COMPARATIVE STUDY ON THE EFFECT OF PROBIOTICS AND SILYMARIN ON RENAL CORTEX IN A RAT MODEL OF HIGH-FAT, HIGH-FRUCTOSE DIET

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ABSTRACT:

Background*: obesity has emerged as a major global health issue. It is recognized as a significant risk factor for chronic kidney disease.*

Aim: *To determine the effects of a high-fat high-fructose diet (HFHF) on the structure of renal cortices of rats and to evaluate the effects of probiotics versus silymarin on the cortices histologically and biochemically.*

Material and methods*: Forty albino rats were randomly divided into four equal groups; group I (control). The rest of groups received HFHF diet daily for eight weeks then divided into: group II: they were given 1 ml physiological saline daily, group III: they received probiotics daily, and group IV: received silymarin daily, all for further four weeks.*

Results: *Data showed that feeding HFHF diet significantly altered the structure of renal cortex in the form of cytoplasmic vacuolations, separation of epithelial cells lining the tubules, and subsequent sloughing in tubular lumen. Significant increase in TNF-α expression. Renal interstitium showed cellular infiltration with significant increase in collagen deposition. Additionally, qRT-PCR analysis showed significant increase in expression of miR-125b and DRAM1 gene in HFHF fed rats. There was improvement of all histological aspects in probiotics and silymarin treated groups. Both drugs caused significant increase in miR-125b expression and significant decrease in expression of DRAM1.However results were more evident in silymarin treated group.*

Conclusion: *HFHF diet is accompanied by renal tissues damage. We concluded that both probiotics and silymarin have therapeutic role in ameliorating HFHF-associated renal injury. However, silymarin has better effects on all aspects.*

Keywords: *Kidney, Probiotics, Silymarin, miR-125b, DRAM1, TNF-α, Fat, Histology*

INTRODUCTION:

One of the most prevalent medical conditions is obesity. It has been shown to cause changes in renal structure and function [1]. High-fat and/or high-carbohydrate diets are the main contributors to the development and progression of metabolic syndrome (MS). Epidemiological research has shown a connection between high rates of overweight, central obesity, and MS, and intake of high-fat diets (HFDs) [2].

A significant risk factor for obesity is eating HFDs, which can speed up increased production of reactive oxygen species (ROS) by activating NADPH oxidase [3&4]. Increased ROS can disturb cellular equilibrium and

exacerbate the symptoms of the MS by damaging proteins, lipids, and nucleic acids [5]. A previous investigation had shown that prolonged HFD intake decreases superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels, increases lipid peroxidation, and ultimately causes cell apoptosis [6].

It was discovered that using rats fed HFD was an excellent way to study the effects of dietary fat on humans [7]. Due to their tendency for weight gain when fed HFD, rat models are thus ideal methods for the induction of obesity [8].

Silymarin, a flavonoid complex isolated from the plant milk thistle, is extensively used in cases of cirrhosis and viral hepatitis. It is made up of two flavonoids, taxifolin, and quercetin, as well as three isomers of flavonolignans called silybin, silydianin, and silychristin[9]. There are two primary mechanisms that are hypothesized to be responsible for silymarin's hepatoprotective effects. Its dose-dependent antioxidant activity is the first. This effect is influenced by the scavenging of free radicals, a decrease in the production of ROS, and the suppression of fatty acid peroxidation. In order to produce anti-inflammatory and antiapoptotic effects, the second technique inhibits nuclear factor kappa-B (NF-kB), modifies inducible nitric oxide, and lowers cyclooxygenase-2 expression. Moreover, silymarin possesses antiviral and antifibrotic effects [10].

Silymarin's anti-inflammatory and antioxidant characteristics may be able to protect against photocarcinogens [11] and renal pathologies [12]. Similar to how it stimulates liver cells, silybin has been found to stimulate kidney cells. In kidney cells that have been harmed in vitro by paracetamol, cisplatin, or vincristine, silybin and silychristin have been found to speed up the rate of proliferation, protein and DNA synthesis, and lactate dehydrogenase activity. Additionally, silybin has been demonstrated to block or lessen nephrotoxic effects when administered prior to or following chemically induced kidney injury [13].

Through molecular interactions with the liver and kidneys, which serve as organs of detoxification, the gut microbiota is viewed as a special type of endocrine organ that regulates immunity and homeostasis in the host [14&15]. According to previous studies [16&17], probiotics are "living micro-organisms" that boost immunity, lessen inflammatory cytokines, and stop cholesterol from accumulating in the blood and hepatocytes [18]. Specific probiotic strains can affect pro-inflammatory chemokines and cytokines, modulate oxidant/antioxidant levels, and aid the bowel by creating mucus [19].

Thousands of gene targets are known to be regulated by a family of non-coding RNAs known as microRNAs (miRNAs), which post-transcriptionally inhibit the expression or translation of target mRNAs [20&21]. Various miRNAs have been identified as markers for chronic diseases, including diabetes, cardiovascular disease, and cancer. Research has also demonstrated a clear link between human obesity and aberrant miRNA expression [22&23].

In a number of disorders, miR-125b has demonstrated disease-suppressing properties [24]. It inhibits osteoblastic differentiation, prevents hepatocellular carcinoma metastasis, and controls cancer cell proliferation and lipogenesis through modulating the expression of its target genes [25,26,27,28&29]. miR-125b enhances murine white adipocyte development in 3T3 preadipocyte cells, most likely through inhibiting SMAD4[30].

miR-125b has a role in regulation of adipocyte differentiation, where its expression level is adversely related to the mRNA expression of uncoupling protein 1 (UCP1) in both white and brown fat of mouse and human origin [28]. miR-125b overexpression by estrogen was found to protect against non-alcoholic fatty liver disease (NAFLD) in female mice [31]. Additionally,

miR-125b expression has been associated with obesity in both mice and humans [32&33]. Furthermore, miR-125b was shown to be downregulated in adipose tissue of HFD-induced obese mice [34].

The complicated breakdown system known as autophagy is in charge of removing harmed and defective organelles. Obesity brought on by a HFD has been found to change autophagy across the body in a tissue-specific way [35]. The TP53 target gene DNA damage regulated autophagy modulator 1 (DRAM1), which codes for a lysosomal membrane protein, is crucial for TP53-mediated autophagy activation and apoptosis [36&37].

In the present investigation, we used biochemical, molecular, histological, and immunohistochemical methods to examine the effects of probiotics versus silymarin on the renal cortices of rats and to evaluate the effects of an HFHF diet on their cortices.

AIM OF THE STUDY:

To determine the effects of a high-fat high-fructose diet (HFHF) on the structure of renal cortices of rats and to evaluate the effects of probiotics versus silymarin on the cortices histologically and biochemically.

MATERIAL AND METHODS:

Experimental design and animals used:

This study included forty adult male albino Wistar rats of an average weight of 150-200 g aged about eight weeks. Clean plastic cages with mesh wire covers were used for housing of study animals that were given a free access to tap water and standard rat chow diet for the experimental period. Animals were maintained under appropriate conditions of light, temperature, and humidity.

After seven-day acclimatization period, rats were randomly divided into four groups: *I (control group)*: 10 rats were fed standard chow diet for the period of the experiment. (12 weeks). *Group II (HFHF group)*: 10 rats were fed high-fat, high-fructose (HFHF) diet daily for eight weeks from the beginning of the experiment, then they were given 1 ml physiological saline by oral gavage daily for further four weeks, then they were sacrificed. According to a previous study[38] **,** the HFHF diet consisted of 35.5% fat, 32.4% CHO and 18% proteins, in 100 grams of dry food with a total caloric value of 521 kcal/100 grams dry food; *Group III (HFHF treated with probiotics)*: rats were fed HFHF diet daily for eight weeks, then they received probiotics daily (1.2 ×109 cfu/ml of lactobacillus per gram of rat body weight) by oral gavage for further four weeks[39&40] then they were sacrificed; *Group IV (HFHF) treated with silymarin)*: rats were fed HFHF diet daily for eight weeks, then received silymarin daily by oral gavage (50 mg/kg/day) for further four weeks [41] and then they were sacrificed. Silymarin was provided by (CHEMICAL INDUSTRIES DEVELOPMENT GIZA - Under License of: MEDA Pharma GmbH &Co. KG- Germany). Silymarin capsules were dissolved in physiological saline.

Histological and Immunohistochemical Studies:

At the end of the experiment, the rats were anesthetized with ether inhalation. The left kidneys were used for biochemical investigation, while the right kidneys were preserved in 10% buffered formalin before being cut into 5 μm thick paraffin slices for histological analysis, using H&E, PAS and Mallory's trichrome stains [42].

For TNF-α immune staining, sections were incubated with the primary antibodies for immunohistochemistry overnight at 4 °C: anti-TNF (rabbit polyclonal IgG, 100 g/ml, 1:50 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and anti-IL 10 (rabbit polyclonal IgG, 1 mg/ml, 1:600 dilution; Abbiotec, San Diego, CA, USA). The primary antibody was then identified using avidin biotin peroxidase detection solution (Dakocytomation labelled streptavidin biotin reagent; Dako, Glostrup, Denmark and

System horseradishperoxidase; Dako), and the signal was seen using diaminobenzidine (Dakocytomation) and Substrate Chromogen System (Dako). Slides were mounted, dehydrated, cleaned, and counterstained with hematoxylin [43].

Morphometric studies:

Animals from all groups were subjected to histomorphometric study. An image analyzer Leica Q win V.3 program installed on a computer in the Department of Histology, Faculty of Medicine, Ain Shams University, was used for histomorphometric studies. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany). Measurements were taken from three different slides obtained from each animal of all groups. Five selected non-overlapping fields were examined from each slide for measurement of the area percentages of collagen and TNF-α.

Detection of the Relative Expression of miRNA-125b and DRAM1:

miRNeasy Mini kit (Qiagen, Hilden, Germany) was used to extract total mRNA and miRNAs from homogenized renal tissues according to the manufacturer's protocol. Utilizing the miScript RT reverse transcription kit (Qiagen, Hilden, Germany), cDNA was synthesized. The quantitative reverse transcription real-time PCR (qRT-PCR) was performed using the miScript Syber Green master mix and QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany) for amplification of miR-125b and DRAM1 respectively. Primers were purchased from Qiagen, Germany; miScript primer assay [miR-125b], cat no: 218300, SNORA11 Primer Assay, cat no: 218300, QuantiTect primer assay [DRAM1], cat no: 249900, and ACTB_ 1_SG QuantiTect Primer Assay (β-actin) cat no: 249900.

The relative expression levels (fold change) for miR-125b and DRAM1 were normalized to an internal control (SNORA11 and β-actin respectively) and relative to calibrator (negative control sample) and were calculated using the equation $2^{-\Delta\Delta Ct}$. [44]

Determination of Total Antioxidant Capacity (TAC):

Tissue homogenate was prepared by washing 0.05 g tissue with phosphate-buffered saline (PBS). The tissue was suspended in homogenization medium. The tissue/medium mixture was homogenized by mechanical homogenization using ultra-sonication method. The cells were treated with ultrasonic cell disruptor. Finally, the supernatant was used for analysis. TAC was measured in renal tissue using the Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit, **(**Elabscience Biotechnology, cat no: E-BC-K136-M, USA) [45].

Ethical consideration:

The experiment took place in the Medical Research Centre, Faculty of Medicine, Ain Shams University (MASRI). All the experimental procedures were performed according to the guideline of animal care and the Scientific Research Ethical Committee of the Faculty of Medicine Ain Shams University and Followed the guidelines for the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 2011). The study was conducted with the approval of Ain Shams Faculty of Medicine Ethical Committee, authorization number "FMASU R25/2023".

RESULTS:

Histological results

In the present study, H&E-stained sections of the renal cortex of the control group revealed the presence of renal corpuscles, the proximal convoluted tubules (PCTs), the distal convoluted tubules (DCTs) and cortical collecting tubules. The renal corpuscle was composed of a glomerulus surrounded by Bowman's capsule. The glomerulus was formed of a lobulated tuft of

capillaries. The Bowman's capsule was formed of an interior visceral layer and exterior parietal layer separated by Bowman's space. The PCTs formed the main elements in the renal cortex. They had a narrow lumen and were lined by pyramidal cells with apical brush border and indistinct cell boundaries. These cells had strongly eosinophilic cytoplasm and basal open face nuclei. In the renal cortex, the DCTs appeared to be less abundant than the PCTs. Compared to PCTs, they had a relatively wider lumen. They were lined by cubical cells with faint acidophilic cytoplasm and apical rounded vesicular nuclei (Fig. 1).

H&E-stained sections of the renal cortex of group II showed atrophy of glomerular capillaries with dilatation of Bowman′s space. Some tubules had fat vacuoles. Other tubules showed pyknotic nuclei and sloughing of their epithelium (apoptosis). Inflammatory cells were seen invading the epithelial lining of tubules from interstitium, mostly intraepithelial lymphocytes. Other renal tubules showed homogenous acidophilic hyaline casts in their lumen. The interstitial tissues showed cellular infiltrate, dilation, and peritubular capillary congestion (Fig. 2).

H&E-stained sections of the kidney of group III showed apparent normal structure of renal corpuscle. Some tubules showed some vacuolations, other tubules showed pyknotic nuclei. The lumen of some tubules revealed acidophilic casts. In group IV there was closely packed tubules and apparent normal structure of renal corpuscle, it was comparable to control group (Fig. 3).

Examination of PAS staining showed, the basement membranes of the renal tubules, the parietal layer of Bowman's capsule, and the glomerular capillaries displayed a distinct PAS positive reaction in the control group. The PCT cells had apical brush borders that were PAS positive (Fig. 4A). PAS stain of group II revealed thickened parietal layer of Bowman′s capsule. However, loss of PAS positive reaction was detected in the disrupted basement membranes of some renal tubules. Additionally, (Fig. 4B). Examination of PAS stain of both groups III and IV showed well defined basement membrane seen surrounding renal corpuscles, many tubules, and glomerular capillaries. They compared to the control group favorably (Fig. 4C & D).

Examination of immune staining for TNF-α showed weak positive brown cytoplasmic reaction in renal mesangium epithelial cells and PCT epithelial cells of control group and group IV, while group III showed mild cytoplasmic reaction. Strong positive brown cytoplasmic reaction was evident in group II for TNF-α immune staining (Fig. 5).

Examination of Mallory's trichrome-stained sections of renal cortex of the control group showed few scattered collagen fibers in the renal interstitium while group II showed accumulation of the collagen fibers in the renal interstitium. In group III, interstitial collagen fibers were slightly more than the control group. Collagen of group IV was comparable to control (Fig.6)

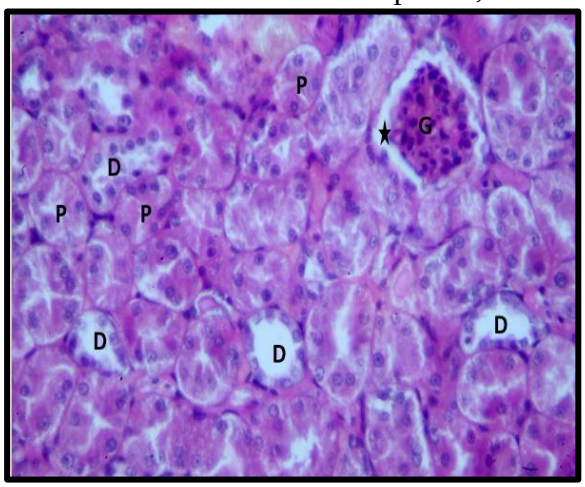

Fig. 1. A photomicrograph of a section of the renal cortex of a rat of group I (control group) showing the renal corpuscle and closely packed renal tubules. The renal corpuscle is formed of a glomerulus (G) surrounded by Bowman's capsule and Bowman's space (✶). The PCTs have narrow lumen and are lined by pyramidal cells with apical brush border and dark acidophilic cytoplasm (P). The DCTs have wider lumen and are lined with cubical cells with less acidophilic cytoplasm (D).
(H&E x 400)

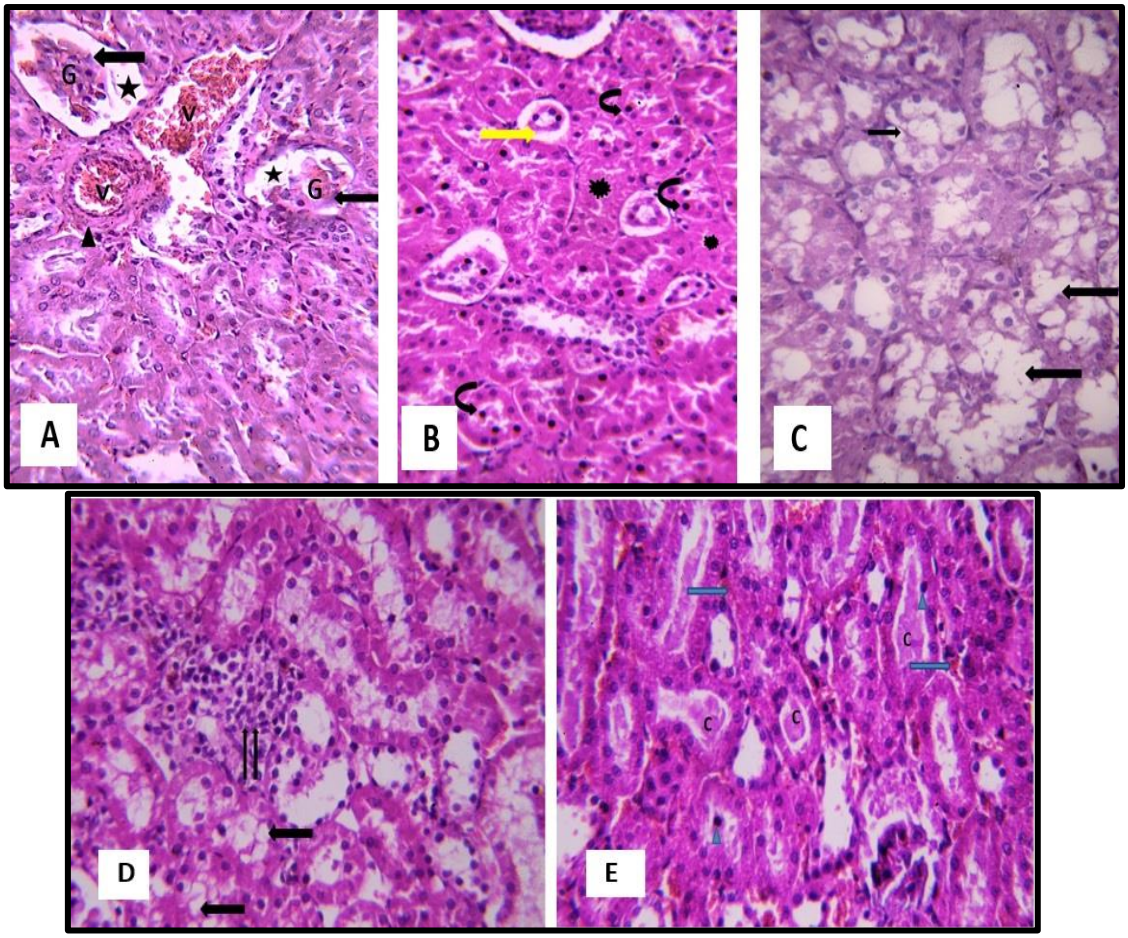

Fig. 2. A Photomicrograph of a section of renal cortex of a rat of group II showing: (A) dilatation and congestion of blood vessels (V) with thickened wall (▲). Degeneration of glomerular cells (G) with dilatation of Bowman's space (✦) (B) some tubules are collapsed and formed of spherical acidophilic masses with ill-defined nuclei (*). Separation of some tubular epithelial cells from their basal lamina (yellow↑) and infiltration of tubular epithelium by small cells with darkly stained nuclei ( ) (C) multiple vacuoles (↑)in some tubules (D) Renal interstitium containing mononuclear cellular infiltrate (↑↑). (E) homogenous acidophilic hyaline cast in the lumen of some tubules (C), sloughing of their epithelial lining (BlueΔ) and peritubular congestion (Blue↑). *(H&E x 400)*

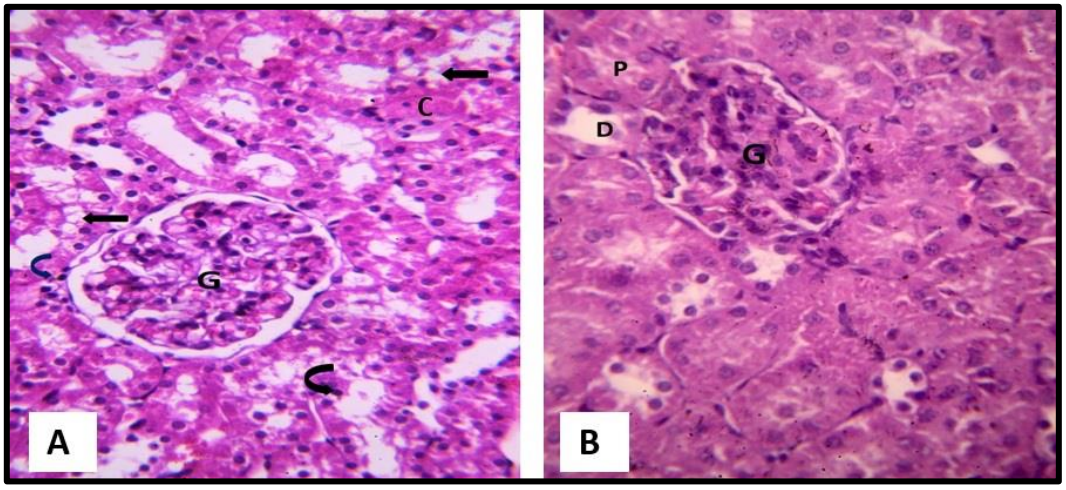

Fig. 3. A Photomicrograph of a section of renal cortex: (A) group III showing apparent normal structure of renal corpuscle (G). Tubules still show some vacuolation (↑), some tubules show pyknotic nuclei ( ) the lumen of some tubules still show acidophilic casts (C). (B) group IV showing closely packed tubules and apparent normal structure of renal corpuscle (G), PCTs (P) and DCTs (D). *(H&E x 400)*

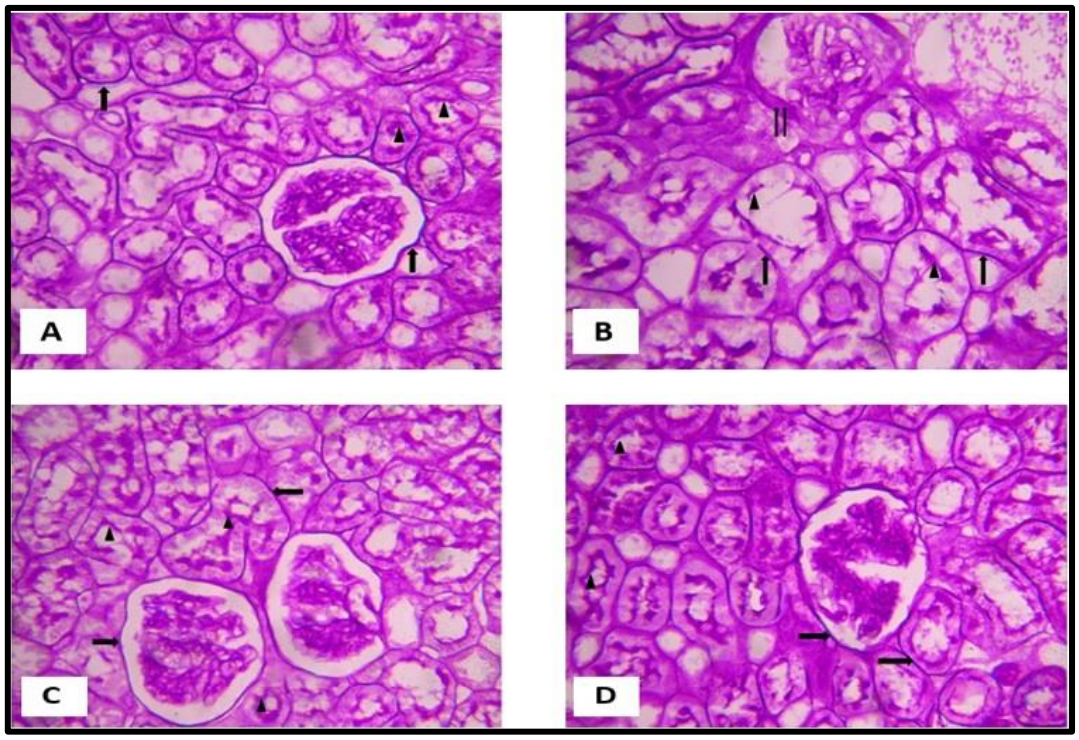

Fig. 4. A photomicrograph of a section of renal cortex of a rat: (A) Control group showing PAS positive apical brush borders of the cells lining the PCTs (▲). The basement membrane of the renal tubules (↑), glomerular capillaries and parietal layer of Bowman′s capsule (↑) are PAS positive. (B) Group II showing interrupted brush border of proximal tubular cells (Δ), and apparent thickened parietal layer of Bowman′s capsule (↑↑). Decreased PAS positive reaction of basement membrane of some tubules is also detected (↑). (C) Group III and (D) Group IV are nearly similar to the control group. *(PAS x 400)*

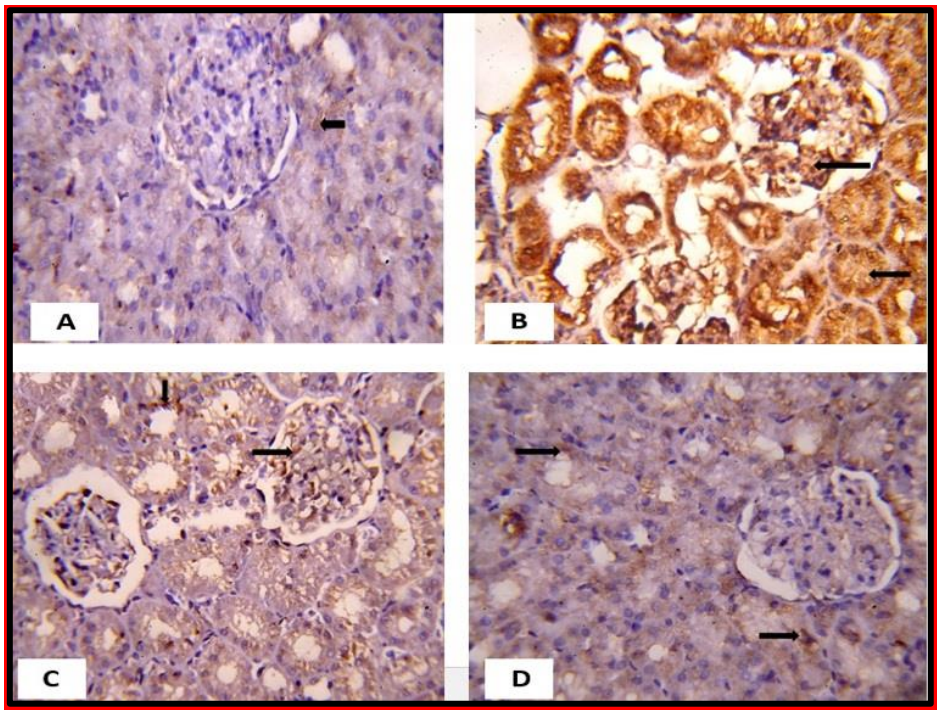

Fig. 5. A photomicrograph of a section of the renal cortex both (A) Control group and (D) Group IV showing weak positive cytoplasmic reaction. (B) group II showing strong positive cytoplasmic reaction in both glomeruli and tubular cells (↑). (C) group III showing mild positive cytoplasmic reaction in glomerular &tubular cells.

(TNF-α x 400)

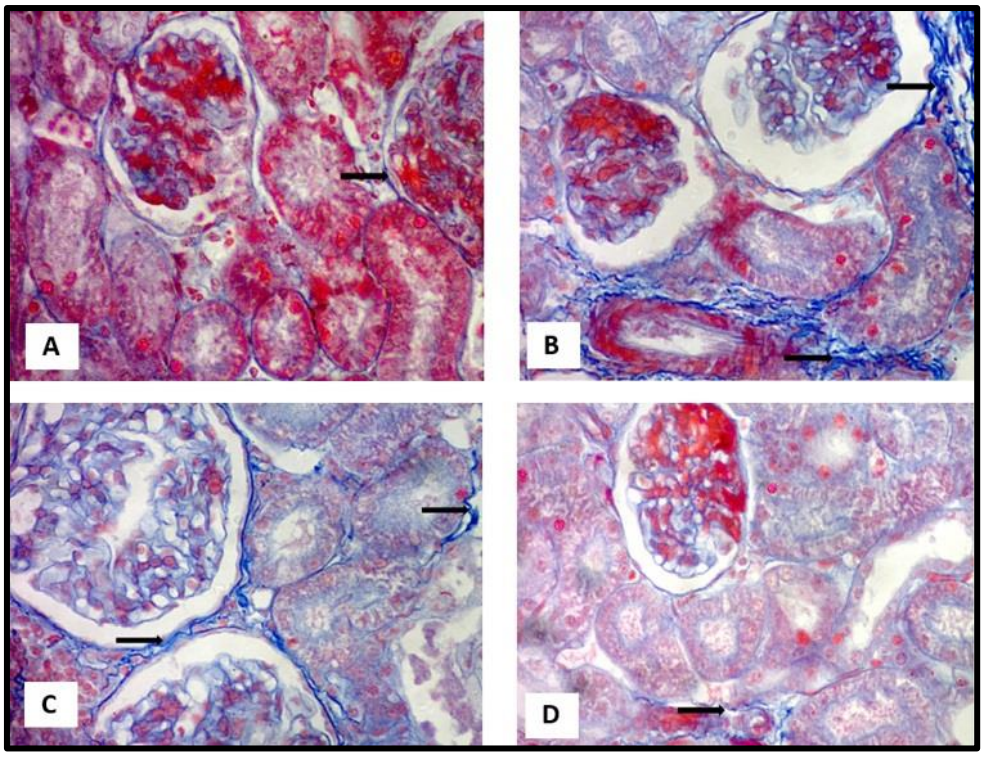

Fig. 6. A photomicrograph of a section of renal cortex of a rat both (A) Control group and (D) Group IV showing few scattered collagen fibers in renal interstitium (↑). (B) Group II showing apparent increase of collagen fibers in renal interstitium (↑) and in between the glomerular capillaries. (C) Group III showing slight increase of collagen fibers (↑) in renal interstitium *(Mallory's trichrome x 400)*

Morphometric results:

Statistical analysis using one-way ANOVA test revealed a notable (P< 0.05) elevation in the mean area percentage of collagen fibers and TNF-α in groups II .While probiotic and silymarin treatments (group III and IV respectively) showed a significant decrease compared to group II. Results of group IV were more comparable to control group (Table 1).

Table 1: Differences of the mean of area percentage of collagen fibers and TNF-α between the studied groups.

	I	II	III	IV
Mean area percentage of collagen fibers	4.8±1.1	37.78±6.2 (■)(♣)(●)	8.3±2.4	6.2±0.9
Mean area percentage of TNF-α	6.9±1.8	49.3±8.9 (■)(♣)(●)	11.3±4.8	8.7±2.3

Values are presented as mean ±SD
(■) significant compared to group I (P< 0.05)
(♣) significant compared to group III (P< 0.05)
(●) significant compared to group IV (P< 0.05)

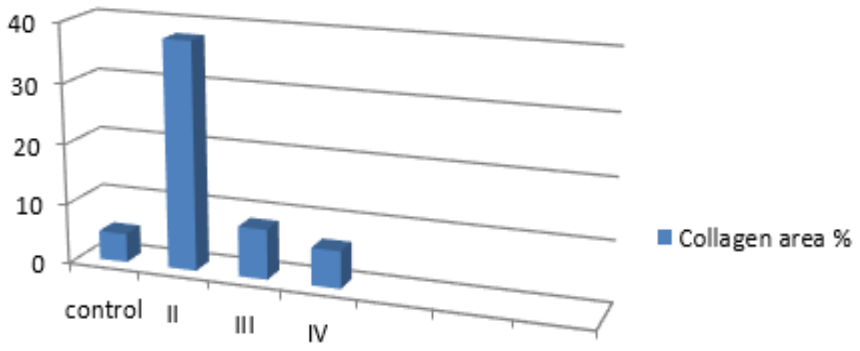

Fig. 7. A histogram showing differences in the area percentage of collagen fibers.

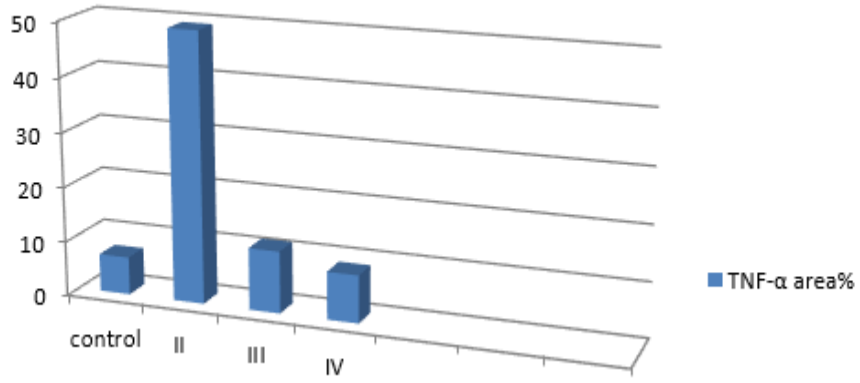

Fig. 8. A histogram showing differences in the area percentage of TNF-α.

Biochemical and Molecular Results:

Assessment of the effects of HFHF diet and the treatment with probiotics and silymarin on miR-125b and DRAM1 expression levels was done. Table 2 shows that there was a highly significant increase in the fold change (RQ) of miR-125b in groups III and IV in comparison to group I and II (P<0.01). Using post-hoc test, there was a highly significant difference in miR-125b RQ values between group III and IV where silymarin caused greater increase in miR-125b expression (P<0.01).

When compared to the control group, group II showed a significant rise in DRAM1 RQ values, while there was a significant decrease in DRAM1 gene expression in group III and IV in comparison to both HFHF diet group and the control group (P<0.01). There was a highly significant difference in DRAM1 relative expression between the two treated groups with more reduction in DRAM1 expression in silymarin-treated group (P<0.01), table 3.

TAC was used to assess the antioxidant status of renal tissues. As shown in table 4, there was a highly significant decrease of TAC in group II as compared to control group (P<0.01). Moreover, both probiotic and silymarin-treated rats showed increase in TAC in comparison to HFHF rats. This increase was more evident in silymarin-treated group (P<0.01).

Regarding the correlations between the different studied parameters between the groups, there was a strong inverse relationship between TAC and renal tissue DRAM1 relative expression. Despite being statistically insignificant, there was a negative connection between tissue miR-125b and

DRAM1 RQ levels among the various groups. However, there was no significant correlation between fold change (RQ) of miR-125b and TAC among the studied groups (Table 5).

Table 2: Relative quantification (RQ) of renal tissue miR-125b expression among the study groups.

miR-125b RQ				
		Mean±SD	N	P
Groups	I	1±0.17	10	0.000 [a]
	II	1.72±0.22	10	
	III	14.47±2.17	10	
	IV	25.89±2.68	10	

[a]**One-Way ANOVA test**

Table 3: Relative quantification (RQ) of renal tissue DRAM1 gene expression among the study groups.

DRAM1 RQ				
		Mean±SD	N	P
Groups	I	1.03±0.2	10	0.000 [a]
	II	4.83±0.34	10	
	III	3.03±0.19	10	
	IV	2.03±0.18	10	

[a]**One-Way ANOVA test**

Table 4: TAC in renal tissue of the study groups.

TAC				
		Mean±SD	N	P
Groups	I	3.46±0.03	10	0.000[a]
	II	0.1±0.04	10	
	III	0.78±0.1	10	
	IV	2.94±0.14	10	

[a]**One-Way ANOVA test**

Table 5: Correlation between miR-125b, DRAM1 and TAC among study groups.

Correlations				
		miR-125b	DRAM1	TAC
miRNA-125b	Pearson Correlation	1	-0.204	0.231
	Sig. (2-tailed)		0.208	0.157
DRAM1	Pearson Correlation	-0.204	1	-0.936**
	Sig. (2-tailed)	0.208		0.000
TAC	Pearson Correlation	0.231	-0.936**	1
	Sig. (2-tailed)	0.157	0.000	
**Correlation is significant at the 0.01 level (2-tailed).				

DISCUSSION:

Obesity is one of the major risk factors that affects roughly 30% of adults in developed nations. It has been shown that a sedentary lifestyle and rising dietary fat intake are related to obesity [46]. The metabolic syndrome, which is characterized by oxidative stress, elevated blood pressure, atherogenic dyslipidemia, a proinflammatory and pro-thrombotic condition, central obesity and cardiovascular disease, and kidney disease, is typically brought on by a HFD [47&48].

Although the heart, kidney, and liver are additional tissues where extra fat may be stored, adipose tissue is the primary site of fat

storage in obesity. HFD changes renal lipid metabolism by establishing an imbalance between lipogenesis and lipolysis in the kidney, which leads to systemic metabolic irregularities, renal lipid accumulation, and lipid peroxidation that lead to renal injury [1].

In the current work, we investigated the effect of HFHF diet on glomeruli, kidney tubules and interstitium. Declèves et al. [49] demonstrated that the kidney is a quick responder to the challenge of feeding HFD. The kidney exhibited an initially planned inflammatory response, followed by a subsequent robust inflammatory and fibrotic response.

In HFHF fed group of the present study, there was a dilatation of Bowman's space, vacuolations of epithelial cells of some tubules. Some tubules showed cytoplasmic vacuolations. The tubular lumina contained acidophilic casts and pyknotic nuclei of sloughed cells. This was supported by significant increase expression of TNF-α in tubular and glomerular cells. These results were concomitant with those of a previous study [50], which found that rats fed HFD exhibited atrophied glomeruli with increased Bowman capsular space, tubular epithelial cytoplasmic vacuolations and tubular necrosis.

HFHF diet induces oxidative stress and activate lipid peroxidase leading to fat droplet accumulation in the tubular cells [51&52]. Thus, the cytoplasmic vacuolations observed in HFHF fed group in the present study might be fat vacuoles. Moreover, Briffa et al. [53] found that HFD might alter the membrane surroundings.

Acidophilia of tubular cells and tubule shape modifications were characteristics of hypercholesterolemia, which damages tubules and impairs filtration, resulted in protein loss and renal tubular luminal leak [54]. This explains our findings of presence of homogenous eosinophilic hyaline casts, which were observed in the lumen of some renal tubules of group II.

Li et al. [55] examined the effect of high-fat/high-sucrose diet on kidney structure and found renal tubular cell damage and significantly increased apoptosis in the kidney cells. While some renal tubule showed blocking of their lumen by casts led to increase back pressure & decrease the glomerular filtration rate.

Destruction of blood renal barrier by TNF- α expression of glomerular cells led to proteinuria. Additionally, by lowering blood flow and filtration rates and altering barrier functions in the capillary wall brought on by obesity, TNF- α insufficiency protects against albuminuria and renal damage. In vitro, research has shown that free fatty acids stimulate the synthesis of TNF- α. Also, high-fat diet caused increased TNF- α expression, which in turn caused inflammation, oxidative stress, apoptosis, higher levels of renal fibrosis, and glomerulosclerosis in the kidney [56].

Furthermore, Cirillo et al. [57] stated that consuming chronic amounts of fructose caused renal microvascular dysfunction, which changed renal autoregulation and caused glomerular hypertension.

Also, Futatsugi et al. [58] found that rats on the HFD diet showed glomerular capillaries and other blood vessels dilatation, mononuclear cell infiltration in the renal interstitium and tubular cells. That was similar to our findings.

Moreover, Chagnac et al. [59]. stated that abnormalities in renal hemodynamics, endothelial dysfunction, glomerular basement membrane thickening and expansion, tubular atrophy, interstitial fibrosis, and a progressive loss of renal function leading to end-stage renal disease were associated with obesity-related kidney disease.

The observed small cells with dark nuclei invading the epithelial lining of some

renal tubules, mostly intraepithelial lymphocytes, were demonstrated by a previous study [60], which stated that these lymphocyte subpopulations can activate macrophages and effector immune cells causing potent tissue damage.

In the current investigation, Mallory's trichrome -stained sections were used to determine collagen fiber proliferation or fibrosis. The HFD-treated group had significant increase in area percentages of collagen in contrast to other groups. This finding is supported by other studies that found that a diet high in fructose increased oxidative stress and ROS, which in turn upregulated CD36. This in turn promoted fibrosis and inflammation in rats with metabolic syndrome [61&62].

In our study, PAS stain of group II revealed interrupted brush border of proximal tubular cells, thickened parietal layer of Bowman′s capsule and lost, disturbed PAS positive reaction in the basement membranes of some renal tubules. Li et al. [55] also noticed deep PAS staining in parietal layer of Bowman′s capsule in high fat high sugar fed group.

Bülow and Boor [63] added that the thickening of the basement membrane, which is frequently accompanied by fibrosis, brought on by cytokines secreted by inflammatory cells like TGF-B.

In our study we found that probiotics-treated group showed apparently normal structure of renal corpuscle. However, tubules still show some vacuolations, some tubules show pyknotic nuclei. The lumen of some tubules still shows acidophilic casts.

Following fermentation of the undigested food in the colon, phenols, amines, and indoles were produced [64]. These endogenous toxins worsen the chronic kidney illnesses linked to oxidative stress, inflammation, and increased intestinal permeability. Through converting the undigested food into short chain fatty acids, probiotics decreased the levels of IL17A and TNF- α [65], which reduced inflammation [66]. This explains the decreased TNF-α immune-staining expression in probiotics-treated group in our study.

Wang et al. [67] proved that in patients with peritoneal dialysis (PD), proinflammatory cytokines TNF-α, IL-5, and IL-6 as well as endotoxins significantly decreased following six months of probiotic therapy. Anti-inflammatory cytokine IL-10 levels also increased. After probiotic administration, uric acid, creatinine, and urea levels remained unchanged.

In our study we found that silymarin-treated group showed apparently normal structure of renal corpuscle and tubules. They were comparable to control group.

Silymarin speeds up regeneration by promoting RNA and protein synthesis [68]. It also modulates TNF-α associated inflammatory pathway. Additionally, in the silymarin-treated group's urine and serum TNF-α and MDA levels were considerably low [69].

Also, chronic treatment with silymarin decreased the expression of inflammatory markers in a dose-dependent way, indicating that it may be successful in lowering inflammatory markers to further mediate kidney damage in a rat model of diabetic nephropathy [69].

In our study examination of PAS stain of group IV showed well defined basement membrane seen surrounding renal corpuscles, glomerular capillaries, and many tubules. Also, the area percentage of collagen was significantly decreased. Similar findings were documented by Feng et al. [62] who found that silymarin treatment improved these HFD induced glomerular alterations and decreased deposition of collagen. They also added that silymarin can reduce oxidative stress and maintain mitochondrial dysfunction in the kidney, possibly by limiting the buildup of renal lipids and the α-oxidation of fatty acids.

Several biological activities, involving metabolism, growth and function of adipose tissue, can be influenced by microRNAs [70,71]. For example, microRNA-221 was found to be up-regulated in obesity and influences fat metabolism downstream of leptin and TNF-α[72]. Another study found that exosomal miR-125b-5p derived from mesenchymal stem cells enhances tubular healing in ischemia acute kidney damage by suppressing p53[73]. Hypoxia induces miR-125b, which suppresses the production of P53 and reduces the amount of cell death that is caused by hypoxia [74].

Our results showed increased expression in miR-125b in the HFHF group as compared to the control group. In line with our results, Wei and his colleagues [33] demonstrated the relevance of miR-125b in lipogenesis in their 2020 work by knocking out miR-125b in mice on HFD. miR-125b-knock out mice given a HFD had higher liver weight, lipid droplet formation, and triglyceride content than mice fed HFD. They concluded that miR-125b-2 is a unique potential target for controlling fat accumulation, as well as a viable target for developing innovative obesity treatment options.

In the current study, there was a significant increase in the miR-125b expression in groups treated with probiotics and silymarin in comparison to control group and HFHF fed group. However, silymarin-treated group revealed a significant expression more than probiotics treated group. In a previous study [75], silymarin-treated group in mice liver and kidney were protected from mitochondrial depolarization-induced cytochrome c release in the cytosol**.** This explained the absence of apoptosis in our study. This is also correlated with another study [62], that illustrated that alterations that occur in obese mice; systemic and renal, were efficiently prevented by administration of silymarin.

On the other hand, increased expression of miR-125b in probiotics-treated group was matching with the study of Allegra et al. [76] which proved that microbiota increase expression of tumor suppressive miRNAs including miR-125b and decrease hepatocellular carcinogenesis.

In cases of acute kidney injury (AKI), autophagy exerts protective benefits at the proper point in the cell cycle, however aberrant autophagy can result in cell death. An important part of the pathophysiological process of kidney damage and repair during AKI is played by the inflammatory response. In the etiology of AKI, autophagy and inflammation have been shown in several investigations to interact [77].

In the current study, relative expression of DRAM1 was assessed as it is reported to be an important player in autophagy activation and has been linked to obesity and kidney injury. As our results reported, there was a significant increase in DRAM1 expression in HFHF fed rats in comparison to the control group. This correlates with a previous study [78], which reported that DRAM1 is important for insulin signaling and that, in vivo, this has implications for glucose tolerance in mice on HFD. The increase in DRAM1 expression is significantly reduced with treatment by probiotics and silymarin. This reduction is more obvious with silymarin treatment. Our results were confirming the importance of silymarin in improving the effect of HFHF diet on different tissues.

The development of many diseases is greatly influenced by oxidative stress [79]. Besides, the functional importance of antioxidant is to halt damage to cellular components that might develop because of chemical reactions involving free radicals [80]. Total antioxidant capacity (TAC) is a common analyte used to determine the antioxidant status of biological samples and can measure how well the body fights off free radicals caused by a particular illness [81].

In alignment with these facts, we measured the TAC in the four groups of our study. There was a significant decrease in TAC level in HFHF fed group in comparison to control group. In contrast, a previous study [82] showed no significant difference between control group and HFD fed group regarding TAC. In the current study, we aimed at highlighting the effect of silymarin on TAC and found a significant increase in TAC level in the treated groups as compared to group II. Considering these findings, Rashid et al. [50] reported that in a rat model, probiotic administration lowers the oxidative stress brought on by a high fat, high sugar diet at intestinal epithelial cells, nephrons, and hepatocytes. It is worth mentioning that the improvement in TAC is better in silymarin-treated group than probiotics-treated group. The effect of silymarin on TAC was proven by another study [83] which declared that silymarin has a preventive effect against the liver and kidney damage that cisplatin can cause.

Conclusion:

We concluded that both probiotics and silymarin have therapeutic role in ameliorating HFHF-associated renal injury. As it inflicts, probiotics improve the total antioxidant capacity, increase miR-125b and decrease DRAM1 expression, and also improve histopathological architecture of the renal cortices. However, silymarin has better effects on all aforementioned aspects. It is possible to prevent and cure metabolic syndrome linked to the HFHF-diet affecting the renal axis using silymarin and probiotics. We recommend using probiotics of different strains and different doses to examine better effects.

Declarations

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Conflicts of interest/Competing interests:

The authors have no conflicting financial or competing interests.

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دراسة مقارنة حول تأثير البروبيوتيك والسيليمارين على القشرة الكلوية للجرذان في نموذج لنظام غذائي عالي الدهون والفركتوز

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المقدمة: برزت السمنة كقضية صحية عالمية رئيسية. ومن المسلم به كعامل خطر كبير لأمراض الكلى المزمنة.

الهدف: لتحديد آثار اتباع نظام غذائي عالي الدهون عالي الفركتوز على بنية القشرة الكلوية للجرذان وتقييم آثار البروبيوتيك مقابل سيليمارين على القشرة الكلوية نسيجيا وكيميائيا حيويا.

المواد والطرق: تم تقسيم أربعون جرذ عشوائيا إلى أربع مجموعات متساوية. المجموعة الأولى (المجموعة الضابطة) ، المجموعة الثانية: تلقى نظام غذائي عالي الدهون عالي الفركتوز يوميا لمدة ثمانية أسابيع ، ثم تم إعطاؤهم 1 مل محلول ملحي فسيولوجي يوميا لمدة أربعة أسابيع أخرى ، المجموعة الثالثة: تلقوا حمية نظام غذائي عالي الدهون عالي الفركتوز يوميا لمدة ثمانية أسابيع ، ثم تلقوا البروبيوتيك يوميا لمدة أربعة أسابيع أخرى ، والمجموعة الرابعة: تلقوا حمية نظام غذائي عالي الدهون عالي الفركتوز يوميا لمدة ثمانية أسابيع ، ثم تلقوا سيليمارين يوميا (50 مجم / كجم / يوم) لمدة أربعة أسابيع أخرى

النتائج: أظهرت البيانات أن اتباع نظام غذائي عالي الدهون عالي الفركتوز غير بشكل كبير أنسجة الكلى في شكل توسع في مساحة بومان، والتغيرات التنكسية في الأنابيب مع زيادة كبيرة في التعبير عن TNF-α. أظهر الخلالي تسلل خلوي وزيادة كبيرة في ترسب الكولاجين. بالإضافة إلى ذلك ، أظهر تحليل qRT-PCR أن التعبير النسبي ل miR-125b و DRAM1 قد زاد في الفئران التي تغذت على نظام غذائي عالي الدهون عالي الفركتوز مقارنة بالفئران الضابطة. كان هناك تحسن في جميع الجوانب النسيجية في البروبيوتيك والمجموعات المعالجة سيليمارين. كان هذا التحسن أكثر وضوحا في المجموعة المعالجة بالسيليمارين. علاوة على ذلك ، تسبب كل من البروبيوتيك والسيليمارين في الإفراط في التعبير عن miR-125b وانخفاض التعبير عن DRAM1. كان هذا التغيير في التعبير النسبي أكثر وضوحا مع علاج سيليمارين.

الاستنتاج: النظام الغذائي عالي الدهون عالي الفركتوز يرافقه تلف الأنسجة الكلوية. خلصنا إلى أن كلا من البروبيوتيك والسيليمارين لهما دور علاجي في تحسين الإصابة الكلوية المرتبطة بالنظام الغذائي عالي الدهون عالي الفركتوز. ومع ذلك ، سيليمارين له آثار أفضل على جميع الجوانب.