ORIGINAL ARTICLE

Turmeric as a Preventive Agent of Oxidative Stress and Diabetic Nephropathy in Alloxan Induced Wistar Rats

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ABSTRACT

Background: Hyperglycemia, a common effect of uncontrolled diabetes, causes auto-oxidation which may lead to oxidative and organ damage such as diabetic nephropathy. Curcumin in turmeric has anti-oxidative, anti-inflammatory, and anti-hyperglycemic effect.
Objective: to prove the effects of turmeric powder and extract on the prevention of oxidative stress and diabetic nephropathy in alloxan induced Wistar rats.
Method: This was an experimental study using posttest only control group design. Twenty male Wistar rats aged 8-12 weeks were randomly divided into four groups: C1 was the normal control group; C2 (diabetic) group was induced by single intraperitoneal injection of alloxan (160 mg/kg); TP and TE group was induced by alloxan and orally administered by 200 mg/kg of turmeric powder and 200 mg/kg of turmeric extract for 21 days. The indicator of examination were glucose level, the percentage of renal injury from histopathological assessment, plasma malondialdehyde (MDA) and renal supernatant MDA level using modification of conventional TBARS spectrophotometry. One way ANOVA and Kruskal-Wallis were used for statistical analysis.
Results: Plasma MDA level in the C2 group (28.5±4.49 nmol/ml) was significantly higher than C1 (14.5±2.62 nmol/ml; p=0.008), TE (6.5±3.56 nmol/ml; p=0.008) and TP group (5.9±5.15 nmol/ml; p=0.008). Histopathological assessment showed that the percentage of renal glomerular injury in the C2 group (56±18.17%) was significantly higher than C1 (14±10.11%, p=0.001), TE (35.3±13.87%; p=0.05) and TP group (26.7±18.85%; p=0.009). There was no difference of glucose level, plasma MDA level, renal supernatant MDA level, and percentage of glomerular damage between TE and TP group.
Conclusion: Turmeric powder and extract may prevent oxidative stress and diabetic nephropathy in alloxan induced Wistar rats.
Keywords: Curcumin, turmeric, oxidative stress, diabetic nephropathy

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia and carbohydrate metabolism, fat and protein abnormalities caused by impairment of insulin production or glucose uptake failure. DM is a chronic disease that requires ongoing medical therapy. The disease is growing in number of cases, as well as in terms of diagnosis and therapy. Among the wider community, this disease is better known as diabetes. From various studies, there is a tendency for the prevalence of DM to increase, both in the world and in Indonesia. The high prevalence of type 2 DM is caused by unchanging risk factors, such as gender, age, and genetic factors, as well as modifiable risk factors such as smoking, education, occupation, physical activity, smoking, alcohol consumption, body mass, and waist circumference[1].

Type 2 diabetes that is not handled properly will lead to various complications, namely acute and chronic complications. Chronic complications of type 2 diabetes can be microvascular and macrovascular complications that can decrease the quality of life of the patient. The main cause of death of type 2 DM is macrovascular complications. Macrovascular complications involve large blood vessels i.e. coronary blood vessels, cerebral blood vessels, and peripheral blood vessels. Microvascular complications focus on a specific lesion of diabetes that attacks capillaries and retinal arterioles (diabetic retinopathy), renal glomerulus (diabetic nephropathy) and peripheral nerves (diabetic neuropathy)[2]. Diabetes mellitus is the most common cause of chronic renal disorder and end stage kidney disease in developed countries. It is the major cause of dialysis and transplantation. The development of diabetic nephropathy is associated with several factors such as genetic susceptibility, hemodynamic and biochemical changes. The pathogenic of diabetic nephropathy is associated with the duration and efficiency of hyperglycemia and blood pressure treatment in diabetes mellitus[3,4,5]. The clinical manifestations of diabetic nephropathy are strongly related to structural changes, especially with the degree of mesangial expansion. However, several other important structural changes are involved. Histopathology assessment using light microscopic show the renal structural changes and the structural functional relationships of diabetic nephropathy[6].

Some markers have been used to assess oxidative stress in diabetic patients. One of the markers that can be used is malondialdehyde (MDA). Malondialdehyde is an organic compound with CH (CHO) formula. Malondialdehyde is a lipid peroxidation product that has been recognized as one of the reliable biological markers of oxidative stress and is used to describe the increase of oxidative stress in chronic DM and kidney disease. Lipid peroxides will induce endothelial damage and inflammatory responses, inhibit vasodilatation and activate

macrophages. Malondialdehyde is the most widely used marker[7].

Turmeric is one of the ingredients that can be used as a prevention of diabetes complications. Curcumin is a compound contained in turmeric rhizome and has antioxidant effect. This effect can be useful in preventing the progression of microvascular complications of DM, especially in chronic renal failure[8]. Preparation that is easier to be applied by the community, the preparation of powder. The objective of this research is to prove the effects of turmeric powder and extract on the prevention of oxidative stress and diabetic nephropathy in alloxan induced Wistar rats.

MATERIAL AND METHODS

Experimental animals and study design: Twenty male Wistar rats (Rattus norvegicus) aged 8-12 weeks, weighing 200-300 grams, had been acclimated for 7 days, and randomly divided into 4 groups. Each group consisted of 5 rats. All rats carried out the induction of diabetes with alloxan[9]. Furthermore, groups II and III were treated with turmeric powder and turmeric extract for 21 days orally. Blood glucose monitoring was performed on day 0, 4, 7, 10, 14 and 21.

Each group was given different treatments. Group I (C1), a normal control group, was administered CMC Na for 21 days. Group II (C2), a diabetic control group, was induced by single intraperitoneally injection of alloxan (160 mg/kg BW) and orally administered by CMC Na for 21 days. Group III (TE) was a diabetic rat group that was orally administered by 200 mg/kgBW/day turmeric extract and CMC Na for 21 days. Finally, group IV (TP) was a diabetic rat group that were orally administered by 200 mg/kg BW/day turmeric powder and CMC Na for 21 days.

Reagents and chemicals: Alloxan, CMC Na, 96% ethanol (C_2H_5OH), haematoxylin, eosin, paraffin wax, distilled water, formaldehyde, sodium chloride, chloroform were used in this study.

Curcumin extract: Turmeric rhizomes were weighed ±500 grams, then washed, drained, and dried with 50ºC oven to obtain dry weight. Dry weights were weighed and powdered (milled and sieved). Dry powder weighed ±50 mg then put into filter paper and incorporated soxhlet flask. Next, soxhletation process was done with 96% alcohol solvent±500mL. Turmeric rhizome extract then was obtained in the form of thick (paste). To facilitate administration to white mice, the extract was diluted with aquadest[10].

Curcumin powder: The fresh rhizomes were cut and peeled with a knife, washed, and blanched for 10 minutes using a steamed pan with a temperature of 60ºC. Then turmeric was sliced with a thickness of approximately 3 mm using a knife. Next, turmeric was dried in the 50ºC oven for 24 hours, then mashed with a blender and sifted with a sieve of 80 mesh[11].

Termination and preparation of sample for histopathological analysis: After 21 days, rats subsequently terminated by chloroform vapour. The blood was collected by retroorbita puncture (under light anaesthesia) and centrifuged at 3000 revolution per minute for 30 minutes to get the plasma. Plasma was separated using sterile syringes and stored under refrigerated condition before biochemical analysis were carried out. The kidneys were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixated in 10% formal saline. Tissue processing was done manually in two days. On day 1, the tissues were dehydrated in graded levels of alcohol. On the second day, the tissues were chemically cleared into xylene, then were infiltrated and embedded in paraffin. Next, the paraffin block tissues were cut with a rotary microtome and orientated perpendicularly to the long axis of the kidney, liver and pancreas. Serial sections of 3 µm thick were obtained and placed on clean albuminized glass slides. Slides were then stained with routine Haematoxylin Eosin (HE) staining. Microscopic analysis was done under Leica DM 750 microscope using LZ series software[12].

Measurement of plasma and renal supernatant malondialdehyde level: The plasmas were used for measurements of MDA level using modification of conventional TBARS spectrophotometry. Data were read at 450 nm wavelength and MDA levels were expressed as nmol/ ml protein. A total of 0.5 grams of rat kidney organs were crushed with quartz sand using mortar until smooth. Then 200 µL NaCl-physiologically added into the mortar. Homogenate was incorporated into polypropylene tubes and added with 550 µL of distilled water, 100 µL TCA, and 250 µL HCl 1N. The mixture was then rehomogenized. The mixture was added with 100 µL Na-Thio 1% and centrifuged at 500 rpm for 10 min. Supernatants were taken and filtered using glass wool. The obtained supernatant was heated for 20 minutes. The heated supernatant then cooled in room temperature. After that, the absorbance value of sample was determined using UV-Vis spectrophotometer at maximum wavelength[7].

Statistical analysis: One-way Anova and Kruskal-Wallis were used for statistical analysis. Statistical analyses were done using SPSS version 21.0 for Windows.

Ethical clearance: The study protocol has received ethical approval from the Medical Research Ethics Committee of Faculty of Medicine, Diponegoro University/ Dr. Kariadi Semarang with ethical clearance no. 41/EC/H/FK-RSDK/V/2018.

RESULTS

As shown in figure 1, the level of plasma MDA in the C1 group (14.49±2.6 nmol/ml) was significantly lower than C2 group (28.5±4.49 nmol/ml; p=0.009). The plasma MDA level decreased significantly in the TE group (6.49±3.56 nmol/ml; p=0.009) and TP group (5.998±5.15 nmol/ml; p=0.009) compared with C2 group. There was no difference between TE and TP group (p=0.917).

Figure 2 showed that renal supernatant MDA in TE group (34.83±14.9 nmol/ml) is not different with TP group (59.55±5.9 nmol/ml; p=0.399). The post hoc LSD test showed that renal supernatant MDA level in C2 group (39.48±3.5 nmol/ml) was significantly higher than C1 group (26.46±5.12 nmol/ml; p=0.029). In contrast, no differences were found between the C2 group and TE group (34.83±14.9 nmol/ml; p=0.405), and between the C2 group and TP group (39.55±5.9 nmol/ml; p=0.990).

Histopathological images of rat kidney cortex between groups with HE staining can be seen from figure 3. It described cellular regenerations in TE and TP groups compared to C1 and C2 groups. Histopathological analysis was shown in figure 4. It showed that the percentage of glomerular injury in the C2 group (56±18.17%) was significantly higher than C1 (14±10.11%; p=0.001), TE (35.3±13.87%; p=0,053) and TP group (26.7±18.85%; p=0.009). However, there was no difference between the TE (35.33±13.86%) and the TP groups (26.7±18.85%; p = 0.394).

Fig 1: Mean comparison of plasma MDA level between groups

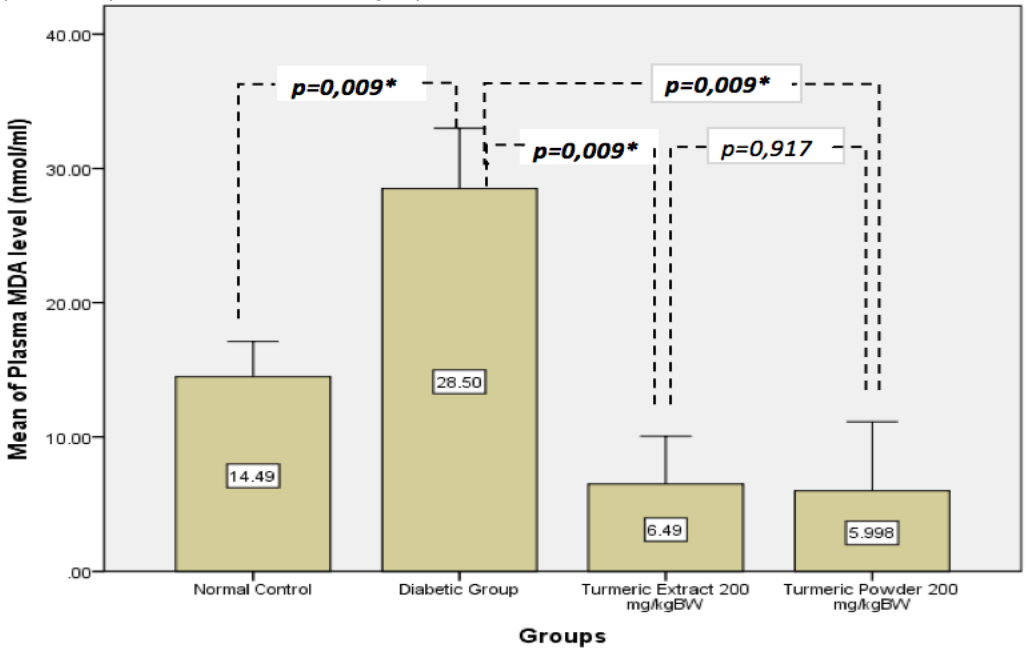


Fig. 2: Mean comparison of renal supernatant MDA level between groups

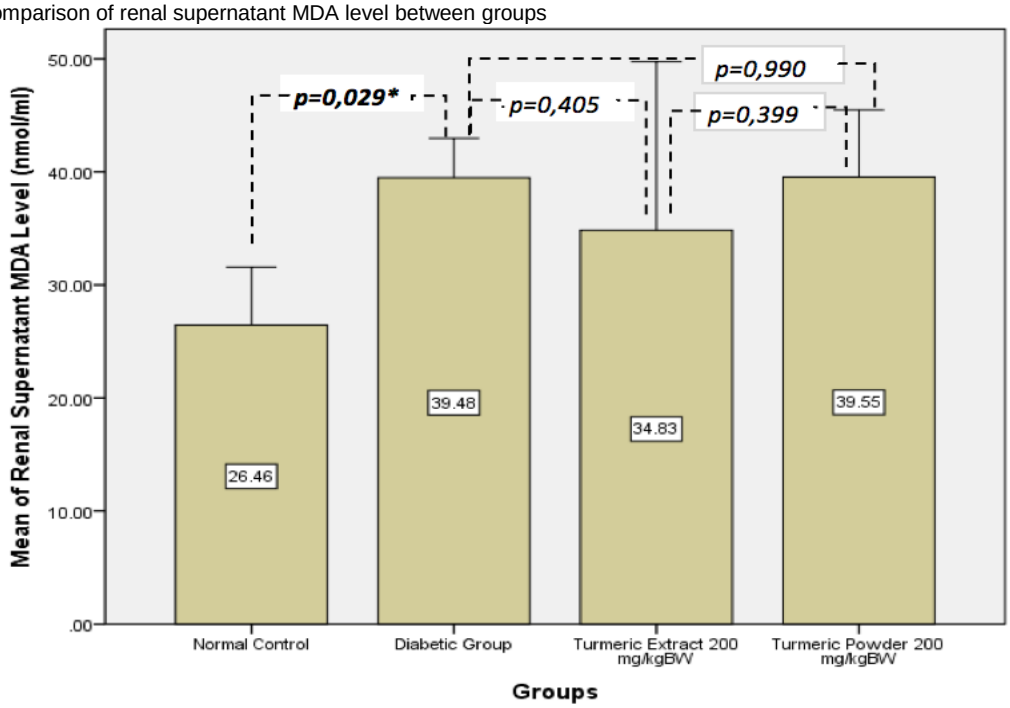


Fig. 3: Histopathology images of rat kidney cortex with HE staining. (A) Normal control, showing normal cellular architecture of glomeruli (100x and 400x magnification). (B) Diabetic control, showing glomeruli abnormalities with areas of vascular degeneration, mesangial expansion and widening of bowman's space (100x and 400x magnification). (C) Diabetic rats treated with turmeric extract 200 mg/kgBB/day p.o, showing cellular regeneration (100x and 400x magnification). (D) Diabetic rats treated with turmeric powder 200 mg/kgBB/day p.o, also showing cellular regeneration.

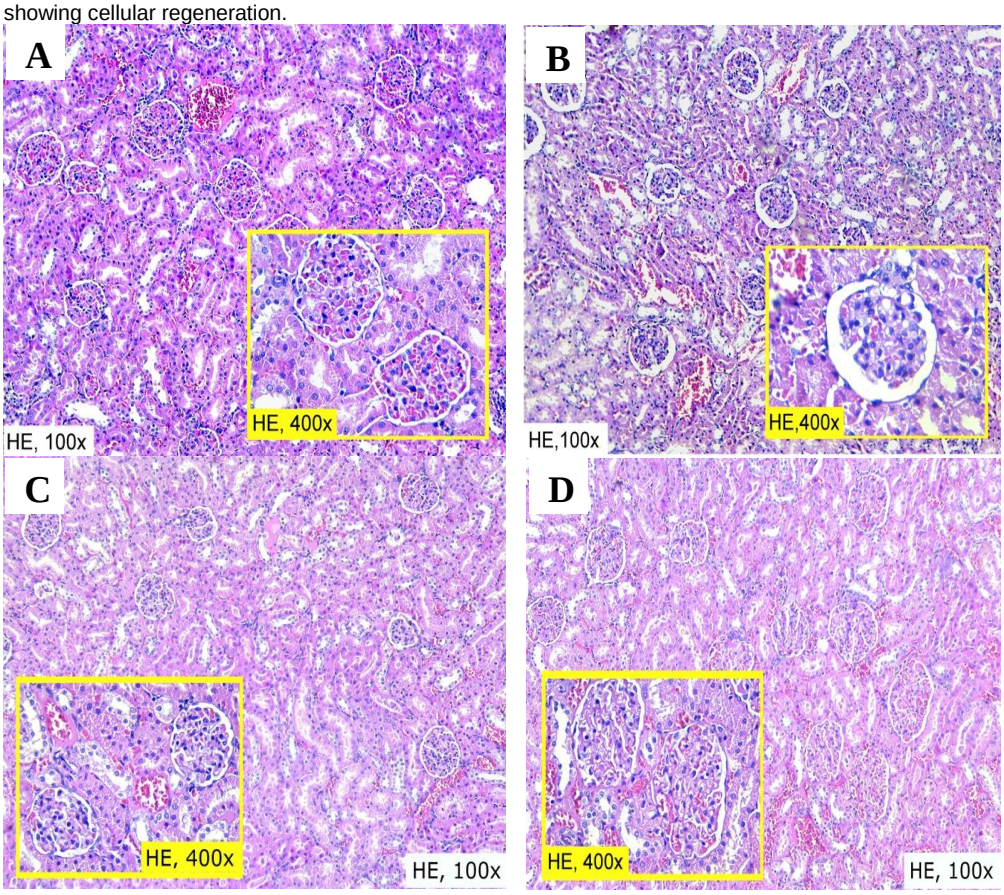
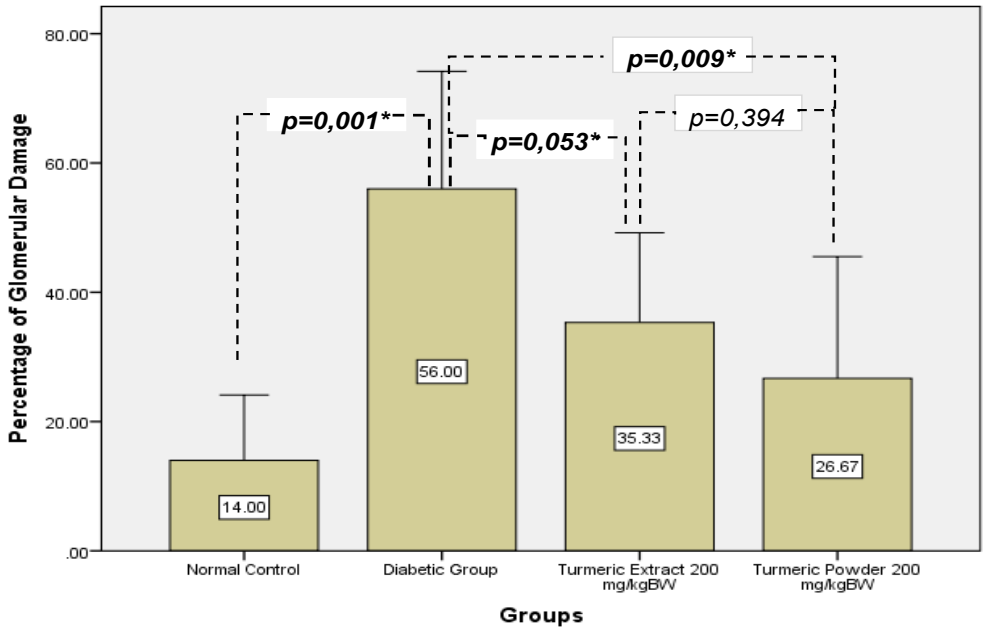


Fig 4: Mean comparison of glomerular injury percentage between groups



DISCUSSION

The result of this study indicated that rats in the TE and TP groups had lower glucose level than normal control group and diabetic group. This is consistent with previous studies which stated that curcumin in turmeric has antihyperglycemic and insulin sensitizer effects[8,13,14,15]. The most active component of turmeric, curcumin, has scientific attention as a potential therapeutic agent in experimental diabetes and for the treatment of the complications of diabetes patients[15,16,17]. Curcumin can reduce blood glucose by reduction in hepatic glucose production and glycogen synthesis and stimulation of glucose uptake by increasing GLUT4, GLUT2 and GLUT3 gene expressions, increasing activation of AMP kinase, promoting PPAR γ ligand-binding activity, suppressing hyperglycemia-induced inflammatory state, stimulation of insulin secretion from pancreatic tissues, improvement in pancreatic cell function, increasing phosphorylation of AKT (PKB), insulin receptor β and reduction of insulin resistance[14].

The experimental animals induced by alloxan are known to be widely used in diabetic model, especially in type 2 diabetic animal model. The results indicated that rats in the diabetic group induced by alloxan had a higher glucose level than normal control group. This is consistent with previous studies in which 160 mg/kg BW single intraperitoneally injection of alloxan may cause hyperglycemia and showed cellular abnormalities with area of vascular degeneration, tubular necrosis, glomerular inflammation, epithelial lining degeneration and desquamation as compared with normal control group[12].

Oxidative stress and inflammatory processes are known to play an important role in the pathogenesis of diabetes mellitus and the complication, such as diabetic nephropathy[18,19]. Several studies have shown that curcumin in turmeric, has antioxidant, anti-inflammatory, antihyperglycemic, and nephroprotective effect[8,15,16,17,20-24].

Malondialdehyde (MDA) is a dialdehyde compound which is the final product of lipid peroxidation in the body. Curcumin treatment decreased plasma and renal supernatant MDA in diabetic rats, resulting in a decreased level of oxidative stress. In the present study, plasma MDA was significantly increased in the C2 group compared to those in the control group, indicating antioxidant defense system impairment. The activity of SOD was enhanced and may contribute to the protective effect of curcumin in diabetic rats by scavenging oxygen free radical or enhancing the antioxidant capacity[16]. Our study demonstrated that curcumin treatment decreased plasma and renal supernatant MDA in diabetic rats, resulting in a decreased level of oxidative stress.

The present study was conducted to assess the nephroprotective and reversible effects of cytoarchitectutural changes after administration of 160 mg/kg BW of alloxan maintained over a given period of time. 21 days of daily treatment with turmeric extract and powder caused a significant histopathological effect on the micro-morphological appearance of the constituents as well as reversible effect ranging from mild to complete restoration in kidney treated with the turmeric after the establishment of diabetics in the rats. Normal control (C1) rats in the kidney tissues were found to be stable. However, diabetic control group (C2) showed high level of cellular abnormalities including glomerulus damages and widening of bowman's space. Alloxan induced rats causes pancreatic β-cell membrane disruption and cytotoxicity after its intracellular accumulation[12]. Turmeric powder and extract have antihyperglycaemic activity due to the presence of flavonoids.

CONCLUSIONS

From this research we could conclude that turmeric powder and extract may prevent oxidative stress and diabetic nephropathy in alloxan induced Wistar rats

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REFERENCES

1. Perkeni. Pengelolaan Dan Pencegahan Diabetes Melitus Tipe 2 Di Indonesia 2015. Jakarta: PB Perkeni; 2015.
2. World Health Organization. Global Report on Diabetes [internet]. Geneva: WHO Press; 2016 [March 2018]. Available at: https://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf;jsessionid=AB8E347DF36836592D6BBB8368E7E944?sequence=1
3. Ziyadeh FN. Mediators of diabetic renal disease: the case for tgf-Beta as the major mediator. J Am Soc Nephrol. 2004; 15 Suppl 1: S55-7.
4. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 1993; 329(20):1456-1462. doi:10.1056/NEJM199311113292004.
5. Mogensen CE. How to protect the kidney in diabetic patients: with special reference to IDDM. Diabetes. 1997; 46 Suppl 2: S104-11.
6. Fioretto P, Mauer M. NIH Public Access. 2009; 27(2): 195-207. doi:10.1016/j.semnephrol.2007.01.012.HISTOPATHOLOGY
7. Yustika AR, Aulannia'am, Prasetyawan S. Kadar malondialdehid (mda) dan gambaran histologi pada ginjal tikus putih [Malondialdehyde (MDA) level and histological images of white mice kidneys] . Student J. 2013; 1(2): 222-228.
8. Chuengsamarn S, Rattanamongkolgul S, Luechapudiporn R, Phisalaphong C, Jirawatnotai S. Curcumin extract for prevention of type 2 diabetes. Diabetes Care. 2012; 35(November): 2121-2127. doi:10.2337/dc12-0116.
9. Sulistiowati F, Tanti SA, Trisharyanti I, Wahyuni AS. Kemampuan perbaikan fungsi ginjal setelah pemberian oral ekstrak etanol bekatul beras hitam pada tikus nefropati diabetik [The ability of kidney function repair after oral administration of etanol black rice extract on nephropathy diabetic rats]. J Chem Inf Model. 2014;53(9):1689-1699. doi:10.1017/CBO9781107415324.004.
10. Hartono, Nurwati I, Ikasari F, Wiryanto. Pengaruh ekstrak rimpang kunyit (curcuma domestica val.) terhadap peningkatan kadar SGOT dan SGPT tikus putih (rattus norvegicus) akibat pemberian asetaminofen [Effects of turmeric extract on the increase of SGOT and SGPT level in the white rats]. Biofarmasi. 2005;3(2):57-60.
11. Ginting C. The effect of amount of turmeric powder on quality of tofu at room temperature storage. 2014;9(2):108-114.

12. Bassey E. Histopathological assessment of the kidney of alloxan induced diabetic rat treated with macerated Allium sativum (garlic). Asian J Biomed Pharm Sci. 2014; 4(35):13-17. doi:10.15272/ajbps.v4i35.214.
13. Kuroda M, Mimaki Y, Nishiyama T, et al. Hypoglycemic effects of turmeric (Curcuma longa L. rhizomes) on genetically diabetic KK-Ay mice. Biol Pharm Bull. 2005; 28(May): 937-939. doi:10.1248/bpb.28.937.
14. Ghorbani Z, Hekmatdoost A, Mirmiran P. Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. Int J Endocrinol Metab. 2014; 12(4): 1-9. doi:10.5812/ijem.18081.
15. Zhang D-W, Fu M, Gao S-H, Liu J-L. Curcumin and diabetes: A systematic review. Evidence-based Complement Altern Med. 2013; 2013:636-53. doi:10.1155/2013/636053.
16. Xie Z, Wu B, Shen G, Li X, Wu Q. Curcumin alleviates liver oxidative stress in type 1 diabetic rats. Open Life Sci. 2017; 12: 452-459. doi:10.3892/mmr.2017.7911.
17. He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: How are they linked? Molecules. 2015; 20(5): 9183-9213. doi:10.3390/molecules20059183.
18. Sasaki S, Inoguchi T. The role of oxidative stress in the pathogenesis of diabetic vascular complications. Diabetes Metab J. 2012; 36(4): 255-261. doi:10.4093/dmj.2012.36.4.255.
19. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev. 2004; 25(4): 612-628. doi:10.1210/er.2003-0019.
20. Abdul-Hamid M, Moustafa N. Protective effect of curcumin on histopathology and ultrastructure of pancreas in the alloxan treated rats for induction of diabetes. J Basic Appl Zool. 2013; 66(4): 169-179. doi:10.1016/j.jobaz.2013.07.003.
21. 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): 448-456. doi:10.1016/j.redox.2013.09.003.
22. Meng B, Li J, Cao H. Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. Curr Pharm Des. 2013; 19: 1-13. doi:CPD-EPUB-20121023-12 [pii]. Kim BH, Lee ES, Choi R, et al. Protective effects of curcumin on renal oxidative stress and lipid metabolism in a rat model of type 2 diabetic nephropathy. Yonsei Med J. 2016; 57(3): 664-673. doi:10.3349/ymj.2016.57.3.664
23. Xie Z, Wu B, Shen G, Li X, Wu Q. Curcumin alleviates liver oxidative stress in type 1 diabetic rats. Mol Med Rep. 2017: 452-459. doi:10.3892/mmr.2017.7911