Full Length Article

The effect of high dietary fructose on the kidney of adult albino rats and the role of curcumin supplementation: A biochemical and histological study

CrossMark

Samraa H. Abdel-Kawi [a,*], *Kamel M.A. Hassanin* [b], *Khalid S. Hashem* [c]

[a] *Department of Histology, Faculty of Medicine, Beni-Suef University, Beni-Suef, 62511 Egypt*
[b] *Department of Biochemistry, Faculty of Veterinary Medicine, Minia University, El Minya, 61519 Egypt*
[c] *Department of Biochemistry, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, 62511 Egypt*

Background: Consumption of fructose, in the form of added sugars such as high fructose corn syrup (HFCS) or sucrose, has increased markedly in the last few years, which is strongly correlated with the prevalence of metabolic syndrome. It is widely used as a food ingredient and has potential to increase oxidative stress. Curcumin is a phenolic compound, and it exhibits protective effects against oxidative damage.

Objective: The aim of the study was to investigate the effects of curcumin on renal injury in fructose-fed rats and the possible underlying mechanism.

Methods: Eighty male rats were randomly divided into control group, fructose group, 200 mg/kg curcumin group and curcumin-fructose group. The histopathological changes in the kidney of rats were observed using hematoxylin and eosin and Masson's trichrome stains. The expressions of renal reduced glutathione (GSH) concentration, glutathione reductase (GR), super oxide dismutase (SOD), catalase activities, lipid peroxidation (LPO), DNA fragmentation %, inducible nitrous oxide (INOS) and homooxygenase 1 (HO-1) mRNA in renal tissue homogenates were assessed. Immunohistochemical detection of alpha-smooth muscle actin (α-SMA) and tumor necrosis factor alpha (TMF-α) were also investigated.

Results: Compared to the control group, GSH, GR, SOD and catalase activities were significantly decreased in the fructose group, while there was a significant increase in LPO, DNA fragmentation %, INOS and HO-1. These changes were accompanied by renal tubular injury, increased collagen deposition and lipid accumulation. Immunohistochemical results revealed increased expression of both α-SMA and TNF-α. Curcumin at 200 mg/kg evidently improved renal tubular injury, suppressed the expressions of renal LPO, DNA fragmentation %, INOS and HO-1, and decreased the expression of both α-SMA and TNF-α in kidney tissue.

Conclusion: Curcumin administration protected the kidney cells from fructose induced oxidative stress by increasing the antioxidant defence mechanism of the kidney cells and its ability to act as a free radical scavenger.

* *Corresponding author.* Department of Histology, Faculty of Medicine, Beni-Suef University, Beni-Suef, Kornish El-Nile, Borg El-Safa, Egypt. Tel.: +00201001916480; fax: +20822321352.
E-mail address: samraahussein@yahoo.com (S.H. Abdel-Kawi).

1.   Introduction

Fructose is a highly lipogenic sugar that has intense metabolic effects. Fructose becomes a major constituent of our modern diet. Fructose consumption has steadily increased over the past 30 years in parallel to the growth of the obesity/metabolic syndrome epidemic. Fructose and high-fructose corn syrup are ingredients in many commercially produced food products (Chen et al., 2004). Excess consumption of fructose is an important contributor to the metabolic syndrome (Ahangarpour et al., 2012). Metabolic syndrome is a well-established risk factor for diabetes, cardiovascular disease and mortality. Recently, studies have suggested that the metabolic syndrome may also contribute to the development of chronic kidney disease (Chen et al., 2004; Kurella et al., 2005). It has been hypothesized that fructose consumption in our diet may be among the factors that contribute to the epidemic of the metabolic syndrome and, consequently, to the epidemic of chronic renal disease (Chen et al., 2004; Elliott et al., 2002; Nakagawa et al., 2006). This hypothesis is supported by the preliminary evidence demonstrating that high fructose consumption induces kidney damages in both rats (Kizhner and Werman, 2002; Oudot et al., 2013) and mice (Aoyama et al., 2012).

Increase catabolism of fructose is associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation (Punitha et al., 2005) Furthermore, it has been postulated that increased catabolism of fructose can accelerate free radical production similar to glucose and impairs the free radical defense system leading to oxidative stress (HS Kumar and Anandan, 2007; Reddy et al., 2009).

Curcumin is a phenolic compound extracted from *Curcuma longa* rhizome commonly used in Asia as a spice, pigment and additive. In traditional medicine of India and China, curcumin is considered as a therapeutic agent used in several foods. Numerous studies have shown that curcumin has broad biological functions particularly antioxidant and anti-inflammatory. In fact, it has been established that curcumin is a bifunctional antioxidant; it exerts antioxidant activity in a direct and an indirect way by scavenging reactive oxygen species and inducing an antioxidant response, respectively (Trujillo et al., 2013).

Curcumin is considered to be a potent cancer chemopreventive agent plus it has a protective effect against oxidative damage (Aggarwal et al., 2005; Duvoix et al., 2005). A protective effect of curcumin has been previously shown on hepatic lipid peroxidation in mice and rats (Eybl et al., 2006).

2.   Materials and methods

2.1.   Chemicals

Fructose was obtained from Sigma Aldrich, Egypt.

Curcumin was obtained from Sigma Chemical Company, USA.

2.2.   Experimental animals

Eighty male albino rats, 16 weeks of age weighing average 150–200 grams were gathered for this experiment and housed in the laboratory of Biochemistry department, Faculty of Veterinary, Beni-Suef University. All rats were placed in a stable environment maintained at 22 ± 1 °C with 12-h light/day cycle. After an acclimatization period of 1 week, the rats were randomly assigned to 4 groups with twenty rats (n = 20) in each group:

Group I: control group was received 0.5 ml of corn oil via gavage for 5 weeks
Group II: fructose group was received 10% fructose in drinking water for 5 weeks (Gao et al., 2012; Wang et al., 2013) daily.
Group III: curcumin group was received curcumin at a dose of 200 mg/kg/day (Buyuklu et al., 2014) as a suspension in corn oil via gavage for 5 weeks.
Group IV: fructose and curcumin group was received both curcumin at a dose of 200 mg/kg/day as a suspension in corn oil via gavage and fructose 10% in drinking water daily for 5 weeks.

2.3.   Sampling

Sampling was performed 24 h after the last dose of fructose and curcumin.

2.4.   Blood sampling

Blood was collected from the medial canthus blood capillaries of the eye in dry centrifuge tubes. The tubes were placed in an inclined position for 5 min, allowing the blood to coagulate, and then placed in an incubator at 37 °C for 10 min. Centrifugation at $1000 \times g$ for 20 min was performed and clear sera were separated and kept in the deep freezer (–80 °C) till use according to the instruction of assay kits of each measured parameter.

2.5.   Specimen collection

At the time of sacrifice, all animals were killed under light anesthesia. Kidneys were dissected out and divided into 3 pieces (0.5 g each): the first part was kept in the deep freezer for oxidative, antioxidant parameters evaluation, the second part was kept for molecular parameters, and the third part was immediately fixed in 10% formol saline for 24 h for histological study.

2.6.   Tissue homogenates

After scarification of rats, kidneys were collected and rinsed with physiological saline for removing any clotted blood or blood cells. 0.5 g of kidney was homogenized in 5 ml of physiological saline by using homogenizer (Ortoalresa, Spain). The homogenates were centrifuged at $1000 \times g$ for 15 min. The supernatant was collected in Eppendorf tubes that were kept in the deep freezer (at –80 °C) for further biochemical investigations or according to the instructions of the biochemical assay kits.

2.7.   Measured parameters

2.7.1.   Serum biochemical parameters
Creatinine and urea concentrations in serum were determined colorimetrically as described by Bartles et al. (1972) and

Table 1 – Primer sequences used for RT-PCR.

Primer	Sequence	Code	Description
INOS	Forward: 5′-AAGAGTTCCCATCATTGCGT-3′ Reverse: 5′ –TCCTCAACCTGCTCCTCACT-3′	Gene ID: 18126 Accession number of Chromosome: NC_000077.6 Accession number of gene transcript: NM_010927.3	Nitric oxide synthase 2, inducible [Mus musculus(house mouse)]
HO1	Forward 5′- GGA AAG CAG TCA TGGTCA GTC A -3 Reverse 5′- CCC TTCCTG TGT CTT CCT TTG T -3	Gene ID: 15368 Accession number of Chromosome: NC_000074.6 Accession number of gene transcript: NM_010442.2	Heme oxygenase (decycling) 1 [Mus musculus (house mouse)]
β-actin	Forward 5′ GGGAAATCGTGCGTGACATT 3′ Reverse 5′- GCGGCAGTGGCCATCTC-3′	Gene ID: 11461 Accession number of Chromosome: NC_000071.6 Accession number of gene transcript: NM_007393.3	Actin, beta [Mus musculus (house mouse)]

Patton and Crouch (1977), respectively, using diagnostic kits (Biodiagnostics, Egypt).

2.7.2.   Oxidative/antioxidant parameters

The kidney homogenate was used for the estimation of reduced glutathione (GSH) according to the method described by Beutler et al. (1963), of glutathione reductase (GR) according to the method described by Long and Carson (1961), and of catalase according to the method described by (Aebi, 1984). Superoxide dismutase (SOD) according to the method described by Nishikimi et al. (1972) and malondialdehyde (MDA) according to the method described by Satoh (1978). DNA fragmentation was measured by the method described by Burton (1956).

2.7.3.   Molecular parameters

2.7.3.1. *Detection of HO 1 and INOS gene expression by real time-polymerase chain reaction (real time-PCR).* Total RNA was isolated from kidney tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer's instruction. The concentration of RNA was measured using a UV spectrophotometer.

2.7.3.2. *cDNA synthesis.* Five-microgram RNA was reverse transcribed using oligonucleotide (dT)18 primer (final concentration, 0.2 mM) and was denatured at 70 °C for 2 min. Denatured RNA was placed on ice and reverse transcription mixture containing 50 mM KCl, 50 mM Tris HCl (pH 8.3), 0.5 mM of deoxyribonucleotide triphosphate (dNTP), 3 mMMgCl2, 1 U/mL RNase inhibitor, and 200 units of moloney murine leukemia virus reverse transcriptase. The reaction tube was placed at 42 °C for 1 h, followed by heating at 92 °C to stop the reaction.

2.7.3.3. *Real-time quantitative polymerase chain reaction (PCR).* For real-time quantitative PCR, 5 μL of first-strand cDNA was used in a total volume of 25 μL, containing 12.5 μL 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer which shown in Table 1. PCR reactions consisting of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles) were performed on step one plus Real Time PCR system (Applied Biosystems). Data were ana-

lyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta actin genes, all these steps were described by Livak and Schmittgen (2001).

2.8.   *Table 1: primers of HO-1 and iNOS*

2.8.1.   Histological study

Specimens were processed to prepare 5-μm-thick paraffin sections for hematoxylin and eosin and for Masson's trichrome (Bancroft and Gamble, 2007) to verify the histological details.

2.8.2.   Immunohistochemical study

Sections of kidneys of all groups were stained with mouse monoclonal antibodies directly against rat TNF-α (diluted 1:20; BioLegend, San Diego, CA, USA), and α-smooth muscle actin (diluted 1:500; DakoCytomation, Glostrup, Denmark). Briefly, formalin fixed paraffin-embedded tissue sections were deparaffinized and endogenous peroxidase activity was blocked with 2% hydrogen peroxide in methanol for 40 min at room temperature. Sections were incubated with 5% fetal calf serum for 20 min at room temperature to block nonspecific binding, and then with primary antibodies overnight at 4 °C. On the next day, secondary biotinylated mouse antibodies were applied to mouse immunoglobulin at a dilution of 1:100 in fetal calf serum for 30 min at room temperature. Sections were incubated with streptavidin-biotin complex/horseradish peroxide at a dilution of 1:100 for 30 min at room temperature. After washing in phosphate-buffered saline (PBS) buffer with 0.05 M Tris buffer, the sections were stained using fresh 3.3 diaminobenzidine tetrahydrochloride as a chromogen with 0.03% hydrogen peroxide for 7 min. Finally, the sections were counter-stained with Mayers hematoxylin (Kierman, 2000).

2.8.3.   Statistical analysis

Statistical analysis was carried out using GraphPad Instate software (version 3, ISS, Rome, Italy), and one-way analysis of variance followed by Tukey–Kramer multiple comparison post

Table 2 – The changes in serum urea and creatinine concentrations.

Groups	Parameters	
	Serum urea concentration (mg/dL)	Serum creatinine concentration (mg/dL)
Control	29 ± 3^a	0.87 ± 0.06^a
Fructose group	87 ± 6.1^b	3.12 ± 0.28^b
Curcumin group	33.2 ± 0.86^a	0.99 ± 0.04^a
Fructose and curcumin group	39.76 ± 6.9^c	1.01 ± 0.032^c

The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.05$.

hoc test were used to establish significant differences between groups.

3. Results

3.1. Biochemical results

3.1.1. *Table 2: the changes in serum urea and creatinine concentrations*

This table indicated that fructose group showed a significant increase in serum urea and creatinine concentration as compared to that in the control group. Curcumin group showed no significant changes in serum urea and creatinine concentration as compared to that in the control group. Administration of curcumin and fructose showed a significant decrease in serum urea and creatinine concentration as compared to that in the fructose group.

Table 3 – The changes in Ho 1 and INOS mRNA expression.

Groups	Parameters	
	HO I	INOS
Control	0.08 ± 0.012^a	0.67 ± 0.03^a
Fructose group	0.98 ± 0.11^b	3.23 ± 0.17^b
Curcumin group	0.1 ± 0.06^a	0.86 ± 0.05^a
Fructose and curcumin group	0.099 ± 0.02^a	1.6 ± 0.03^c

The results are expressed as mean values of the relative expression level and standard errors. The different superscript letters indicate a significant difference at $P > 0.05$.

Table 5 – The changes of hepatic LPO and DNA fragmentation %.

Groups	Parameters	
	LPO (mg/g tissue)	DNA fragmentation %
Control	21.8 ± 1.04^a	19.3 ± 1.22^a
Fructose group	89.66 ± 4.94^b	67.33 ± 3.22^b
Curcumin group	28.14 ± 2.3^a	22.36 ± 1.97^a
Fructose and curcumin group	30.56 ± 1.31^c	33.31 ± 2.42^c

The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.05$.

3.1.2. *Table 3: the changes in Ho 1 and INOS mRNA expression*

Results in Table 3 showed a significant increase in HO 1 and INOS mRNA expression as compared to that in the control group. Curcumin group and fructose/curcumin group showed a significant decrease in HO1 and INOS mRNA expression as compared to that in the fructose group and a nonsignificant difference as compared to that in the control group.

3.1.3. *Table 4: the changes of hepatic GSH, GR, SOD and CAT*

The results showed a significant decrease of GSH concentration, GR, cat and SOD activities in fructose group as compared to those in the control group. Curcumin group showed a nonsignificant differences in GSH concentration, CAT and SOD activities as compared to those in the control group while there was a significant decrease in GR as compared to that in the control group. Fructose/curcumin group showed a significant increase in GSH concentration, GR, CAT and SOD activities as compared to those in the fructose group.

3.1.4. *Table 5: the changes of hepatic LPO and DNA fragmentation %*

The table showed a significant increase in LPO concentration and DNA fragmentation % in fructose group as compared to those in the control group. Curcumin group has a nonsignificant difference in LPO and DNA fragmentation % as compared to those in the control group. Fructose/curcumin group showed a significant decrease in LPO and DNA fragmentation as compared to that in the fructose group.

3.2. Histological results

3.2.1. *Hematoxylin and eosin stain*

In the control group (group I), H&E staining revealed a normal architecture in the cortex and medulla, with the normal

Table 4 – The changes of hepatic GSH, GR, SOD and CAT.

Groups	Parameters			
	GSH (mg//g tissue)	GR (U/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Control	35.7 ± 1.14^a	88.54 ± 1.33^a	50.09 ± 3.69^a	6.23 ± 1.2^a
Fructose group	9.76 ± 0.51^b	44.19 ± 1.84^b	10.02 ± 0.76^b	1.55 ± 0.09^b
Curcumin group	30.22 ± 0.75^a	79.19 ± 2.01^c	48.11 ± 3.7^a	5.9 ± 0.88^a
Fructose and curcumin group	28.98 ± 0.77^c	75.09 ± 1.87^c	41.1 ± 3.28^c	4.66 ± 1.25^c

The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.05$.
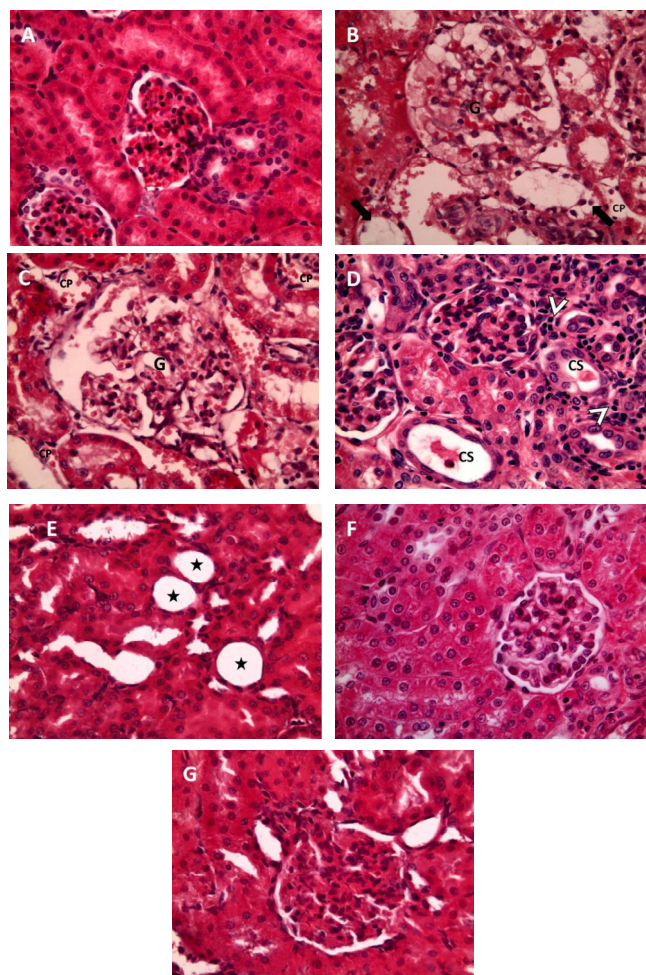
Fig. 1 – histopathological changes of different treated groups (H&E stain). Histological examination of the kidney with hematoxylin and eosin (H&E) staining in rats of group I (control group) (A), group II (fructose-fed) (B, C, D, E), group III (curcumin treated) (F) and group IV (curcumin and fructose treated) (G). Groups I and III (A, G) showed no morphological changes. Group II (B, C, D, E) showed degeneration of tubular epithelial cells with tubular dilatation (black arrows), peritubular dilated and congested blood capillaries (CP) with congested dilated glomerular capillaries (G). Inflammatory infiltration (arrow heads) and tubular cast formation (CS) also observed. The asterisk (*) indicates the presence of fatty infiltration. (Magnification: 400×).

Fig. 2 – Changes in collagen deposit in different groups by Masson's trichrome stain. Masson's trichrome-stained interstitial collagen deposit (Blue) in kidneys in rats of group I (control group) (A), group II (fructose-fed) (B), group III (curcumin treated) (F) and group IV (curcumin and fructose treated) (G). Group II (B) showed minimal peritubular (black arrow) and moderate intraglomerular deposits of collagen (white arrows), while groups I, III and IV (A, C, D) showed minimal deposits. (Magnification: 400×).

histological profile of group III animals was almost identical to that of the control group I (Fig. 1F).

3.2.2. Masson' trichrome

Groups I (control group), III (curcumin treated) and IV (fructose and curcumin treated) showed minimal deposits (Fig. 2A, C and D), while group II (fructose-fed) showed increased peritubular and intraglomerular collagen deposits and significantly increased compared with the control group ($P < 0.01$) (Fig. 2B) (Table 6).

3.2.3. Immunohistochemical stain of α-SMA

In the control group (group I), curcumin group (group III) and fructose and curcumin treated group (group IV) the expression of α-SMA was only observed on smooth muscle cells of the vessel wall in renal tubular interstitium (Fig. 3A, C and D). However, α-SMA was mainly expressed in the renal tubular epithelial cells in addition to the vessel wall in the fructose group and significantly increased compared with the control group ($P < 0.01$) (Fig. 3B) (Table 7).

appearance of the renal corpuscles and tubules (Fig. 1A). The majority of cells of the proximal and distal convoluted tubules and the collecting tubules were normal. In the fructose group (group II), the following are shown: degeneration of tubular epithelial cells with tubular dilatation, peritubular dilated and congested blood capillaries and congested dilated glomerular capillaries, inflammatory infiltration, tubular cast formation and fatty infiltration (Fig. 1B–E). In curcumin and fructose treated group (group IV), it showed a picture nearly similar to the control group kidney except some dilated tubules (Fig. 1G). The
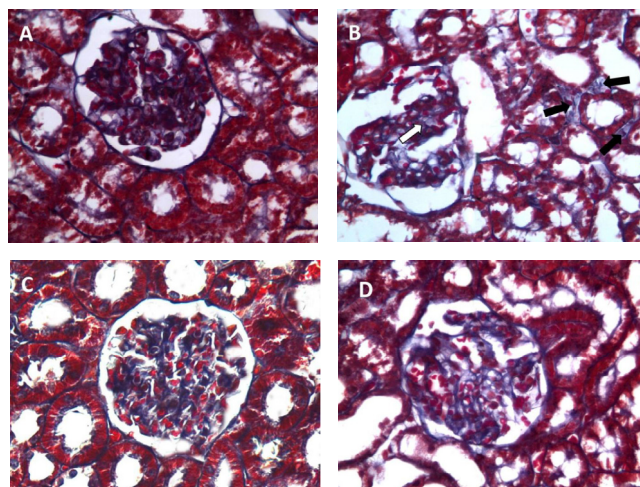
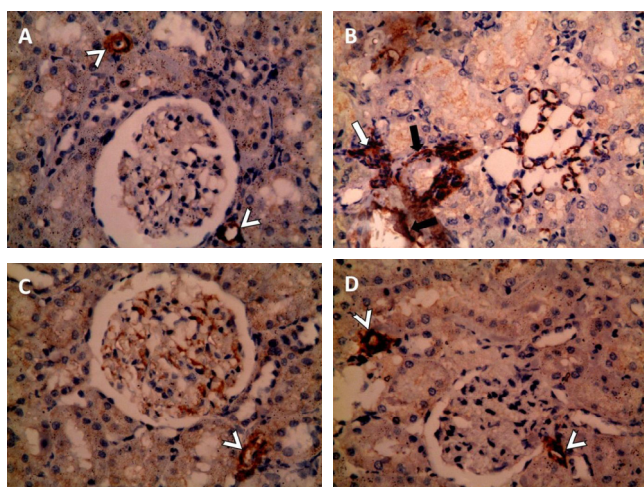| Table 6 – Area % of collagen fibers distribution.	
Groups	Parameter: collagen area percent
Control	0.5 ± 0.34^a
Fructose group	3.9 ± 0.48^b
Curcumin group	0.7 ± 0.45^a
Fructose and curcumin group	0.75 ± 0.48^a
The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.01$.	

Fig. 3 – Immunohistochemistry for α-smooth muscle actin (α-SMA) in different groups. Immunohistochemistry for α-smooth muscle actin (α-SMA) in rats of group I (control group) (A), group II (fructose-fed) (B), group III (curcumin treated) (C) and group IV (curcumin and fructose treated) (D). Positive signal is shown by brown color. α-SMA was expressed in vascular smooth muscle of arteriole (arrowheads) In groups I, III and IV (A, C, D), and was negative for tubular epithelial cells. However, some tubular cells expressed α-SMA in cytoplasm of renal tubules in group II (B) (black arrows) and interstitial area (white arrow). (Magnification: 400×)

3.2.4. Immunohistochemical stain of TNF-α

In the control group (group I), curcumin group (group III), and curcumin and fructose treated group (group IV), immunohistochemical staining revealed weak reaction (Fig. 4A, C and D) while in the fructose group it showed strong reaction and significantly increased compared with the control group ($P < 0.01$) (Fig. 4B) (Table 8).

4.   Discussion

Fructose is widely used as a food ingredient and has potential to increase oxidative stress. Our current study showed that fructose administration in fructose group showed a signifi-
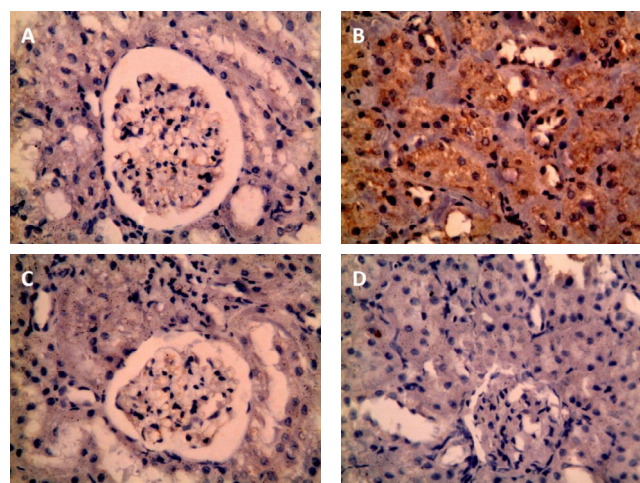

Fig. 4 – Immunohistochemistry for tumor necrosis factor alpha (TNF- α) in different groups. Immunohistochemistry for tumor necrosis factor alpha (TNF- α) in rats of group I (control group) (A), group II (fructose-fed) (B), group III (curcumin treated) (C) and group IV (curcumin and fructose treated) (D). Positive signal is shown by brown color. Groups I, III and IV (A, C, D), showed weak reaction to TNF, while group II (B), showed strong positive reaction in tubular epithelium. (Magnification: 400×)

cant increase of serum urea and creatinine concentration as compared to control group. These findings come in agreement with the data that suggest that the increase of fructose consumption is one factor that contributes to the occurrence of metabolic syndrome and consequently to the rise in incidence of chronic renal disease (Nakagawa et al., 2005). From the current results, it was so clear that fructose caused a significant decrease in GSH concentration, GR, CAT and SOD activities and these findings could be attributed to the ability of fructose to accelerate reactive oxygen species (ROS) production. ROS can reduce the activity of antioxidant enzymes such as CAT, GR and SOD (Datta et al., 2000) and our findings come in agreement with those of Rajasekar et al. (2008). The increase in ROS and the decrease of GR activity lead to depletion of GSH concentration. Furthermore, fructose caused significant increase in lipid peroxidation and this agreed with results showed by Pasko et al. (2010). The increase in lipid peroxidation could be attributed to the ability of fructose to

Table 7 – Area % of tumor necrosis factor alpha (TNF-α) distribution.	
Groups	Parameter: TNF-α area percent
Control	0.52 ± 0.27^a
Fructose group	14.23 ± 1.65^b
Curcumin group	0.67 ± 0.26^a
Fructose and curcumin group	8.23 ± 0.69^a

The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.01$.
[a] Significant compared to control group.
[b] Significant compared to fructose group.

Table 8 – Area % of alpha smooth muscle actin distribution.	
Groups	Parameter: α-SMA area percent
Control	0.35 ± 0.29^a
Fructose group	1.34 ± 0.52^b
Curcumin group	0.39 ± 0.23^a
Fructose and curcumin group	0.55 ± 0.33^a

The results are expressed as mean values and standard errors. The different superscript letters indicate a significant difference at $P > 0.01$.
[a] Significant compared to control group.
[b] Significant compared to fructose group.

reduce cellular energy and increase thesusceptibility of cells to lipid peroxidation during its catabolism (Rajasekar et al., 2005). Our results showed that fructose caused a significant increase in pro-inflammatory cytokines including INOS expression, which consequently increases the formation of nitric oxide (NO). The increased levels of NO can react with O^- leading to the formation of the peroxynitrite anion ($ONOO^-$), which oxidizes sulfhydryl groups and generates OH (Kaur et al., 2006).

HO-1 is an endogenous, cytoprotective enzyme that is upregulated under conditions of oxidative stress (Choi et al., 2011). The transcription factor, nuclear factor erythroid 2-related factor (Nrf) 2, plays an important role in transcriptional regulation of HO-1 (Ishii et al., 2000). Nrf-2 breaks free from Kelchlike ECH-associated protein1 (Keap1) and then it will be translocated from the cytosol to the nucleus, where it sequentially binds with the antioxidant response element (ARE). This binding develops a cis-acting enhancer sequence that mediates the transcriptional activation of genes in response to oxidative stress (Itoh et al., 2003). These changes lead to a cytoprotective response, which is characterized by an upregulation of antioxidant enzymes such as HO-1 and decreased sensitivity to oxidative stress damage (Dhakshina and Jaiswal, 2001).

The use of natural dietary antioxidants to prevent oxidative and chromosomal damage is currently eliciting considerable interest. Curcumin is used worldwide for medicinal, as well as food, purposes. Curcumin exhibits potent antioxidant, antitumor and anticancer properties (Sharma et al., 2005). The current study showed that administration of curcumin with fructose modulated the fructose induced renal functions alterations, whereas curcumin group showed a significant decrease of urea and creatinine concentration as compared to the fructose group. Moreover, curcumin group showed a significant increase of GSH concentration and GR activity, and decrease of CAT, SOD activities, LPO, DNA fragmentation % and INOS mRNA expression as compared to fructose group. Curcumin has a potent antioxidant activity (Venkatesan et al., 2000).

One of the most acceptable hypotheses of the antioxidant activity of curcumin is its ability to scavenge reactive oxygen and nitrogen free radicals (Khopde et al., 1999). It is a diferuloyl methane having two O-methoxy phenolic OH groups attached to α, β-unsaturated β-diketone (heptadiene-dione) moiety. The free radical scavenging activity of curcumin can arise either from the phenolic OH group or from the CH2 group of the β-diketone moiety (Priyadarsini et al., 2003).

Administration of curcumin caused a significant decrease of HO-1 mRNA expression. This finding is contradicting with those reported by Balogun et al. (2003) as they have found that HO-1 mRNA expression significantly increased with curcumin administration.

HO-1 is a ubiquitous and redox-sensitive inducible stress protein (Motterlini et al., 2002). HO-1 is regulated by NRF2 in response to oxidative stress (Choi et al., 2011; Ishii et al., 2000). The decrease of HO -1 mRNA expression could be explained due to free radical scavenger effect of curcumin, which diminishes the oxidative stress action of fructose, leading to inhibition of ROS formation and so prevent the separation of NRF2 from Keap1 in cytoplasm and entrance to the nucleus (Itoh et al., 2003). Persistence of NrF2 in the cytosol causes no alterations or changes in the cellular antioxidant enzymes and defense

mechanisms such as HO-1 activity in curcumin and fructose treated group and control group.

The present results demonstrated that fructose induced kidney remodeling as characterized by slough and dilatation of tubular epithelial cells in the cortex, cast formation and interstitial collagen deposit in rats. Supplementing with curcumin (200 mg/kg) attenuated the tubular damage and interstitial fibrosis in the kidneys, suggesting that curcumin posses protective effect against fructose induced renal injury. The present study showed lipid accumulation within kidney of fructose fed group (group II). Lipid accumulation in nonadipose tissue has been increasingly recognized to contribute to organ injury through a process termed lipotoxicity. There is substantial evidence that excess renal lipids can cause injury in animal models of metabolic disease (obesity, metabolic syndrome and diabetes mellitus), chronic kidney disease, acute renal injury of several etiologies, as well as aging. Lipotoxic cellular dysfunction and injury occur through several mechanisms such as release of proinflammatory and profibrotic factors (Bobulescu, 2010). There are many evidences that fructose consumption may induce excessive lipid accumulation especially in the liver cell (Tappy and Lê, 2010). Curcumin treatment decreased renal lipid content in fructose fed rats.

Renal inflammation is known to play an important role in the initiation and progression of tubulointerstitial injury in the kidneys (Eardley and Cockwell, 2005). Fructose has been demonstrated to induce the production of macrophage associated MCP-1 in kidney proximal tubular cells (Cirillo et al., 2009) that promotes monocyte and macrophage migration and activation. The activated macrophages produce numerous proinflammatory cytokines such as TNF-α (Galkina and Ley, 2006), which have been shown to mediate inflammation in several models of renal injury, including tubulointerstitial injury (Guo et al., 2001). The present study found that curcumin was able to suppress fructose-induced overexpression of TNF-α in the kidneys of curcumin treated group (group IV). These findings are consistent with the attenuation of tubular injury. Thus the renoprotective effect of curcumin supplement is associated with suppression of renal overexpression of macrophage-associated proinflammatory cytokines. Oudot et al. (2013) showed the same findings but by using salt restriction and betaine respectively, which evidently improved renal tubular injury, suppressed the expressions of renal TNF-α.

The present study showed a significant increase in the interstitial collagen deposition in fructose group (group II) as demonstrated with Masson's trichrome. Palanisamy and Venkataraman (2011) found that collagen fibers increased in fructose fed rats. Palanisamy et al. (2011) found that proliferation of connective tissue was evident from increased collagen deposition in perivascular and intraglomerular regions. Aoyama et al. (2012) also showed that rats fed with fructose displayed tubulointerstitial fibrosis localized on the outer cortex of the kidney. The activated macrophages produce proinflammatory cytokines, such as IL-6, PAI-1 and TGF-β1 (Galkina and Ley, 2006), which may enhance renal fibrosis. These results were reversed in curcumin treated group (group IV), which showed minimal collagen deposition.

In our study, fructose is shown to produce an increase the expression of α-SMA in the kidney, which coincides with increased collagen fibers as shown with Masson's trichrome.

Similar results were obtained by Palanisamy et al. (2011) and Palanisamy and Venkataraman (2011) who showed increased α-SMA expression in fructose-fed rat kidney. Marotta et al. (2012) also found that fructose-fed rats showed significant increase of α-SMA in the kidney. These results were reversed in curcumin treated group (group IV), which showed minimal expression of α-SMA in the kidney.

Further investigation is needed to broaden our collective knowledge regarding the details surrounding the therapeutic actions of curcumin, specifically the manner in which they suppress proinflammatory cytokines.

5.   Conclusion

Our present results demonstrate that supplement with curcumin attenuate fructose-induced renal injury in rats by suppressing renal overexpression of proinflammatory cytokines. Our findings provide evidence supporting benefit of curcumin supplement for the metabolic syndrome-associated kidney injury.

Acknowledgments

REFERENCES

Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.

Aggarwal BB, Kumar A, Aggarwal MS, Shishodia S. Curcumin derived from turmeric (*Curcuma longa*): a spice for all seasons. In: Bagchi D, Preuss HG, editors. Phytopharmaceuticals in cancer chemoprevention. Boca Raton: CRC Press Inc.; 2005. p. 349–87.

Ahangarpour A, Mohammadian M, Dianat M. Antidiabetic effect of hydroalcholic urticadioica leaf extract in male rats with fructose-induced insulin resistance. Iran J Med Sci 2012;37:181–6.

Aoyama M, Isshiki K, Kume S, Chin-Kanasaki M, Araki H, Araki SI, et al. Fructose induces tubulointerstitial injury in the kidney of mice. Biochem Biophys Res Commun 2012;419:244–9.

Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, et al. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. Biochem J 2003;371:887–95.

Bancroft JD, Gamble M. 2007. Theory and practice of histological techniques. 6th ed. London: Churchill Livingstone.

Bartles H, Bohmer M, Heieri C. Serum creatinine determination without protein precipitation. Clin Chim Acta 1972;37:193–7.

Beutler E, Duron O, Kelly BM. Improved method for determination of glutathione. J Lab Clin Med 1963;61(5): 882–8.

Bobulescu IA. Renal lipid metabolism and lipotoxicity. Curr Opin Nephrol Hypertens 2010;19:393–402.

Burton KA. Study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956;62:315–27.

Buyuklu M, Kandemir FM, Ozkaraca M, Set T, Bakirci EM, Topal E. Protective effect of curcumin against contrast induced nephropathy in rat kidney: what is happening to oxidative stress, inflammation, autophagy and apoptosis? Eur Rev Med Pharmacol Sci 2014;18(4):461–70.

Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, et al. The metabolic syndrome and chronic kidney disease in US adults. Ann Intern Med 2004;140:167–74.

Choi JH, Kang JW, Kim DW, Sung YK, Lee SM. Protective effects of Mg-CUD against D-galactosamine-induced hepatotoxicity in rats. Eur J Pharmacol 2011;657:138–43.

Cirillo P, Gersch MS, Mu W, Scherer PM, Kim KM, Gesualdo L, et al. Ketohexokinase-dependent metabolism of fructose induces proinflammatory mediators in proximal tubular cells. J Am Soc Nephrol 2009;20:545–53.

Datta K, Sinha S, Chattopadhyay P. Reactive oxygen species in health and diseases. Natl Med J India 2000;13:304–10.

Dhakshina S, Jaiswal AK. Functional characterization and role of INrf2 in antioxidant response element-mediated expression an antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. Oncogene 2001;20:3906–17.

Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, et al. Chemopreventive and therapeutic effects of curcumin. Cancer Lett 2005;223:181–90.

Eardley KS, Cockwell P. Macrophages and progressive tubulointerstitial disease. Kidney Int 2005;68:437–55.

Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr 2002;76:911–22.

Eybl V, Kotyzova D, Leseticky L, Bludovska M, Koutensky J. The influence of curcumin and manganese complex of curcumin on cadmium-induced oxidative damage and trace elements status in tissues of mice. J Appl Toxicol 2006;26(3):207–12.

Galkina E, Ley K. Leukocyte recruitment and vascular injury in diabetic nephropathy. J Am Soc Nephrol 2006;17:368–77.

Gao H, Guan T, Li C, Zuo G, Yamahara J, Wang J, et al. Treatment with ginger ameliorates fructose- induced fatty liver and hypertriglyceridemia in rats: modulation of the hepatic carbohydrate response element binding protein-mediated pathway. Evid Based Complement Alternat Med 2012;14:570948.

Guo G, Morrissey J, McCracken R, Tolley T, Liapis H, Klahr S. Contributions of angiotensin II and tumor necrosis factor-alpha to the development of renal fibrosis. Am J Physiol Renal Physiol 2001;280:777–85.

HS Kumar S, Anandan R. Biochemical studies on the cardioprotective effect of glutamine on tissue antioxidant defense system in isoprenaline-induced myocardial infarction in rats. J Clin Biochem Nutr 2007;40:49–55.

Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, et al. Transcriptional factor Nrf2 coordinately regulates a group of oxidative stress inducible genes in macrophages. J Biol Chem 2000;275:16023–9.

Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, Yamamoto M. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells 2003;8(4):379–91.

Kaur G, Tirkey N, Chopra K. Beneficial effect of hesperodin on lipopolysaccharide-induced Hepatotoxicity. Toxicology 2006;226:152–60.

Khopde SM, Priyadarsini KI, Venkatesan P, Rao MNA. Free radical scavenging ability and antioxidant efficiency of curcumin and its substituted analogue. Biophys Chem 1999;80:85–91.

Kierman JA. Histological and histochemical methods: theory and practice. 3rd ed. Oxford: Butterworth-Heinemann; 2000.

Kizhner T, Werman MJ. Long-term fructose intake: biochemical consequences and altered renal histology in the male rat. Metabolism 2002;51:1538–47.

Kurella M, Lo JC, Chertow GM. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. J Am Soc Nephrol 2005;16:2134–40.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 22DDCT. Methods 2001;25:402–8.

Long WK, Carson PE. Increased erythrocyte glutathione reductase activity in diabetes mellitus. Bio. Biophys Res Commun 1961;5(5):394–9.

Marotta F, Kumari A, Catanzaro R, Solimene U, Jain S, Minelli E, et al. A phytochemical approach to experimental metabolic syndrome-associated renal damage and oxidative stress. Rejuvenation Res 2012;15(2):153–6.

Motterlini R, Green CJ, Foresti R. Regulation of heme oxygenase-1 by redox signals involving nitric oxide. Antiox Redox Signal 2002;4:615–24.

Nakagawa T, Tuttle KR, Short RA, Johnson RJ. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. Nat Clin Pract Nephrol 2005;1:80–6.

Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, et al. A causal role for uric acid in fructose-induced metabolic syndrome. Am J Physiol Renal Physiol 2006;290:625–31.

Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Bioph Res Common 1972;46:849–54.

Oudot C, Lajoix AD, Jover B, Rugale C. Dietary sodium restriction prevents kidney damage in high fructose-fed rats. Kidney Int 2013;83:674–83.

Palanisamy N, Venkataraman AC. Soy protein prevents renal damage in a fructose-induced model of metabolic syndrome via inhibition of NF-κB in male rats. Pediatr Nephrol 2011;26(10):1809–21.

Palanisamy N, Kannappan S, Anuradha CV. Genistein modulates NF-κB-associated renal inflammation, fibrosis and podocyte abnormalities in fructose-fed rats. Eur J Pharmacol 2011;667(1–3):355–64.

Pasko P, Barton H, Zagrodski P, Izewska A, Krosniak M, Gawlik M. Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods Hum Nutr 2010;65:146–51.

Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem 1977;49:464–9.

Priyadarsini KI, Maity DK, Naik GH, Kumar MS, Unnikrishnan MK, Satav JG, et al. Role of phenolic OH and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radic Biol Med 2003;35(5):475–84.

Punitha IS, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evid Based Complement Alternat Med 2005;2:375–81.

Rajasekar P, Ravichandran MK, Anuradha CV. Intraperitoneal L-carnitine regulates lipid metabolism and reduces oxidative stress in fructose-induced hyperlipidemic rats. Diabetol Croat 2005;34:87–94.

Rajasekar P, Viswanathan P, Anuradha CV. Renoprotective action of L-carnitine in fructose-induced metabolic syndrome. Diab Obes Metab 2008;10:171–80.

Reddy SS, Ramatholisamma P, Karuna R, Saralakumari D. Preventive effect of Tinospora cordifolia against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. Food Chem Toxicol 2009;47:2224–9.

Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978;90:37–43.

Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer 2005;41:1955–68.

Tappy L, Lê KA. Metabolic effects of fructose and the world wide increase in obesity. Phys Rev 2010;14:23–46.

Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol 2013;1(1):448–56.

Venkatesan N, Punithavathi D, Arumugam V. Curcumin prevents adriamycin nephrotoxicity in rats. Br J Pharmacol 2000;129:231–4.

Wang J, Gao H, Ke D, Zuo G, Yang Y, Yamahara J, et al. Improvement of liquid fructose-induced adipose tissue insulin resistance by ginger treatment in rats is associated with suppression of adipose macrophage-related pro-inflammatory cytokines. Evid Based Complement Alternat Med 2013;14:590376.