Macedonian Journal of Medical Sciences. 2012 Dec 15; 5(4):382-388.
http://dx.doi.org/10.3889/MJMS.1857-5773.2012.0244
Basic Science

OPEN ACCESS

Ocimum Gratissimum Linn Worsens Streptozotocin-Induced Nephrotoxicity in Diabetic Wistar Rats

Adejoke Y. Onaolapo[1], Olakunle J. Onaolapo[2], Stephen O. Adewole[3]

[1]Department of Human Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria; [2]Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria; [3]Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Citation: Onaolapo AY, Onaolapo OJ, Adewole SO. *Ocimum Gratissimum* Linn Worsens Streptozotocin-Induced Nephrotoxicity in Diabetic Wistar Rats. Maced J Med Sci. 2012 Dec 15; 5(4):382-388. http://dx.doi.org/10.3889/MJMS.1957-5773.2012.0244.

Key words: *Ocimum gratissimum;* Streptozotocin; Diabetes Mellitus; Nephrotoxicity; Kidney.

Correspondence: Adejoke Y. Onaolapo. Department of Human Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Phone number: 2348062240434. E-mail: olakunleonaolapo@yahoo.co.uk, adegbayibiy@yahoo.com

Received: 20-Feb-2012; Revised: 20-May-2012; Accepted: 23-May-2012; Online first: 18-Oct-2012

Competing Interests: The author have declared that no competing interests exist.

Abstract

Effects of ethanolic extract of Ocimum gratissimum leaves (*O. gratissimum*) on the kidneys in Streptozotocin-induced diabetic rats were studied. Thirty-six adult rats were assigned into six groups (A, B, C, D, E and F) of six rats each. Diabetes was induced with a single intraperitoneal injection of Streptozotocin. Group A (non diabetic control) and F (diabetic control) received normal saline orally, group B received a Metformin at 25 mg/kg while groups C, D and E had *O. gratissimum* at 400, 600 and 800 mg/kg. Body weight and fasting blood glucose levels were monitored weekly; blood levels of Creatinine (Cr) and Urea (U) were measured on day twenty eight. Animals were sacrificed and kidney sections processed for histological study. Statistical analysis was carried out using a one way ANOVA followed by a post-hoc test. Results showed significant reduction in body weight and blood glucose levels in the groups that received either metformin or *O.gratissimum* as well as dose dependent increase in blood urea and creatinine levels, histopathology revealed varying degrees of renal injury. The study concluded that *O. gratissimum* worsens diabetes induced renal injury.

Introduction

Herbal medicines are naturally occurring plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices [1]. For a long time, herbal medicines or their extracts have been used to cure various diseases [1]. *Ocimum gratissimum* an herbaceous plant belonging to the Labiatae family [2] is indigenous to tropical areas especially India and it is also found in West Africa. In Nigeria, it is found in the savannah and coastal areas. It is known by various names in different parts of the country, in the southern part of Nigeria, the plant is called “effinrin-nla” by the Yoruba speaking tribe. It is called “Ahuji” by the Igbos in the eastern part, while in the Northern part of Nigeria; the Hausas call it “Daidoya” [2]. *O. gratissimum* has been used extensively in the traditional system of medicine in many countries. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea [3]. In the Savannah areas, decoctions of the

leaves are used to treat mental illness while *O.gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile, it is also used in the treatment of fungal infections, fever, cold and catarrh [4].

Diabetic nephropathy (DN) is one of the most severe diabetic microangiopathies and has become a worldwide epidemic, accounting for approximately one-third of all cases of end-stage renal disease [5]. Increased thickness of glomerular basement membrane and augmentation of glomerular extracellular matrix (ECM) are recognized as pathological hallmarks of DN [6]. At present, diabetic kidney disease affects about 15%–25% of type I diabetes patients [7] and 30%–40% of patients with type II diabetes [8]. Diabetic nephropathy is characterized by specific renal morphological and functional alterations.

Research on the various potential scientific uses of *O.gratissimum* is well documented and ongoing. In a previous publication we described the hepatotoxic potential of this extract in Wistar rats [9], however there is very little information on the effects of this herb on the kidney in the diabetic state; our intention here was to study the effects of *O.gratissimum* on kidney biochemistry and microanatomy in diabetic Wistar rats.

Materials and Methods

Plant material

Fresh leaves of *Ocimum gratissimum* Linn were collected from Ogbomoso, Oyo state, Nigeria in August 2010. The plant was identified by Dr Ogunkunle of the Department of Biology, Ladoke Akintola University of Technology, Ogbomoso and a voucher specimen was deposited in the herbarium of the department (LAU2396).

Chemicals and drugs

Normal saline, 5% ethanol, Streptozotocin (STZ (Sigma St. Louis, USA)), 0.1 M citrate buffer pH 4.5, Metformin (Bristol-Myers Squibb.). All chemicals and drugs used were of analytical grade.

Preliminary phytochemical screening of plant fraction

Preliminary screening of the extract was performed for the presence of secondary metabolites, using the following reagents and chemicals: alkaloids with Mayer's and Dragendorff's reagents [10, 11]; flavonoids with the use of Mg and HCl [12, 13]; tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds [13].

Preparation of extract of ocimum gratissimum

O. gratissimum leaves were first separated from the stalk, rinsed with water to remove dirt; air dried at room temperature and ground to fine powder using an electric blender (Christy and Norris - 47362, England) at the Department of Pharmacognosy of the Obafemi Awolowo University, Ile – Ife. Extraction was performed by adding 800 mg of ground powdered in 5 liters of ethanol in a sterile flask, mixture was swirled to ensure effective mixing and a stopper used to avoid loss of volatile liquid at ambient temperature (28 ± 2°C).

The mixture was extracted by agitation on a rotary shaker. After 48 hrs, the mixture was decanted. The filtrate was then poured into stainless trays and the extract was allowed to evaporate to dryness at room temperature (28 ± 2°C) for 2-3 days by using a vacuum evaporator (RE 100B Bibby Sterilin, United Kingdom).The dry residue was stored until ready to use. The percentage yield of extract was 10.33%w/w.

Animals

Thirty-six healthy adult male Wistar rats purchased from the Empire Animal Farms in Osogbo, Osun State, Nigeria were used with weight in the range of 180 to 200 g. The animals were randomly allocated into six groups. The animals were housed in metallic cages measuring 64" x 36" x 32" (6 rats in each cage). All animals had free access to food and water *ad libitum*.

They were maintained under standard laboratory conditions i.e. a well aerated room with alternating light and dark cycles of 12 hours each and at room temperature of 25°C. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Ladoke Akintola University of Technology, Ogbomoso. All rules applying to animal safety and care were observed.

Acute toxicity test

The 50% lethal dose determination for each of the fractions was conducted separately using modified method of Lorke [14]. Details as previously published [9].

Induction of diabetes mellitus

Diabetes mellitus was experimentally induced in 36 rats by a single intraperitoneal injection of [15] of

Streptozotocin (STZ) (Sigma St. Louis, USA) dissolved in 0.1M sodium citrate buffer pH 4.5 [16]. An equivalent volume of citrate buffer instead of Streptozotocin was administered to a group of six animals which served as control. Seventy-two hours post induction of diabetes, blood glucose was determined from the tail vein after an overnight fast. Rats with blood glucose levels of ≥ 18 mmol/L were considered diabetic and used.

Experimental method

The animals were assigned into six groups A, B, C, D, E and F of six rats each. Group A, the non diabetic control received normal saline orally. Animals in group F served as the diabetic control and were also administered normal saline orally. Animals in group B received Metformin daily at a dose of 25 mg/kg body weight [17]. Groups C, D and E received daily oral doses of ethanolic extracts of *Ocimum gratissimum* leaves at 400, 600 and 800 mg/kg [3, 15]. All treatments were administered for a period of 28 days. Samples for blood glucose estimation were determined weekly using the glucose oxidase method [18]. Creatinine and Urea (Cr, U) levels were measured on the 28th day. Furthermore, sections of the right kidney were obtained, processed, sectioned and stained with Hematoxylin & Eosin (H&E). An Olympus BX50 digital light microscope was used to examine the slides and acquire photomicrographs.

All the data for all biochemical parameters were analyzed by analysis of variance (ANOVA), and post-hoc tests (Student Newman Keuls) were used to determine the source of a significant effect. Results are expressed as Mean ± S.E.M., $p<0.05$ is taken as accepted level of significant difference from control.

Collection and analysis of blood samples for kidney biochemistry

Blood was collected from each diabetic and non diabetic rat on the 29th day by intracardiac puncture; rats were fasted for 8 hours before samples were collected. Samples were collected into lithium heparinised bottles, blood was allowed to clot and plasma separated by centrifugation at 3500 rpm for 10 minutes using a hematocrit centrifuge (JICA, Japan). The supernatant was assayed either immediately or stored at –20^{o}C. Blood creatinine was determined by a colometric reaction (Jaffe's Method), [19, 20], using an autoanalyser (Astra 8 autoanalyzer; Beckman Instruments, Fullerton, CA), and urea was measured using a colometric reaction (DAM Method) [21, 22].

Results

Preliminary phytochemical screening

Freshly prepared extracts subjected to preliminary phytochemical screening test revealed the presence of carbohydrates, reducing sugars, lipids, flavonoids, alkaloids, steroids, tannins, cardiac glycosides and resin.

Physical examination of the animals

The animals in group A appeared healthy, active and gained weight. The animals in groups B, C, D and E appeared ill looking, were polyuric evidenced by increased micturition and the soiling of their animal coat. They also exhibited poor grooming and were lethargic, however all these symptoms abated by the end of the second week of the study (day 14). Food consumption was noticed to have increased despite continued reduction in body weight. Animals in groups D and E were noticed to have developed total haematuria, first noticed on day 16 and persisted till sacrifice. The animals in group F appeared ill looking, were polyuric as evidenced by increased micturition and also the soiling of their animal coat. They also exhibited poor grooming and as the weeks progressed there was loss of hair and worsening lethargy.

Effects of ocimum gratissimum on body weight

The mean body weight monitored weekly for a period of 28 days in all groups are presented in Fig 1. There was a statistically significant ($F(5, 35) = 11.63$, $p<0.05$) reduction in body weight in the groups that

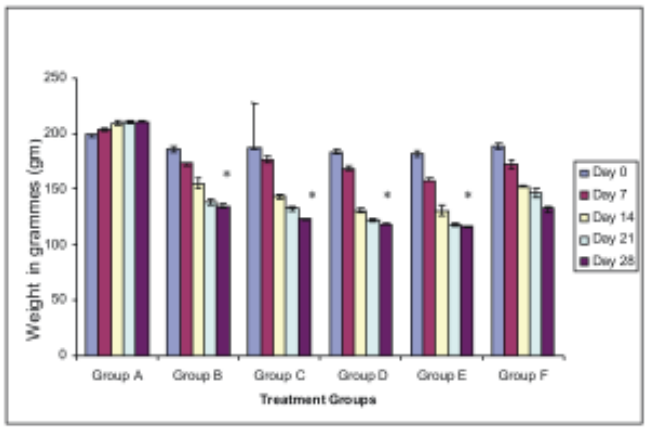

*Figure 1: The effect of O.gratissimum on Body Weight. Each bar represents Mean ± S.E.M *p<0.05 group by group comparison, n = 6. Groups A- non diabetic control, B- Metformin, C- 400 mg, D- 600 mg, E-800 mg, F- Diabetic control.*

received *O. gratissimum* compared with the non- diabetic control (Group A). Administration of the extract caused a 65.6%, 64.4% and 63.62% reduction in body weight in groups C, D and E respectively at day 28 compared with their initial body weight. Metformin administration also resulted in a significant reduction in body weight when compared to non- diabetic control; its administration resulted in a 72% reduction in body weight between days 0 and 28. Compared to *Ocimum gratissimum*, metformin caused more reduction in body weight although the effect was not statistically significant.

Effects of ocimum gratissimum on blood glucose

The mean blood glucose levels monitored weekly for a period of 28 days in all experimental groups are presented in Fig. 2. There was a statistically significant ($F(5, 35) = 42.23$, $p<0.05$) reduction in blood glucose following administration of *O. gratissimum* (C, D and E) compared to the diabetic control (group F). Administration of extract caused a 17.2%, 17.06%, 15.87% reduction in blood glucose levels in groups C, D and E respectively at day 28 compared to initial blood glucose levels. Metformin administration also resulted in a significant drop in the blood glucose level as seen by a 28% reduction in blood glucose between days 0 and 28. Compared to *Ocimum gratissimum*, metformin caused more reduction in blood glucose although the effect was not statistically significant.

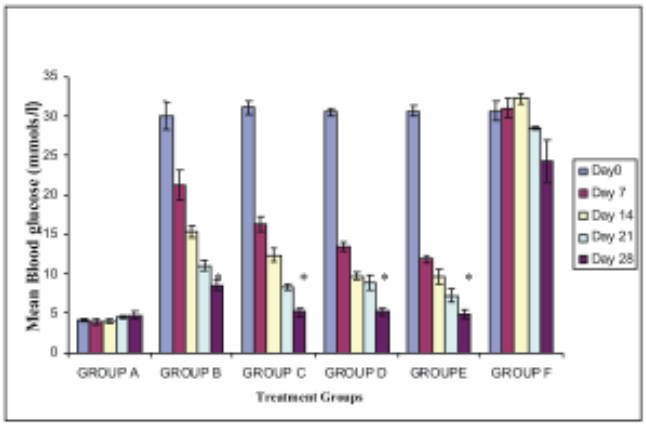

Figure 2: The effect of O.gratissimum *on Blood Glucose. Each bar represents Mean ± *S.E.M p<0.05 group by group comparison, n = 6. Groups A- non diabetic control, B- Metformin, C- 400 mg, D- 600 mg, E-800 mg, F Diabetic control.*

Effects of ocimum gratissimum *on blood creatinine*

Figure 3 shows the mean levels of blood creatinine in experimentally induced diabetic animals following an experimental period of 28 days. There was a statistically significant ($F(5, 35) = 7.43$, $p<0.05$) increase in blood creatinine in the diabetic control (group F) when compared to the non diabetic control (group A). The diabetic state resulted in a 300 % increase in the creatinine level (190 μmol/L vs.63.83 μm/L). Administration of *O. gratissimum* caused dose dependent increases in creatinine levels and this increments ranged from 100 - 260% (66.17 μmol/L, 137 μmol/L, 167 μmol/L) in groups C, D and E respectively compared to the non diabetic control (63.83 μmol/L), however only the increments seen in groups D and E were statistically significant. Administration of Metformin caused a mild increase in blood creatinine compared to non diabetic control (66.17 μmol/L vs.63.83 μmol/L).

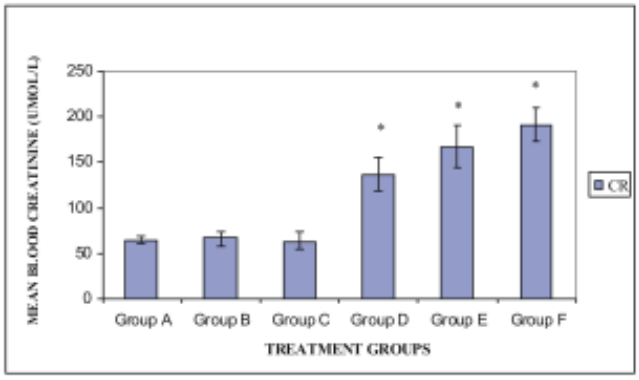

*Figure 3: The effects of O.gratissimum on blood creatinine levels. Each bar represents, Mean ± S.E.M, *p<0.05 group by group comparison, n = 6. Groups A- non diabetic control, B- Metformin, C- 400 mg, D- 600 mg, E-800 mg, F Diabetic control.*

The effect of ocimum gratissimum *on blood urea levels*

Figure 4 shows the mean levels of blood urea in experimentally induced diabetic animals following an experimental period of 28 days. There was a significant ($F(5, 35) = 16.51$, $p<0.05$) increase in blood urea level in the diabetic control group (F) compared to the non diabetic control. The diabetic state resulted in over 650 % increase (34.17 vs. 3.967 mmol/L). *O. gratissimum* caused dose dependent increase in levels of serum urea with increments of between 250 - 300% (7.63, 15 and 19.07 mmol/L) in groups C, D and E respectively compared to the non diabetic control (3.967 mmol/L), however only the increments seen in groups D and E were statistically significant. The Metformin group showed a slight reduction in urea level compared to non diabetic

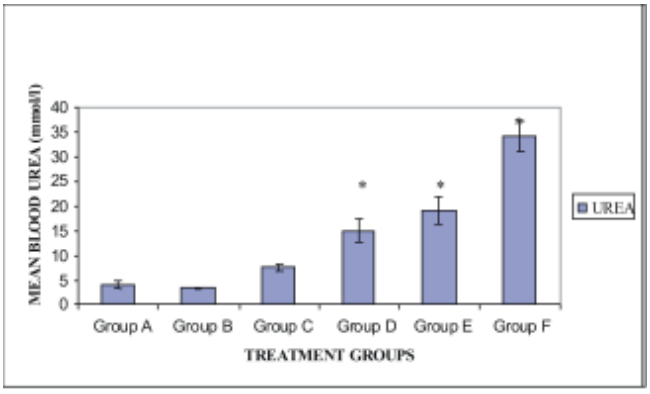

*Figure 4: Effect of O.gratissimum on blood Urea levels. Each bar represents, Mean ± S.E.M, *p<0.05 group by group comparison, n = 6. Groups A- non diabetic control, B- Metformin, C- 400 mg, D- 600 mg, E-800 mg, F Diabetic control.*

control although this difference was only visual (3.25 vs.3.967 mmol/L).

The effect of ocimum gratissimum *on relative weight of the kidney*

Tables 1 shows the mean relative weights of the kidney in all experimental groups. Kidney weight was significantly ($F(5, 35) = 9.08$, $p < 0.05$) higher in the rats in the diabetic control group compared to non diabetic control (3.72 ± 0.22 vs. 2.30 ± 0.21). There was also a statistically significant ($F(5, 35) = 9.08$, $p < 0.05$) dose dependent increase in kidney weight in rats in groups C, D and E that received *O. gratissimum* compared to the

Table 1: Showing of weights of animals and weights of kidney at time of sacrifice Each value represents, Mean ± S.E.M, *p<0.05 group by group comparison, n = 6. Groups A- non diabetic control, B- Metformin, C- 400 mg, D- 600 mg, E-800 mg, F - Diabetic control.

Groups	Mean weight at sacrifice	Mean Kidney Weight	Relative Kidney weight gm/kg body weight
A	210 ± 0.62	0.482 ± 0.32	2.30 ± 0.21
B	134 ± 2.217	0.390 ± 0.33	2.91 ± 0.13
C	122 ± 1.23	0.460 ± 0.28	3.77 ± 0.31*
D	118.3 ± 0.62	0.474 ± 0.24	4.02 ± 0.21*
E	115.8 ± 0.72	0.486 ± 0.33	4.23 ± 0.33*
F	131.8 ± 2.70	0.490 ± 0.33	3.72 ± 0.22*

animals in non diabetic control group (3.77 ± 0.31, 4.02 ± 0.21, 4.23 ± 0.33 vs. 2.30 ± 0.21). Comparing animals in group B that received Metformin to the non diabetic control revealed an increase in kidney weight (2.91 ± 0.13 vs. 2.30 ± 0.21) that was however not statistically significant.

Result of kidney histopathology

Examination of the kidneys of groups A and B animals revealed grossly normal kidneys. Kidneys taken from animals in groups C and F were enlarged. Examination of kidneys taken from group D and E showed pethechial haemorrhages under the Glisson's capsule.

Slides taken from sections of the kidney of animals in group A (Figure 5a) showed normal kidneys with well demarcated cortex and medulla. Glomeruli, renal tubules, collecting ducts and blood vessels all appeared normal. Sections from group B (Figure 5b) showed normal kidney architecture with some increase in inflammatory cells within the glomerulus. Groups C (Figure 5c), D (Figure 5d) and E (Figure 5e) showed loss of normal renal architecture and varying degrees of inflammatory cell infiltration within the glomerula and interstitium. Some of the glomeruli were distorted and slightly expanded with venous congestion. Slides of animals in group F (Figure 5f) revealed distortion of the normal renal architecture, hyperthrophied glomeruli and dilated renal tubules.

Discussion

Diabetic nephropathy is the largest single cause of end-stage renal failure worldwide. Despite the available modern therapies of glycaemic and blood pressure control, many patients continue to show progressive renal damage. Nephropathy is defined as partial loss of function of kidney associated with nephrotic syndrome, glomerulosclerosis, persistent albuminuria, declining glomerular filtration rate (GFR), elevated arterial blood pressure and fluid retention [23]. The intention of this study was to evaluate the ability of *Ocimum gratissimum* to protect against changes that occur in the kidney as a result of diabetes mellitus, however, we observed that *Ocimum gratissimum* is not protective against STZ induced morphological and biochemical changes in the kidney but rather worsens it.

Ocimum gratissimum treatment caused a significant dose dependent reduction in body weight, elevation of renal function indices and loss of normal kidney architecture compared to non diabetic rats. This study also showed that Metformin causes a significant reduction in body weight. Metformin effects on body weight make it the drug of choice for obese diabetes. Several studies have shown that it also helps non-diabetics lose weight by reducing hunger [24]. The mechanisms responsible for weight loss following Metformin use are still subject to investigation. Recent studies however postulate that a decrease in carbohydrate metabolism and an increase in that fat

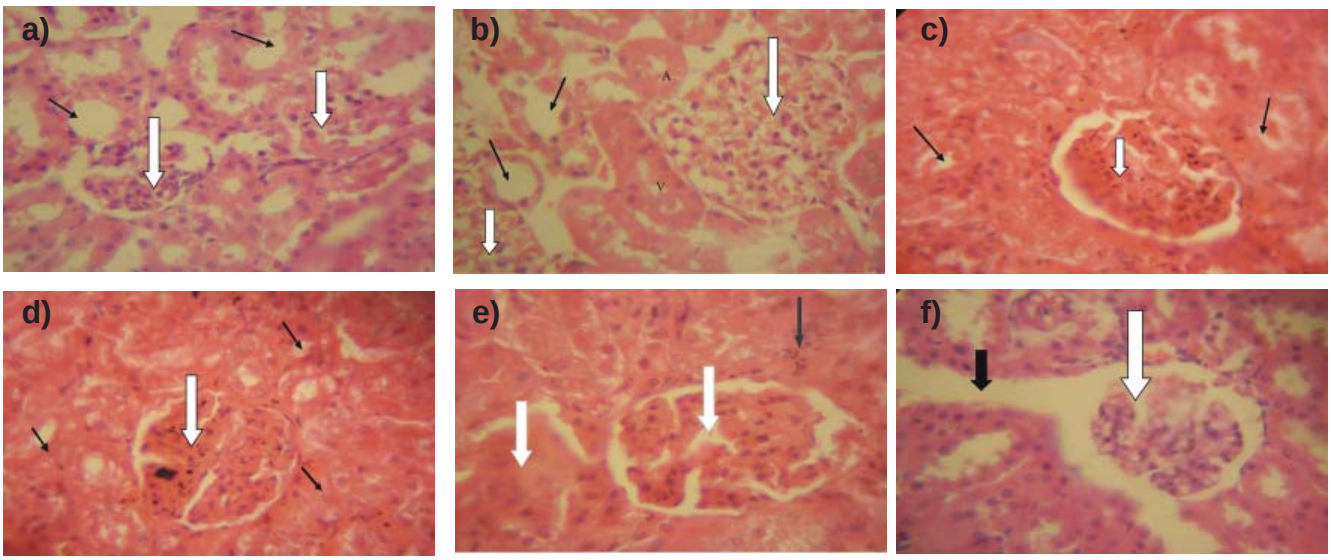

Figure 5: a) Group A showing normal rat kidneys: normal glomeruli with prominent nuclei (white arrows), renal tubule (thin arrows). (H&E x400); b) Group B showing rat kidney: normal glomeruli with prominent nuclei (white arrows), renal tubule thin arrows), arteries (A) and veins (V), increase mononuclear cell infiltration of the glomerulus (H&E x400); c) Group C showing rat kidney with fat infiltration, collapsed necrotic glomeruli with pyknotic nuclei (white arrow), and contracted renal tubule (thin arrows). Mononuclear cell infiltrations. (H&E x 400); d) Group D showing rat kidney: collapsed necrotic glomeruli with pyknotic nuclei (white arrow), Contracted renal tubules (thin arrows). Numerous inflammatory cells and cellular debris seen within renal parenchyma (H&E x400); e) Group E showing rat kidney: hypertrophied and granular glomeruli with pyknotic nuclei (white arrows), congested tubules (thin arrows). Numerous inflammatory cells and cellular debris seen within renal parenchyma (H&E x400); f) Group F showing rat kidney with shrunken glomeruli (white arrow) renal tubular dilatation (black arrow), marked mononuclear cell infiltration. (H&E x 400).

metabolism may be responsible [25]. Reduction in body weight in the STZ diabetic rats in comparison to normal rats indicate excessive break down of tissue proteins [26]. *O. gratissimum* in this study caused more weight reduction in comparison to diabetic control.

The results of kidney biochemistry showed that STZ diabetic rats had a significant elevation in urea and creatinine levels indicative of renal cell injury. The metformin group showed a reduction in the levels of these substances, indicating protection against renal injury or possible recovery. Effects of *O. gratissimum* on urea and creatinine mirrored its effect on the liver transaminases documented in a prior study [9]. There was a dose related increase in urea and creatinine which would indicate renal injury worse with increasing doses. Histopathology of diabetic rats showed hyperthrophied and granular glomeruli with pycnotic nuclei, dilated renal tubules, numerous mononuclear cells infiltrates and cellular debris seen within renal parenchyma. The diabetic rats that received, metformin showed normal glomeruli with prominent nuclei, some renal tubule dilation and increased mononuclear cell infiltration. The kidneys showed mild to moderate signs of renal injury at increasing doses of *O. gratissimum*.

Accumulation of glycogen in the kidney tubules as a result of the ensuing hyperglycaemia is thought to be responsible for the progression of disease in diabetic nephropathy [27]. *O. gratissimum* at the doses of 600 and 800 mg/kg showed worse features of toxicities compared to that seen with the diabetic control. The results of this study show that *O. gratissimum* in its crude state should be used with immense caution if at all in the treatment of diabetes

Conclusion: The study concluded that *O. gratissimum* reduces body weight and blood glucose parameters at all doses but worsens Streptozotocin-induced renal injury at increasing doses.

References

1. Man MQ, Shi YY, Man M. Chinese herbal medicine (Tuhuai extract) exhibits topical anti-proliferative and anti-inflammatory activity in murine disease models. Experimental Dermatology. 2008;17(8):681–687.

2. Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A. *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. The Open Compl Med J. 2009);1: 1-15.

3. Aguiyi JC, Obi CI, Gang SS, Igweh AC. Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapia. 2000;71:444-446.

4. Rabelo M, Souza EP, Soares PMG. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz J Med Biol Res. 2003;36: 521-4.

5. Rossing P. Diabetic nephropathy: worldwide epidemic and effects of current treatment on natural history. Curr Diab Rep. 2006;6:479–483.

6. Makino H, Miyamoto Y, Sawai K, Mori K, Mukoyama M, Nakao K, Yoshimasa Y, Suga S. Altered gene expression related to glomerulogenesis and podocyte structure in early diabetic nephropathy of db/db mice and its restoration by pioglitazone. Diabetes. 2006;55**:**2747–2756.

7. Hovind P, Tarnow L, Rossing K. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. Diabetes Care. 2003;26(4):1258–1264.

8. Yokoyama H, Okudaira M, Otani T. Higher incidence of diabetic nephropathy in type 2 than in type 1 diabetes in early-onset diabetes in Japan. Kidney International. 2000;58(1):302–311.

9. Onaolapo AY, Onaolapo OJ, Adewole OS. Ethanolic Extract of *Ocimum Grattissimum* Leaves (Linn.) Rapidly Lowers Blood Glucose Levels in Diabetic Wistar Rat. Maced J Med Sci. 2011; 4(4):351-357.

10. Farnsworth NR. Biological and Phytochemical screening of plants. J Pharm Sci. 1966;35:225-276.

11. Harborne JB. Phytochemical analysis: A Guide to Modern Techniques of Plant analysis, 3rd edition. Chapman and Hall. London, 1998.

12. Silva LG, Lee IS, Kinghom DA. Special problems with the extraction of plants; In: Methods in Biotechnology Natural product isolation. Cannell JPR (ed.) Humana, press Inc., Totowa, New Jersey, USA, 1993;4:329-363.

13. Houghton PJ, Raman A. Laboratory handbook for fractionation of natural extracts. Chapman and Hall: London, 1998:199.

14. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275-287.

15. Tanko MY, Okasha MA, Magaji RA, Yaro AH. Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozotocin induced diabetic wistar rats. Afr J Biotechnology. 2007;6: 2087-90.

16. Zafar M, Naeem-ul-Hassan Naqvi S, Ahmed M, Kaim Khani Z. Altered liver morphology and enzymes in streptozotocin-induced diabetic rats. Int J Morphol. 2009; 27(3):719-25.

17. Yanardag R, Ozsoy-Sacan O, Bolkent O, Orak H, Karabulut-Bulan O. Protective effects of metformin treatment on the liver injury of streptozotocin-diabetic Rats. Human & Exp Tox. 2005;24:129 -135.

18. Trinder P. Determination of blood glucose using 4- amino phenazone as Oxygen acceptor. J Cli Path. 1969;22:246-248.

19. Jaffé M. Uber den Niederschlag, welchen Pikrinsäure in normalem Harnerzeugt und über eine neue Reaktion des Kreatinins. Z Physiol Chem. 1986;10:391 400.M.

20. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest. 1965;17(4):381-7.

21. Fearon WR. The carbamido diacetyl reaction: a test for citrulline. Biochem J. 1939;33(6):902-7.

22. Wybenga DR, Di Giorgio J, Pileggi VJ. Manual and automated methods for urea nitrogen measurement in whole serum. Clin Chem. 1971;17(9):891-5.

23. Blickle JF, Doucet J, Lrummel T, Hannedouche T. Diabetic nephropathy in the elderly. Diabetes Metab. 2007;33:S40-S55.

24. Paolisso G, Amato L, Eccellente R, Gambardella A, Tagliamonte MR, Varricchio G, Carella C, Giugliano D, Donofrio F. Effect of metformin on food intake in obese subjects. Eur J Clin Investig. 1998;28: 6:441-446.

25. Pedersen J, Olesen ES. Observations on the mechanism of increased weight loss during metformin administration in obesity. Acta Endocrinol (Copenh). 1968;57(4):683-8.

26. Andallu B, Varadacharyulu N. Antioxidant role of mulberry (Morus indica L. cv.anantha) leaves in streptozotocin-diabetic rats. Clin Chim Acta. 2003;338: 3-10.

27. Ramesh C, Gopal V, Sembulingam K, Nappinnai M. Therapeutic management strategies for type 2 diabetes. Curr Diabetes Rev. 2006;2(3):339-42.