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Curcumin reduced diabetic nephropathy in a rat model

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ABSTRACT. This study aimed to examine the effects of curcumin, a phytochemical antioxidant, on the treatment and care of diabetic nephropathy and to contribute to alternative treatment strategies for diabetes. Male Wistar albino rats (8–10 weeks old) were divided into five groups of seven. Experimental diabetes was induced in all rats except for those in Group 1 (placebo group) by administration of 110 mg/kg nicotinamide, followed by intraperitoneal administration (after 15 min) of 55 mg/kg streptozotocin. Groups 1, 3, 4, and 5 were treated with 0.1 ml normal saline (0.9% NaCl), 150mg/kg/day metformin, 10 mg/kg/day glycazide (diamicron), and 200 mg/kg/day curcumin, respectively. Group 2 did not receive any treatment. Kidney tissues of rats were collected for histopathological examination. There were no significant differences in the kidney dimensions of the rats. In the histopathological evaluation of kidney tissues with diabetic nephropathy, glomerular congestion and destruction were observed. Rats treated with

curcumin had significantly less kidney damage, based on histopathological analysis, than those treated with the diabetes drugs. We conclude that curcumin has protective effects in kidneys due to its antioxidant properties. It has potential for use, in addition to antidiabetic drugs, for diabetes treatment.

Key words: Antioxidant effect; Curcumin; Diabetic nephropathy; Phytochemical agent

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine system-related diseases and is characterized by hyperglycemia due to impaired insulin secretion mechanisms (Kurt et al., 2004; World Health Organization, 2016). Oxidative stress has been reported to play a crucial role in the pathophysiology of several immunodeficiency disorders, cancers, and chronic diseases, including diabetes (Aydın et al., 2012). In addition, an increase in blood glucose levels further increases oxidative stress and causes secondary complications. Therefore, the literature reports the use of antioxidants in the treatment of diabetes (Özcan et al., 2015). Consequently, several plants have antioxidant properties. Previous studies have reported that the interstitial area of the kidneys diminished, and creatinine levels improved after pretreatment with curcumin (Aggarwal et al., 2007; Shoskes et al., 2000). Another study showed that curcumin plays a role in the removal of superoxide anions, nitrogen dioxide radicals, and hydroxyl radicals (Reddy and Lokesh, 1994).

This study aimed to investigate the possible protective and therapeutic effects of curcumin on the treatment and care of diabetic nephropathy as a potent antioxidant agent and to determine whether it is superior to other antidiabetic drugs.

MATERIAL AND METHODS

Study design

Male Wistar rats, weighing 250-300 g, were used in this study. The animals were placed in stainless steel cages at 22 ± 2°C under a 12-12 h light-dark cycle and given a normal diet and tap water without restriction. The procedures were governed by the Directive 2010/63/EU of the European Parliament and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Ethics approval was obtained from the Animal Experiments Local Ethics Committee of the Dicle University.

Streptozotocin prepared in citrate buffer in animals is a method applied to create experimental diabetes by destroying the β cells of the pancreas and stopping insulin production and secretion. In doing so, streptozotocin has been shown to cause alkylation of DNA bases. When this occurs, since nicotinamide is used for the repair of DNA damage, the stores are depleted, and a deficiency develops in the body. To prevent this, nicotinamide is given with it. (Irer and Alper, 2004)

The rats were divided into five groups, with seven rats in each group. Group 1 was considered a placebo group that received only 0.1 ml normal saline (0.9% NaCl). A single dose

of nicotinamide (110 mg/kg) was administered to the remaining four groups to induce experimental diabetes. After 15 min, a single dose of 55 mg/kg streptozotocin solution prepared in citrate buffer was administered into the abdominal cavity. After 72 h, the rats were weighed. Afterwards, fasting blood glucose was measured in a blood sample taken from the tail vein of the rats. Those with fasting blood glucose levels of 14 mmol/dL = 250 mg/dL and above were included in the diabetic groups.

After the rats developed diabetes, they received different antidiabetic drugs: the 3rd group received 150mg/kg/day metformin, the 4th group received 10 mg/kg/day glycazide (Diamicron), and the 5th group received 200 mg/kg/day curcumin via gastric gavage for 8 weeks. Group 2 did not receive any treatment.

Weekly blood glucose measurement and weight monitoring were performed from the tail blood of the rats. At the end of eight weeks, the rats were sacrificed by performing cardiac puncture under mild ketamine anesthesia after 12 h of fasting, and the abdomen was opened.

Weekly monitoring was performed for fasting blood glucose levels (tail vein) and weight. At the end of 8 weeks, the rats were sacrificed by cardiac puncture under light ketamine anesthesia after 12 h of fasting, and the abdomen was opened. The kidney tissues of the rats were collected and stored in 10% formaldehyde solution for tissue fixation. Tissue fixation and follow-up were performed on the tissues whose fixation process was completed using a Thermo Scientific brand tissue tracking device. Paraffin blocks were embedded in the same brand of tissue embedding device. Tissue sections of 5 µm thickness were taken from the obtained paraffin blocks on the slide in a Thermo Scientific microtome device and stained with Hematoxylin and Eosin staining in the same brand staining device. Microscopic diagnosis and evaluation were performed under Nikon brand light microscope.

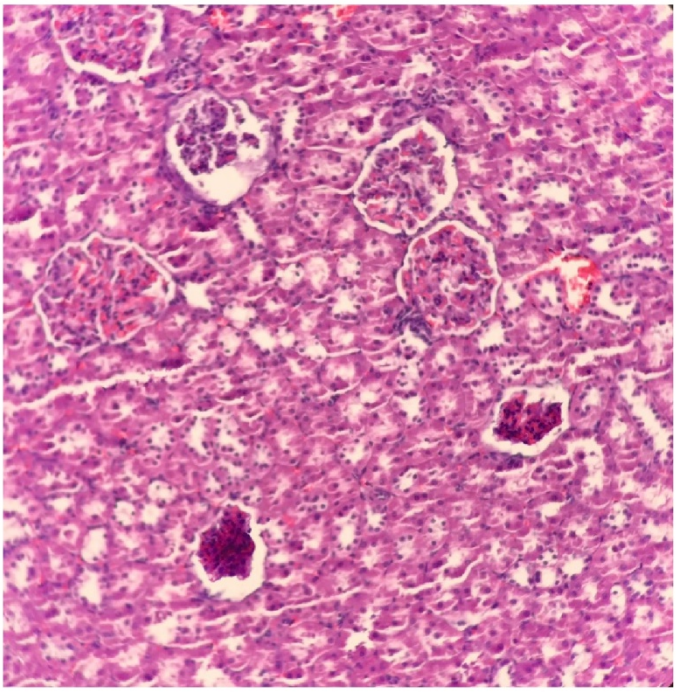

Figure 1. Damaged glomerular structures with and signs of congestion in histopathological sections of kidney tissue (Hematoxylin and Eosin staining ×100)

Histopathological examination was performed using Hematoxylin and Eosin staining (Figure 1) and observed under a light microscope (Figure 2).

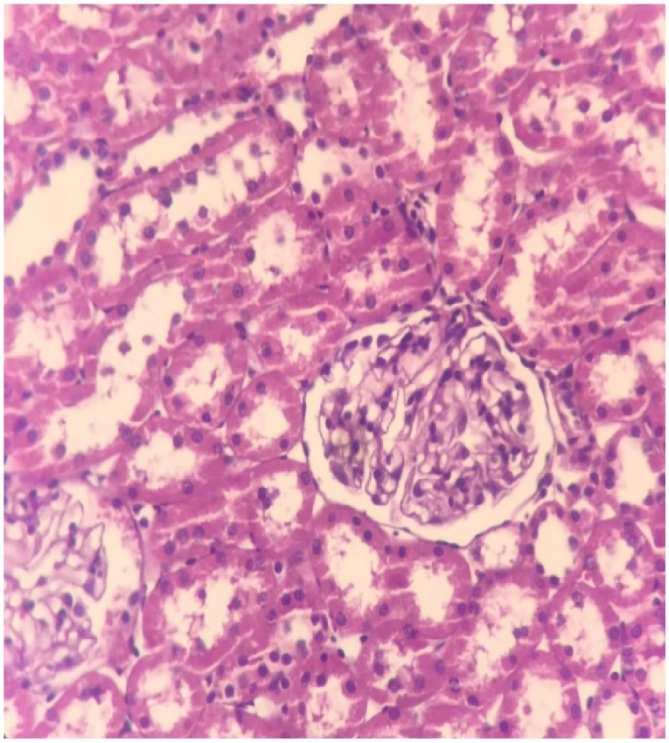

Figure 2. Close view of normal glomerular structure observed in the histopathological sections of rat kidney tissue after curcumin treatment (Hematoxylin and Eosin staining ×200)

Statistical analysis

The rat tissues were examined for the presence or absence of congestion in some vascular structures, which formed glomerular ball-like structures. The statistical model was based on the presence or absence of congestion. The Kruskal–Wallis test was used to distinguish differences in the glomerular damage (GD) scores of the rats among the groups. The Mann–Whitney U test was used to identify between-group differences in glomerular damage and GH scores of the groups. Differences were considered significant at $P < 0.05$. The results were investigated using Statistics Package for Social Sciences for Windows®22.0 (IBM Corporation, Chicago, Illinois) software.

RESULTS

The number of damaged glomeruli observed on a cross-section isolated from each kidney tissue was counted, and the total value was statistically evaluated. The results are presented in Tables 1-3. As advanced glomerulosclerosis was not observed, it was not statistically evaluated.

The arithmetic means, standard deviations, and minimum and maximum values for GD of the groups included in the study are shown in Table 1.

Table 1. Descriptive statistics of glomerular damage and congestion of the rat groups included in the study.

		Group 1	Group 2	Group 3	Group 4	Group 5
Glomerular Damage	N	7	7	7	7	7
	Arithmetic mean	7.571	12.142	13.000	10	3.857
	Standard Deviation	4.429	6.669	3.742	5.568	3.185
	Minimum Value	2	5	8	3	0
	Maximum Value	16	23	19	17	10
Congestion		present	present	present	present	absent

Figure 3 presents the GD scores of the groups. The highest GD scores were observed in Group 2, which was not subjected to any treatment. Group 5, which was treated with curcumin, had the lowest GD score.

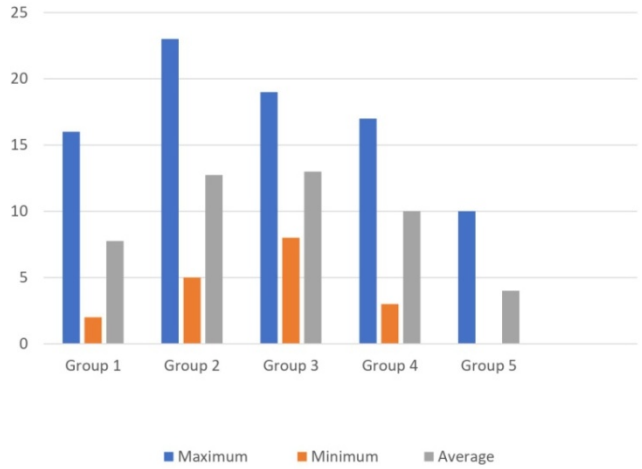

Figure 3. Descriptive statistics of the groups regarding glomerular damage.

Kruskal-Wallis test showed that there was a significant difference in the GD scores of the rats among the groups (Table 2).

Table 2. Kruskal–Wallis test results showing the intergroup differences in glomerular damage.

	Group	N	Mean rank	Chi-Square	Sd	p
Glomerular Damage	Group 1	7	15.14	13.222	4	0.010
	Group 2	7	22.57			
	Group 3	7	25.50			
	Group 4	7	19.29			
	Group 5	7	7.5			
	Total	35				

In addition, the Mann–Whitney U test showed a statistically significant difference in the GD scores of rats between Groups 1 and 3 (U = 7, z = −2.243, P < 0.05), Groups 2 and 5 (U = 4, z = −2.631, P < 0.05), Groups 3 and 5 (U = 1.500, z = −2.2949, P < 0.05), and Groups 4 and 5 (U = 7, z = −2.243, P < 0.05; Figure 4). The GD scores of the rats in Group 3 were significantly higher than those in Group 1, and the GD scores of Groups 2, 3, and 4 were significantly higher than those of Group 5.

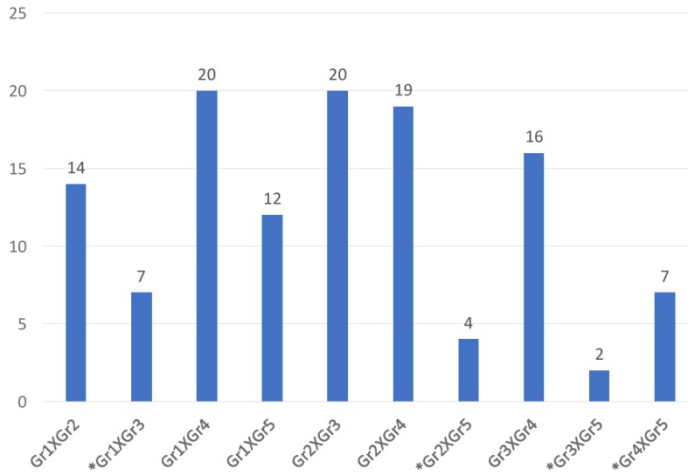

Figure 4. Mann -Whitney U test results comparing the GH scores of the groups. (*P < 0.05)

DISCUSSION

In this study, it was found that nephropathic damage was reduced by curcumin treatment in experimental diabetic rats, and better results were obtained compared with other antidiabetic drugs such as Metformin and Diamicron. These drugs have limited efficacy in preventing or reducing the development of oxidative damage. However, curcumin was found to have a useful role in preventing and reducing this damage. Consequently, curcumin was found to provide an additional benefit or be an alternative to antidiabetic drugs for the treatment of diabetes.

Reactive oxygen radicals (RORs) are heterogeneous molecules produced by mature myeloid cells during the innate immune response and play a role in normal intercellular signaling. When activated, phagocytes produce sufficiently high levels of ROR to kill bacteria. The disruption of glucose metabolism in diabetes triggers the production of superoxide radicals in the mitochondria (Hamamcioğlu, 2017). This has a crucial impact on cardiac and vascular complications due to diabetes (Johansen et al., 2005). In addition, excessive production of nicotinamide adenine dinucleotide phosphate hydrogen oxidase, which is the primary source of ROR, increases oxidative stress and complications due to diabetes (Nishikawa et al., 2000).

Baynes and Thorpe (1999) emphasized that tissue damage resulting from metabolic stress, hypoxia, and ischemia-reperfusion due to disturbances in energy metabolism increases free radical production and changes the antioxidant defence system. Moreover,

this condition has been reported to cause damage to the beta cells of the pancreas due to hyperglycemia.

According to the experimental and clinical results of a study investigating the role of oxidative stress during the development of diabetic nephropathy, increased levels of lipid peroxide and 8-hydroxydeoxyguanosine in the kidneys of experimental diabetic rats with albuminuria indicate oxidative tissue damage (Drews et al., 2010). This study also reported that increased glucose levels further stimulate damage due to diabetic nephropathy and induce mRNA release from transforming growth factor-beta 1 and fibronectin in glomerular mesangial cells.

Several studies have indicated the role of curcumin in vascular disorders caused by diabetes. For example, in an experimental study by Bulboaca et al. (2019), vascular disorders caused by diabetes improved with curcumin treatment. In another experimental study, curcumin had a stabilizing effect on protein structure (Belviranli et al., 2012).

In another study, it was shown that the antioxidant and radical scavenging effect of curcumin was formed through the lineloic acid system. (Ak and Gulcin, 2008).

In this study, Wistar albino rats were divided into normally fed placebo control, untreated diabetic, metformin-treated diabetic, diamicron-treated diabetic, and curcumin-treated diabetic groups. Then, data related to nephropathy, which might have developed due to diabetes, were compared among these groups to evaluate the antioxidant effects of curcumin. The histopathological examination in our study revealed mild signs of GD and congestion in the kidneys of placebo control rats. Although these changes were similar to those observed in the kidneys of the rats in the groups that developed diabetes and received antidiabetic drugs, they were significantly less abundant. In addition, there were significantly fewer nephropathic findings in the group treated with curcumin than in the placebo group ($P < 0.05$). Stress factors in the placebo group may indicate that GD occurred because of previous food intake. Moreover, the lower GD in the curcumin-treated group showed that curcumin therapy may have a protective effect against such factors. Larger studies are warranted to validate this finding.

In Group 2 experimental diabetic rats that did not receive any treatment, GD in renal nephrons due to oxidative stress caused by increased blood glucose levels were higher than those in the other groups. This observation is important in terms of demonstrating the effect of hyperglycemia on GD. In addition, in this study, nephropathic findings in the experimental diabetic rats in Group 5 after curcumin treatment were compared with those in the experimental diabetic rats in Group 2 without any treatment. The curcumin-treated group developed significantly fewer nephropathic findings than the no-treatment group ($P < 0.05$) (See Figure 3). These results demonstrate the therapeutic effects of curcumin.

Metformin and diamicron drugs are used in the treatment of diabetes because of their hyperglycemia-reducing effects. It is known that these drugs prevent oxidative damage caused by hyperglycemia by regulating or lowering blood glucose levels. Blood glucose levels vary due to stress factors, insufficient insulin secretion, and decreased glucose uptake into cells. As changes in blood glucose levels cause disturbances in daily life, antidiabetic drugs have limited efficacy in preventing or reducing the development of oxidative damage. The nephropathic changes observed in the kidneys of rats receiving antidiabetic agents were examined and compared with those observed in the kidneys of rats receiving curcumin treatment. The nephropathic effects observed in curcumin-treated rats were significantly lower than those in rats treated with antidiabetic agents ($P < 0.05$). These results showed

that curcumin has a better therapeutic effect than antidiabetic drugs, supporting the hypothesis of our study.

Many studies have suggested the positive effects of curcumin in the treatment of diabetes. For example, Lu et al. (2017) reported that the effects of curcumin in rats with diabetic nephropathy might be due to its anti-inflammatory properties, resulting in reduced kidney damage. In another study, Abe et al. (1999) reported that curcumin reduced inflammation by affecting the number of cytokines released from leukocytes. Similarly, Satin et al. (2016) suggested the favorable effects of curcumin treatment on the livers of experimental diabetic rats. In addition, curcumin has been found to be effective in anticancer therapy as an anti-inflammatory agent (Aqil et al., 2017) and in the treatment of neurodegenerative diseases (Qureshi et al., 2018).

In different studies, curcumin was reported having chemo therapeutic effects (Hatcher et al., 2008), mitochondrial therapeutic effects (Trujillo et al., 2013) and diabetic nephropathy effects (Sharma et al., 2006) on patients. In addition to these studies, Weisberg et al. (2008) reported the anti-inflammatory effects of curcumin treatment on patients with obesity. They also stated that the decrease in macrophages in adipose tissue with curcumin treatment was associated with an increase in adiponectin levels.

Diabetes is a long-term, chronic, and multisystemic disease. It is well established that many side effects of hyperglycemia occur in the human body. As most of these complications progress, they become permanently irreversible, causing the organs and systems to deteriorate or function inadequately. In this study and other studies in the literature, it was observed that curcumin has beneficial effects on diabetes treatment. Therefore, this compound is recommended as a supplement or an alternative to antidiabetic drugs to prevent complications due to hyperglycaemia and prioritize the treatment of diabetes.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abe Y, Hashimoto S and Horie T (1999). Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol. Res.* 39: 41-47. https://doi.org/10.1006/phrs.1998.0404.

Aggarwal BB, Sundaram C, Malani N and Ichikawa H (2007). Curcumin: The Indian solid gold. *Adv. Exp. Med. Biol.* 595: 1-75. https://doi.org/10.1007/978-0-387-46401-5_1.

Ak T and Gulçin I (2008). Antioxidant and radical scavenging properties of curcumin. *Chem-Biol. Interact.* 174: 27-37. doi: 10.1016/j.cbi.2008.05.003.

Aqil F, Munagala R, Jeyabalan J, Agrawal AK, et al. (2017). Exosomes for the enhanced tissue bioavailability and efficacy of curcumin. *AAPS J.* 19: 1691-1702. https://doi.org/10.1208/s12248-017-0154-9.

Aydın M, Selcoki Y, Nazli Y, Yalcin KS, et al. (2012). Relationship between total antioxidant capacity and the severity of coronary artery disease. *J. Clin. Exp. Invest.* 3: 22-28. https://doi.org/10.5799/ahinjs.01.2012.01.0105.

Baynes JW and Thorpe SR (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes.* 48: 1-9. https://doi.org/10.2337/diabetes.48.1.1.

Belviranlı M, Okudan N and Atalık K (2012). Effects of curcumin supplementation on oxidant/antioxidant status of heart tissue in aged rats. *Genel. Tıp. Dergisi.* 22 (2): 61-66.

Bulboacă AE, Porfire AS, Tefas LR, Boarescu PM, et al. (2019). Liposomalcurcumin is better than curcumin to alleviate complications in experimental diabeticmellitus. *Molecules*. 24: 846. https://doi.org/10.3390/molecules24050846.

Drews G, Krippeit-Drews P and Düfer M (2010). Oxidative stress, and beta-cell dysfunction. *Pflügers Arch.* 460: 703-718. https://doi.org/10.1007/s00424010-0862-9.

Hamamcioğlu AC (2017). The role of oxidative stress and antioxidants in diabetesmellitus. *Turk. J. Diabetes Obesity*. 1: 7-13. https://doi.org/10.25048/tjdo.2017.2.

Hatcher H, Planalp R, Cho J, Torti FM, et al. (2008). Curcumin: from ancient medicine to current clinical trials. *Cell. Mol. Life Sci.* 65(11): 1631-1652. doi:10.1007/s00018-008- 7452-4.

Irer SV and Alper G (2004). Experimental Models of Diabetes Mellitus. *J. Turk. Clin. Biochem.* 2(3): 127-136

Johansen JS, Harris AK, Rychly DJ and Ergul A (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc. Diabetol.* 4: 5. https://doi.org/10.1186/1475-2840-4-5.

Kurt M, Atmaca A and Gürlek A (2004). Diabetic nephropathy. *Acta Medica.* 35: 12-17.

Lu M, Yin N, Liu W, Cui X, et al. (2017). Curcumin ameliorates diabetic nephropathy by suppressing NLRP3 inflammasome signaling. *Biomed Res. Int*. 2017: 1516985. https://doi.org/10.1155/2017/1516985.

Nishikawa T, Edelstein D, Du XL, Yamagishi S, et al. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 404 (6779): 787-790. https://doi.org/10.1038/35008121.

Özcan O, Erdal H, Çakırca G and Yönden Z, et al. (2015). Oxidative stress and its impacts on intracellular lipids, proteins, and DNA. J. Clin. Exp. *Invest.* 6: 331-336. https://doi.org/10.5799/ahinjs.01.2015.03.0545.

Qureshi M, Al-Suhaimi EA, Wahid F, Shehzad O, et al. (2018). Therapeutic potential of curcumin for multiple sclerosis. *Neurol. Sci.* 39: 207-214. https://doi.org/10.1007/s10072-017-3149-5.

Reddy AC and Lokesh BR (1994). Studies on the inhibitory effects of curcumin andeugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol. Cell. Biochem.* 137: 1-8. https://doi.org/10.1007/bf00926033.

Satin K, Petpiboolthai H and Anupunpisit V (2016). Effect of Curcumin on Characterization and Localization of Interleukin-13 and Tumor Necrosis Factor-alpha in Liver of Diabetic Rats. *J. Med. Assoc. Thai.* 99: S187-S195.

Shoskes DA, Jones EA and Shahed A (2000). Synergy of mycophenolate mofetil and bioflavonoids in prevention of immune and ischemic injury. *Transplant. Proceed.* 32: 798-799. https://doi.org/10.1016/s0041-1345(00)00987-8.

Sharma S, Kulkarni SK and Chopra K (2006). Curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats. *Clin. Exp. Pharmacol. Physiol*. 33(10): 940-945. https://doi.org/10.1111/j.1440-1681.2006.04468.x.

Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, et al. (2013). Reno protective effect of the antioxidant curcumin: Recent findings. *Redox biology*. 1(1): 448-456. https://doi.org/10.1016/j.redox.2013.09.003.

Weisberg SP, Leibel R and Tortoriello DV (2008). Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. *Endocrinology*. 149: 3549-3558. https://doi.org/10.1210/en.2008-0262.

World Health Organization (2016). *Global report on diabetes*. Geneva, Switzerland. https://www.who.int/publications/i/item/9789241565257.