Original Article

Effect of Garlic on Rat Diabetic Renal Cortex. A histological and Immunohistochemical Study

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ABSTRACT

Background: Diabetic nephropathy is one of the chief microvascular complications of diabetes and it has turn into the main cause of end-stage renal disease nictitate dialysis.

Aim of work: To observe the effects of garlic supplementation on STZ induced diabetic nephropathy in Albino rats using histological and immunohistochemical stains.

Material and Methods: Three groups were included, control group of 5 rats served as control, experimental group of streptozotocin- induced diabetes with dose of 50 mg/kg body weight intraperitoneal for eight weeks and third group is the diabetic rats supplemented with garlic orally with a dose of (500 mg/kg body weight) for 6 weeks starting two weeks after diabetes induction.

At the end of the experiment, rats were sacrificed then kidneys were dissected out and processed for paraffin blocks. Sections were stained with haematoxylin & eosin and immunohistochemistry for vimentin, nuclear factor kappa and desmin.

Results: Increased diameter of glomeruli in diabetic rats. These changes were decreased in the diabetic group treated with garlic. Desmin, vimentin and NF-κB expression were induced in diabetic group but they decreased after garlic supplementation.

Conclusion: The changes in diabetic group were regressed with garlic supplementation. It is advisable to give natural products like garlic to diabetic patients as they help to ameliorate diabetic complications.

Key Words: Desmin, diabetic nephropathy, garlic, nuclear factor kappa, vimentin.

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INTRODUCTION

The Diabetes mellitus is a metabolic disease due to impaired secretion of insulin or due to insulin insensitivity. Diabetes mellitus affects approximately 4% of the population in world and its prevalence is expected to rise (American Diabetes Association, 2005). Diabetic patients are liable to multiple vascular complications, including atherosclerosis, diabetic nephropathy and neuropathy (Rosario & Prabhakar 2006).

Diabetic nephropathy (DN) has become the main cause for end stage renal disease which requires dialysis (Yang et al., 2010). Renal fibrosis is the main pathological character in DN. Diabetic nephropathy is characterized by early glumerular hypertrophy, hyperfiltration and build-up of extra cellular matrix components as fibronectin. DN is stimulated by transforming growth factor beta1 (TGF-b1) which trigger thickening of the glumerular and tubular basement membrane and develop into glumerulosclerosis and renal fibrosis (Schena & Gesualdo, 2005).

Garlic (Allium sativum) is one of the most significant ingredients in Indian traditional medicine. Garlic has therapeutic properties and has been used in the treatment of several diseases for centuries. Pharmacologically, garlic is suggested to have a hypolipidemic (Sher et al., 2012), anticoagulant and anticancer (Demeule et al., 2004) effects. It exerts antigenotoxic effects by modulating oxidative stress (Kumaraguruparan et al., 2005).

AIM OF WORK

The purpose of the present study is to observe the effects of garlic supplementation on streptozocin (STZ) induced diabetic nephropathy in Albino rats and on the expression of desmin which is reported to be induced markedly in pathological conditions that affects podocytes (Li et al., 2008), vimentin which could detect renal fibrosis (Galichon & Hertig 2011) and nuclear factor kuppa (NF-xB) as the products of hyperglycemia are reported to trigger activation of NF-kB (Piperi et al., 2015).
EFFECT OF GARLIC ON RAT DIABETIC RENAL CORTEX

MATERIAL AND METHODS

Animals used

Twenty-five adult female albino rats weighing 200-250 gm were used. They were obtained from the Faculty of Pharmacy animal house, Mansoura University. Each three animals were housed in a cage at a constant temperature 18°C and humidity 45% on a 12-h light/dark cycle. Rats had a free access to standard diet and drinking water. The experiment was carried out according to the rules and regulations laid down by the Committee of the Animals Experimentation of Mansoura University and obtained approval by Institutional Review Board.

Chemicals and Reagents

Garlic extract (allium sativum) and Streptozotocin (STZ) were obtained from Sigma Aldrich, Egypt.

Experimental design

The animals were fasted for 16 hours then injected intraperitoneally with STZ with a dose of 50 mg/kg body weight dissolved in citrate buffer to induce diabetes (Karabas et al., 2013). After the injection, 5% sucrose was supplemented for 24hrs to prevent animals from fatal hypoglycemia. Blood glucose level was checked after one week by the glucometer. The animals with a blood glucose level more than 300 mg/dl were considered diabetic and were included in the study.

Rats were divided in three groups. The control one contains five rats. The diabetic rats were divided into two groups (10 animals each), diabetic group and garlic supplemented group. Garlic supplemented group received garlic orally with a dose of (500 mg/kg body weight) starting two weeks after STZ injection and continued for six weeks (Sayed, 2013).

Histopathological Examination

At the end of the experiment, rats in all groups were sacrificed by chloral hydrate (300 mg/kg body weight). Then kidneys were dissected out and put in 10% neutral buffered formalin after washing with phosphate buffer saline. They were then processed and embedded in paraffin. Sections were cut at 3-4µm and stained with haematoxylin and eosin for histopathological examination. Glomerular diameters were measured by image J programme in 10 fileds/slide & 10 slides per rat.

Immunohistochemical stains

Paraffin sections were cut at 3 µm, rehydrated then blocked with 5% bovine serum albumin (BSA) in Tris buffered saline (TBS) for 2 hours. They were then immunostained with a primary antibody monoclonal mouse antibody to desmin (abcam) (1:100) (AbdEl-Moniem et al., 2105), with primary antibody to vimentin (abcam) (Gu et al., 2013) and (Rabbit polyclonal IgG to rat NF-κB p65 (abcam) (Shiju et al., 2013), at a concentration of 1 µg/ml containing 5% BSA in TBS. After this, sections were incubated overnight at 4 °C, followed by washing the slides with TBS. Finally, sections were incubated with goat anti-rabbit secondary antibody, then washed with TBS then incubated for 5–10 min in 0.02% diaminobenzidine solution containing 0.01% H2O2, dehydrated and mounted. At the end, slides were visualized under the light microscope.

Measurement of the average diameter of glomeruli:

The diameter of the glomeruli was measured in haematoxylin and eosin stained sections at 100 x magnification by using an ocular micrometer calibrated with a stage micrometer. At least 30 glomeruli were randomly chosen for each animal. Two measurements were taken for each glomerulus (at the maximum transverse diameter perpendicular to the pervious one). The average diameter = (Maximum transverse diameter+ Maximum perpendicular diameter) ÷ 2 (Johara et al., 2014).

Image analysis for immunostained sections:

The percentage of colour density (brown) of the reaction was measured using the colour deconvolution plugin feature of the free software Image J programme to separate colours. Digital images were captured with a digital camera (Olympus SC100) from randomly chosen areas in desmin, vimentin and NF-κB immunostained sections. Three sections per animal were used.

Statistical Analysis

Data were expressed as mean ± S.D (n = 4) and analyzed on Graph Pad Prism 5.01 software. Statistical analysis was performed by One-way ANOVA then independant T test. Results were considered statistically significant if P value was< 0.05.
RESULTS

Control group

Sections of kidney showed normally appearing glomeruli and tubules (Fig. 1a). The mean glomerular diameter was 62.7 ± 7 µm (Fig. 4a). Sections stained with desmin showed negative reaction in glomeruli (Fig. 1b) and the area fraction was 12.67 % (Fig. 4b). Kidney sections stained with vimentin showed negative reaction in glomeruli (Fig. 1c) and the area fraction was 4.2 ± 0.7% (Fig. 4c). Sections of kidney showed no glomerular reaction to the nuclear factor kuppa but it was positive in tubules (Fig. 1d) and the area fraction was 14.9 ± 3.1 % (Fig. 4d).

Diabetic group

Glomerular damage was seen in the kidneys of the diabetic group. Several glomeruli appeared enlarged in size and others were distorted (Fig. 2a). The diameter of the glomeruli of the diabetic rats for 8 weeks showed significant increase 93.6 ± 8.6µm (Fig. 4a). Sections stained with desmin showed significant increase in the positive reaction in glomeruli (Fig. 2b) and the area fraction was 26.16 % (Fig. 4b). Sections of kidney showed increased reaction of the glomeruli to vimentin and the reaction appeared in the interstitium (Fig. 2c) and the area fraction showed significant increase 10.9 ± 3.2% compared to the control (Fig. 4c). Sections of diabetic kidneys showed positive reacting glomeruli to nuclear factor kuppa and it disappeared in tubules which were positive in control group (Fig. 2d) and the area fraction showed significant increase 23.5 ± 4.4% when compared to the control group (Fig. 4d).

Diabetic group treated with garlic:

Sections of kidney showed almost normally appearing glomeruli (Fig. 3a) with decreased bowman’s space. The diameter of the glomeruli in garlic treated diabetic animals showed a non-significant reduction 89.3 ± 14.2µm compared with the diabetic group (Fig. 4a). Sections stained with desmin showed a decrease in the positive reaction in glomeruli (Fig. 3b) and the area fraction was 12.87 % (Fig. 4b). Sections of kidney showed decreased reaction of the glomeruli to vimentin (Fig. 3c) and the area fraction showed significant reduction 4.5 ± 2.1% compared with diabetic group (Fig. 4c). Sections of kidney showed decrease in reaction of the glomeruli to the nuclear factor kuppa (Fig. 3d) and the area fraction showed a significant reduction 18.0 ± 3.8% (Fig. 4d).
Fig. 1: Photomicrographs of kidney of adult control rats showing:
(1a) sections showing average sized glomeruli (G) with narrow Bowman’s space (arrow). (Hx & E 100).
(1b) sections stained with desmin showing negative reaction to desmin in glomeruli (G) with some positive reaction (arrows) in proximal (P) and distal convoluted tubules (D). (X 400).
(1c) sections stained with vimentin showing negative reaction of the glomeruli to vimentin. (X 100).
(1d) sections stained with nuclear factor kuppa showing negative reaction to nuclear factor kuppa in averaged size glomeruli (G) with positive reaction (arrows) in proximal (P), distal convoluted tubules (D) and collecting tubules (C). (X 400).
Fig. 2: photomicrographs of sections of adult rats diabetic kidney showing:

(2a) section showing enlarged sized glumeruli (G) with dilated Bowman’s space (black arrow) and some glomeruli are distorted (white arrows). (Hx & E X 100).

(2b) sections stained with desmin showing positive desmin reaction (arrow) in the enlarged glomeruli (G). (X 400).

(2c) sections stained with vimentin showing positive reaction (black arrows) in the enlarged glomeruli (G) and it appears also in tubules (white arrow). (X 100).

(2d) sections stained with nuclear factor kappa positive reaction (black arrows) to nuclear factor kappa in the enlarged glomeruli (G) and it appears in the interstitium (white arrow) with negative reaction in tubules (T). (X 400).
Fig. 3: photomicrographs of adult rats diabetic kidney supplemented with garlic showing:
(3a) sections showing slightly enlarged glomeruli (G) with slight dilatation of Bowman’s space (arrow). (Hx & E X 100).
(3b) sections stained with desmin showing decrease in the positive reaction to desmin (arrow) in the glomeruli (G).
(3c) sections stained with vimentin showing decreased positive reaction to vimentin (arrow) in the glomeruli (G) with limited reaction in interstitium (white arrow). (X 100).
(3d) sections stained with nuclear factor kappa showing decrease in the positive reaction (arrow) of nuclear factor kappa in the glomeruli (G) with limited reaction in tubules (white arrows). (X 400).
Fig. 4: Charts showing comparison between:
(4a) insignificant difference between diameters of glomeruli of different groups.
(4b) mean area fraction of desmin expression in kidneys of different groups. Significant increased expression in diabetic group (10.9%) and significantly decreased in garlic supplemented diabetic group (4.5%) but did not reach control level (4.2%).
(Fig 4c) mean area fraction of vimentin expression in kidneys of different groups. Significant increased expression in diabetic group (10.9%) and significantly decreased in garlic supplemented diabetic group (4.5%) but did not reach control level (4.2%).
(Fig 4d) mean area fraction of Nfk expression in kidneys of different groups. significantly increased expression in diabetic group (23.5%) and significantly decreased in garlic supplemented diabetic group (18%) but did not reach control level (14.9%).
DISCUSSION

Diabetic nephropathy (DN) caused around 44% of cases of chronic end-stage renal disease (ESRD) in the USA (Shaw et al., 2010). Diabetes contributes to increased mortality from DN that leads to ESRD (Vallon & Thomson 2012).

A recent study has concluded that Arab ethnicities are more prone to have diabetes. They showed a single-nucleotide polymorphisms, especially for type 2 diabetes, which has been identified in Arabs (Al-Rubeaan et al., 2013).

Tervaert et al. (2010) conducted a study that helped in establishing a new classification of diabetic nephropathy according to severity of glomerular lesion as they best reflect the course of progressive DN and easy to be identified. Class I consists of thickening of the glomerular basement membrane (GBM). Class II has mild (IIA) to severe (IIB) mesangial expansion. Class III represents nodular glomerulosclerosis. Finally, Class IV is categorized as advanced DN comprizing more than 50% global glomerulosclerosis associated with podocyte loss.

It was found In this study and in agreement with previous studies (Xiao et al., 2015 & Chen et al., 2016) that diabetes in rats was associated with glomerular injuries. Several glomeruli showed mesangial widening with hypercellularity (mesangial expansion) and increased glomerular diameter.

Oxidative stress is related to changes in the redox state caused by the continues hyperglycemic and increased level of Advanced Glycation End Products (AGEs). This factors affect renin-angiotensin system and the signalling of the transforming growth factor-beta (TGF-β), producing chronic inflammation, glomerular and tubular hypertrophy. On the same hand, renal fibrosis, thickening of the tubular and glomerular membranes, dysfunction of podocytes and appearance of apoptosis (Manda et al., 2015). Oxidative stress in DN has the ability to act as a trigger, modulator and link in the pathological events that occur in DN. It is known that redox state propagates and affects the signals from the cellular membrane to the nucleus (Tiwari et al., 2013). The AGEs could mediate their actions through interaction with Receptor of Advanced Glycation Ends (RAGEs) for inducing the proliferation, apoptosis, autophagy or migration of the cells (Lee & Park 2013). The intracellular production of Reactive Oxygen Species (ROS) is incited by the AGE-RAGEs interaction (Bohlender et al., 2005) through the activation of the peroxisomes proliferator gamma receptor (Matsui et al., 2007). This leads to multiple transcription factors activation, which will induce a large quantity of proinflammatory and profibrotic responses (Miranda-Díaz et al., 2016).

Kidney is the most important organ involved in detoxification of AGEs (He et al., 1999). AGEs are complex group of modifications on proteins, it could be formed inside the body, or absorbed from the diet (Fu et al., 1994). AGEs formation leads to structural and functional alterations of intra- and extra-cellular proteins (Aronson, 2003). On the same hand, AGEs accumulation cause induction of oxidative stress (Bierhaus et al., 1997). Another third pathway reported recently, AGEs exert their pathological effect via cellular receptors (Zhuang & Forbes 2016).

Another mechanism which could share in the development of DN, hyperglycemia induces epithelial-myofibroblast transdifferentiation of renal tubular epithelial cells which ends in renal fibrosis (Simonson, 2007). This transition is associated with increased expression of mesenchymal markers, as vimentin (Shiju et al., 2013) as was found in the present study.

Studies showed that in diabetes, many factors as AGEs, shear stress and oxidative stress share in NF-κB activation (Edwards et al., 2008). Activated NF-κB promotes expression of multiple genes implicated in inflammation as cytokines and adhesion molecules. All of these play an important role in the pathogenesis of renal diseases (Liu et al., 2010). In this study, it has been found that garlic significantly suppresses NF-κB expression in the kidneys of the diabetic rats. This finding suggests that garlic is useful in delaying progress of the renal complication of diabetes by inhibiting NF-κB activation.

Desmin is an intermediate filament protein which has suggested to be a podocyte injury indicator (Zou et al., 2006). Injured podocytes undergo epithelial-to-mesenchymal transition (EMT). This phenotypic conversion causes
podocytes to lose its specialized epithelial markers and acquire new mesenchymal markers as desmin (Li et al. 2008). Increased desmin expression found in the diabetic rats in this study is also reported by (Gu et al., 2013).

The present study found that garlic could help in delaying progression of diabetic nephropathy proven by histological and immunohistochemical stains. These results are supported by Sayed (2013) who showed that aged garlic extracts had the ability to ameliorate kidney damage, by attenuating DN in Wistar rats. They suggested that the protective effect of garlic on DN might be due to its anti-glycation and hypolipidemic effects.

CONCLUSION

In conclusion, the histopathological and immunohistochemical changes seen in the renal cortex of the diabetic group were partly regressed with garlic supplementation. Consumption of garlic might be encouraged in diabetic patients to attenuate the progress of diabetic nephropathy.

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Effect of Garlic on Rat Diabetic Renal Cortex

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Effects of Garlic on Rat Diabetic Renal Cortex: A Study

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Summary

Diabetic nephropathy is one of the complications of diabetes mellitus and can become the main cause of renal failure at the end of the disease.

The objective of the study was to delay the occurrence of diabetic nephropathy resulting from uncontrolled diabetes.

The study was conducted on 25 female albino rats, divided into three groups:

- Group 1: 10 control rats
- Group 2: Diabetic rats
- Group 3: Diabetic rats treated with garlic

Results:

- Increased glomerular diameter in diabetic rats, but these changes were less in the diabetic group treated with garlic.
- Increased immune reaction with immune markers (interferon, interleukin) in the diabetic group treated with garlic, while it decreased in the group treated with garlic.

Conclusions:

- Delaying the changes in diabetic nephropathy can be achieved with garlic treatment.
- Garlic could be used as a natural product to reduce the complications of diabetes.

Keywords: Diabetic nephropathy, Garlic, Diabetes, Interferon, Interleukin

References: