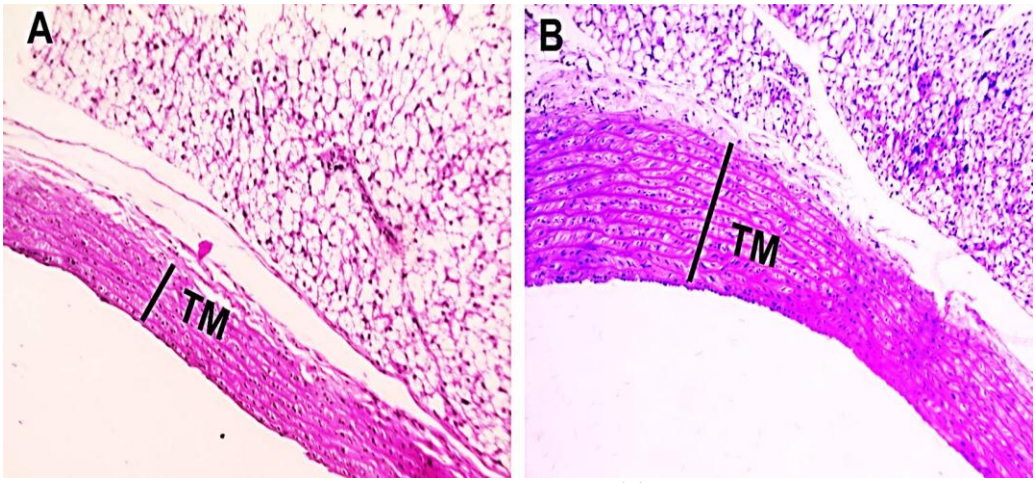

Figure 5 (A&B): Aorta shows increased thickness of tunica media (**TM**) in **B** (fructose group) when compared with **A** (control rats) (H&E, X 200).

DISCUSSION

Alterations in the blood biochemistry and tissue structures of kidneys, heart and aorta were achieved in fructose group after 4 weeks. The increase in levels of urea and creatinine indicates that the majority of renal function is lost (Borges et al., 2008). Renal injury was indicated after 4 weeks by increased serum values of creatinine, urea, GPT, GOT, LDH, Na, NOH, NOB, NOK, cholesterol, TG, LDL, MDAH, MDAB, MDAK and decreased Level of HDL.

Histopathological examination of kidney from fructose group demonstrated characteristic changes involving glomerular, tubular and interstitial lesions similar to those previously mentioned by Yang et al. (2014).

We quantified the corpuscular area, capillary tuft area, glomerular sclerosis and interstitial fibrosis in kidneys. Semi-quantitative measurements of glomerular sclerosis and interstitial fibrosis were considered as classical quantitative assessments of renal damage. In addition, the semi-quantitative method of measuring the degree of interstitial fibrosis gives a good idea about frequency, distribution and variability of the parameter investigated in focal area of the damage. Statistical analysis of the most measured parameters in kidneys, heart and aorta showed significant differences between fructose and control groups without significant changes between 2 time points. In kidneys, high fructose intake decreased glomerular tuft area and corpuscular area, increased percentage of rats affected with interstitial renal fibrosis score 1 and percentage of rats had glomerular sclerosis score 2. Rats maintained on diet rich in fructose developed insulin resistance (Hwang et al., 1987). A number of studies have described prominent glomerular injury in rats maintained on such diet (Boot-Hanford and Heath, 1981). Glomerular volume, fractional interstitial area, fractional mesangial area and width of glomerular basement membrane were all increased by diabetes as mentioned by (Satchell, 2012). Glomerulosclerosis was also reported by de Castro et al. (2013) in adult-fructose fed rats. The highest percentages of rats had interstitial renal fibrosis (score 1) was demonstrated in fructose group (66.7% after 4 weeks and 70% after 8 weeks) in agreement with Yang et al. (2014). Size of cardiomyocytes significantly increased in both groups after 8 weeks when compared with 4 weeks. It was mentioned that left ventricular mass from normal female Wister rats increased over age (Bru¨el et al., 2002; Wulfsohn et al., 2004). High fructose administration decreased size of cardiomyocytes, induced cardiac interstitial fibrosis score and increased aortic wall thickness. Histological findings in heart from fructose group mimic those reported in STZ-induced diabetic rats (Hayat et al., 2004; Thent et al., 2012). Moreover, high fructose diet increased thickness of aorta as previously recorded by Hayat et al. (2004) in diabetic rats.

CONCLUSION

It is concluded that, high fructose administration induces alterations in blood biochemistry, renal and cardiovascular tissue structures in rat.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

RS did the study plan, experiments, measured the serum biochemical profiles and drafted the manuscript. BHM took part in planning the study and revised the manuscript. WA evaluated the histopathological slides and critically corrected the manuscript. All the authors approved the manuscript before final submission.

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