The Egyptian Journal of Hospital Medicine (2009) Vol., 36 : 380- 396

Therapeutic Potential of Green Tea Extract and Low Doses of γ-Irradiation on Diabetic Nephropathy of Rats

Hanafy N.A. and Hanaa F. Waer
Radiation Biology Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

Abstract

Introduction: Diabetic nephropathy is one of the most frequent and serious complications of diabetes mellitus. This study was designed to evaluate the effect of green tea (GT) extract and low doses of 0.5 Gy γ-radiation (R) on diabetic nephropathy (DN) of rats.
Materials and methods: Male Swiss albino rats were used in this study. DN was induced in rats using streptozotocin (45 mg/kg body weight). The rats were divided into five groups DN, DN+R, DN+GT, DN+GT+R and a sham treatment control group. Throughout the experimental period (3and 6 weeks) animals body weight, glucose and insulin levels were evaluated. Kidney functions assay (serum urea and creatinin) were recorded. Histopathological observations in kidney tissue, DNA and glycogen intensity were also detected.
Results: Diabetic rats exhibited many symptoms including loss of body weight, increase in blood glucose level and decrease in serum insulin levels. Increase in serum urea and creatinin levels. Diabetic kidney showed a moderate renal damage, multifocal clarifications and vacuolations. Carbohydrates intensity showed a significant increase and DNA intensity showed many alterations. Improvements in glomerular and tubulointerstitial lesions were demonstrated in the diabetic rat group exposed to low doses of γ-radiation or supplemented by green tea either alone or combined in addition to amelioration in glucose, insulin urea and creatinin levels.
Conclusion: The present study demonstrates the efficacy of low doses of γ- radiation and in reducing diabetes-induced functional and histological alterations in the kidneys. The longterm control of blood glucose levels using low doses of γ-radiation or green tea either alone or combined could prevent the progression of diabetes mellitus, and therefore, nephropathy could be prevented.

Introduction

Diabetic nephropathy is one of the major microvascular complications of diabetes. The renal lesions, whether related to type 1 or 2 diabetes mellitus, are similar (**Fioretto and Mauer, 2007**). At present, patients with diabetes mellitus in the world were increased by millions, and the number is increasing; however, maintaining prolonged dialysis therapy is a great burden on patients both mentally and physically, and social problems, including financial issues, have also been raised. Therefore, the daily consumption of food and drink containing effective agents for the management of onset and/or progression of diabetic complications has been receiving attention to reduce the number of patients with end-stage renal failure. There is much debate over the health benefits of green tea extracts, and it is common to drink tea with meals.

In the previous studies, it has been reported that green tea polyphenols have antioxidant properties, and green tea is a useful agent to protect against protein oxidation- and glycation-associated diseases (**Yokozawa *et al.*, 1996, 1997, 1998; Nakagawa and Yokozawa, 2002 and Nakagawa *et al.*, 2002**). Green tea polyphenols were also indicated as beneficial agents to manage the development of diabetic nephropathy induced by streptozotocin (STZ) injection (**Yokozawa *et al.*, 2005**). Alternatively, of the catechins, (⁻)-epigallocatechin 3-*O*-gallate (EGCg) is known to be the most abundant in green tea. Recently, it has been reported that EGCg had an antioxidant effect on creatinine (Cr) oxidation in rats with chronic renal failure and thus inhibited methylguanidine production in an adenine-induced renal failure model (**Nakagawa *et al.*, 2004**).

Radiation is known to have significant effects on living organisms depending on the dose received. At higher doses, radiation destroys living materials including cells in tissue. At moderate levels, it is generally harmful but is also used for beneficial purposes including radiation therapy of cancer. At low doses, on the other hand, radiation is generally regarded as safe, and its effect, if any, is considered to be negligible. However, there have been several reports of interesting but unexpected effects of low-dose ionizing radiation on living organisms. For example, sublethal low-dose irradiation has been shown to induce an increased life span in insects (**Caratero *et al.*, 1998**), increased resistance to oxygen toxicity (**Lee and Ducoff, 1984**), and enhancement of immune function (**Yoon *et al.*, 1998**). These results have suggested the existence of significant biological effects of low-dose irradiation. Since many diseases have been shown to be caused by pathological oxidative stress, the present study examined the effects of low-dose irradiation and the dietary green tea extract in suppresses the oxidative stress of diabetic neuropathy using streptozotocin (STZ)-induced diabetic rats.

Material and Methods

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Green tea extract

Green tea extract was obtained from Arab Company for Pharmaceuticals and Medicinal plants MEPACO-Egypt (Enshams El Raml - Sharkeiya) in the form of tablets each tablet contain 300 mg of green tea dray extract. In the present study green tea extract was prepared by dissolving the tablets in distilled water at dose level 45mg/1ml/rat/day. It was administered daily by gavage.

Radiation facility:

Whole body gamma irradiation was performed at the National Centre for Radiation Research and Technology, Atomic Energy Authority (NCRRT), Cairo, Egypt, using Caesium -137 in a Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). Animals were exposed to fractionated dose levels of 0.5 Gy/week of γ-radiation. The γ-radiation delivered at a dose rate of 0.61 Gy min^{-1} for 3 and 6 weeks.

At the end of the experimental periods, animals were killed by cervical decapitation. Serum was collected. Kidneys were removed and washed in ice-cold saline.

Experimental Design.

Adult male Swiss albino rats, weighing 120 to 130 g, were obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo) were used in this study. They were allowed free access to laboratory pellet chow (24.0% protein, 3.5% lipid, and 60.5% carbohydrates) and water. After several days of adaptation rats were injected intraperitoneally with freshly prepared streptozotocin (STZ) at the dose of 45 mg / kg b.w. (**Chattopadhyay *et al.*, 1997**) in 0.1 M Citrate buffer of pH 4.5 (**Mitra *et al.*, 1996**). Control animals were received citrate buffer alone. Induction of the diabetic state was confirmed by measuring blood glucose levels 72 h after injection of STZ. The rats whose blood glucose concentrations were $\geq$ 300 mg/dl were randomly divided into four groups: DN (Diabetic group). DN+R (diabetic group exposed to 0.5 Gy/week of γ-radiation for 3 and 6 weeks). DN +GT (diabetic group supplemented by 45mg/1ml/rat/day green tea extract for 3 and 6 weeks). DN+GT+R (diabetic group supplemented 45mg/1ml/rat/day green tea extract and exposed to 0.5 Gy/week of γ-radiation for 3 and 6 weeks). A normal group of rats that underwent sham treatment was also included.

Blood Glucose

The blood glucose level was determined by glucose oxidase method using a one touch basic plus glucometer (Lifescane Ltd., California, USA).

Insulin assay
Serum insulin levels were determined by Biosource –INSEASIA according to Temple *et al.* (1992).

Kidney functions assay
Kidney function was assayed in the form of serum urea and creatinin assay Serum urea was determined by colorimetric test (**Fawcett and Scott, 1960**) using the available reagent kit. Serum creatinine was determined by kinetic test without deproteinization (**Bartels *et al.*, 1672**) using the available reagent kit.

Histological and histochemical studies
Kidneys ware fixed in 10% buffered formalin and embedded in paraffin for the histological and histochemical studies. Kidney sections were cut at 4 mm with a microtome and deparaffined with xylene. They were stained with hematoxylin and eosin (H–E) for the histological studies (**Drury and Wallington, 1976**). Feulgen reaction staining was used for DNA detection (**Sheehan and Hrapchak, 1980**). Periodic acid-Schiff's (PAS) method to demonstrate carbohydrates (**Hotchkiss, 1948**).

Statistical Analysis
The statistical analysis was carried out using SPSS version 10 statistical programs (SPSS Inc., Chicago, IL, USA). Differences among treatments within the experiment were analyzed by one-way analysis of variance (ANOVA). Significant differences between treatment means were determined by student t-test. The results are presented as mean ± SE of five independent experiments unless stated otherwise.

Results

Effect of green tea extract and low doses of γ-irradiation on fasting glucose levels, insulin levels and body weight of diabetic rat.
Rats received STZ became diabetic at a frequency of 70%. The anti-hyperglycaemic effect of the low dose of γ-radiation and green tea supplementation either alone or combined on the fasting blood sugar levels of diabetic rats is shown in table (1). The plasma glucose levels of diabetic-induced rats significantly increased 72 h following the induction. Thus, the initial blood glucose level of the diabetic rats was 300±11.35 mg/dl compared to basal glycaemia of non diabetic control animals (86.67±2.19 mg/dl). Exposure of diabetic rats to 0.5 Gy/week of γ radiation for 3 and 6 weeks (DN +R) significantly decreased the blood glucose compared to DN group. Green tea extract at a dose of 45mg/1ml/rat/day, administered to diabetic rats, immediately after diagnosis of diabetes (DN +GT), produced a significant decrease in glucose level ($P < 0.05$) compared with diabetic controls. In DN + GT+R group, combined treatment of the diabetic rats by green tea and γ-radiation exposure revealed more significant decrease in fasting glucose level compared with the diabetic group.

A significant decrease in insulin level was detected in the group suffered from diabetes mellitus (0.173±0.015) compared to the control level (0.322±0.012). Exposure of the diabetic animals to 0.5 Gy/week of γ-radiation (DN +R) significantly increase insulin level by increasing the period of γ-radiation exposure. Gavage intubations of the diabetic animals by 45mg/1ml/rat/day GT extract produced a more significant increase in insulin level compared to those of DN group either after 3 weeks or 6 weeks. Nearly normal level of insulin was recorded when diabetic animals treated with GT extract and exposed to 0.5 Gy/week of γ-radiation for 3 or 6 weeks of diabetic induction.

Diabetes was associated with reduced body weight when compared with the control rats. Exposure of the diabetic group to 0.5 Gy/week of γ- radiation showing some improvement in animal's body weight either after 3 or 6 weeks compared to DN group. Gavage of the diabetic animals with GT represents a significant increase in the body weight after 6 weeks. Also gavage of the diabetic animals with 45mg/1ml/rat/day GT in addition to the exposure to 0.5 Gy/week of γ-radiation represented more significant increase in body weight after 6 weeks in compression to control and DN groups.

Table1: Effect of green tea extract and low doses of γ-irradiation on fasting plasma glucose levels, insulin levels and body weight of diabetic rat.

Parameters	Period of time	Groups				
		Control	DN	DN +R	DN +GT	DN +GT+R
Glucose (mg/dl)	3Weeks	86.67±2.19[b]	300±11.35[a]	107.33±3.29[ab]	99.33±4.22[b]	102.83±4.72[b]
	6Weeks			111.50±1.50[ab]	95.67±0.92[b]	97.67±1.41[b]
Insulin (ng/ml)	3Weeks	0.322±0.012[b]	0.173±0.015[a]	0.199±0.018[a]	0.247±0.034[a]	0.224±0.006[ab]
	6Weeks			0.332±0.015[b]	0.355±0.029[b]	0.372±0.027[b]
B.WT (gm)	3Weeks	249.5±8.02[b]	203±4.12[a]	212±4.16[a]	185±4.83[ab]	201.67±4.64[a]
	6Weeks		157±11.14[a]	174.67±8.04[a]	221±14.08[ab]	264±15.30[b]

Each value represents the mean of 6 records ± S.E.
Means with different superscripts are significantly different at the 0.05 level.
Control: sham treatment group. *DN: (Diabetic group).*
DN+R: diabetic irradiated group. *DN +GT: diabetic group supplemented by green tea.*
DN +GT+R: diabetic group supplemented by green tea exposed to γ radiation.
a: significantly difference from control level. *b: significantly difference from diabetic level.*

Effect of green tea extract and low doses of γ-irradiation on serum urea and creatinine levels of diabetic rat.

In table 2 low doses of γ-radiation and GT treatments produced declined in urinary function (urea and creatinine excretion rates) of diabetic animals which increased significantly in DN group compared with normal control rats either after 3 or 6 weeks. In DN +GT+R group combined treatment of the diabetes rats by green tea and γ-radiation exposure significantly lower urea and creatinine excretion rates compared with DN grou

Table2: Effect of green tea extract and low doses of γ-irradiation on serum urea (mg/l) and creatinine (mg/d) of diabetic rat.

Parameters	Period of time	Groups				
		Control	DN	DN +R	DN +GT	DN +GT+R
Urea (mg/l)	3Week	148±19[b]	455±35 [a]	296±27[ab]	390±24[a]	343±20[a]
	6Weeks		481±25 [a]	177±16[ab]	280±31[ab]	228.5±33 [ab]
Creatinine (mg/d)	3Weeks	0.33±0.13[b]	0.85±0.10[a]	0.55±0.09[ab]	0.76±0.12[a]	0.66±0.10[a]
	6Weeks		0.96±0.29[a]	0.49±0.08[ab]	0.65±0.07[ab]	0.57±0.13[ab]

Legends as in table (1)

Histological results

Histological examination of the sections of kidney from normal control rat is shown in Fig. A (1). The circular areas observed in this photograph is the renal Malpighian corpuscle (↑). It is composed of glomerulus surrounded by Bowman's capsule with thin glomerular basement membranes and patent capsular space. Numerous tubules (proximal and distal) lie in the area adjacent to the glomerulus.

Kidney section of STZ-diabetic rat showed highly affected cytoplasm and nuclei of the convoluted tubules, some of the distal convoluted tubules cells appeared free from nuclei (curved arrow), others contained marginal chromatin (▼), the remnant contained pyknotict nuclei (p). Debris of ruptured cells could be detected inside the tubules (●). (Fig. A, 2) compared to kidney of non diabetic control rat.

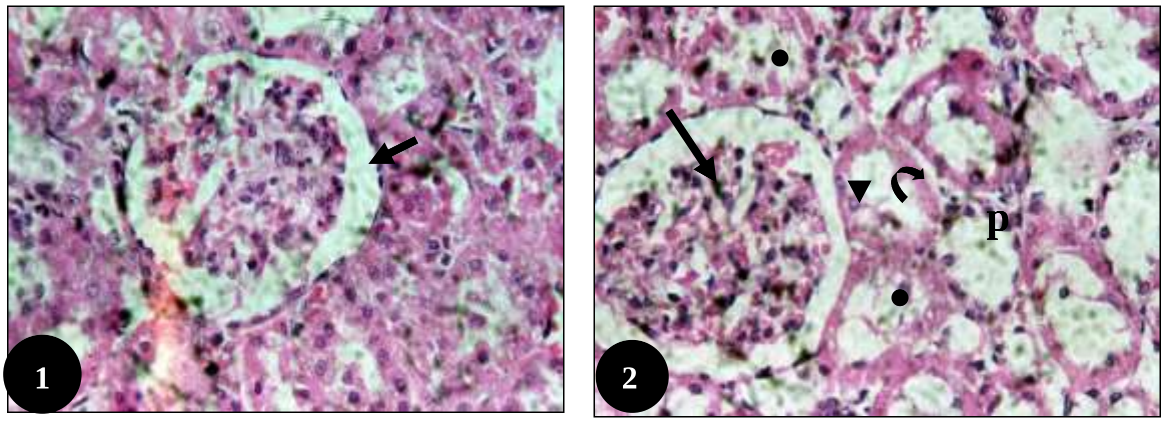

Fig (A): photomicrographs of kidneys sections. 1: Control non diabetic rat. 2: STZ diabetic rat. Sections were stained with H&E. Magnifications X400.

When STZ-diabetic gavage administrated by GT for 3 weeks, a well developed glomerulus (↓) proximal (px) and distal (ds) convoluted tubules in kidney tissue section were detected. (Fig. B, 3) while treatment for 6 weeks showed also a well developed convoluted tubules but the glomerulus showed lobulated structure with deeply stained nuclei (→) (Fig. C, 6). Exposure of the STZ-diabetic rat to 0.5 Gy/week of γ-radiation for 3 weeks represented normal structure of the glomerulus and proximal and distal convoluted tubules (Fig. B, 4). But the exposure for 6 week showed atrophied glomeruli, widened Bowman's space (↑) with high cellularity in the visceral layer of the Bowman's capsule (curved arrow) (Fig. C, 7).

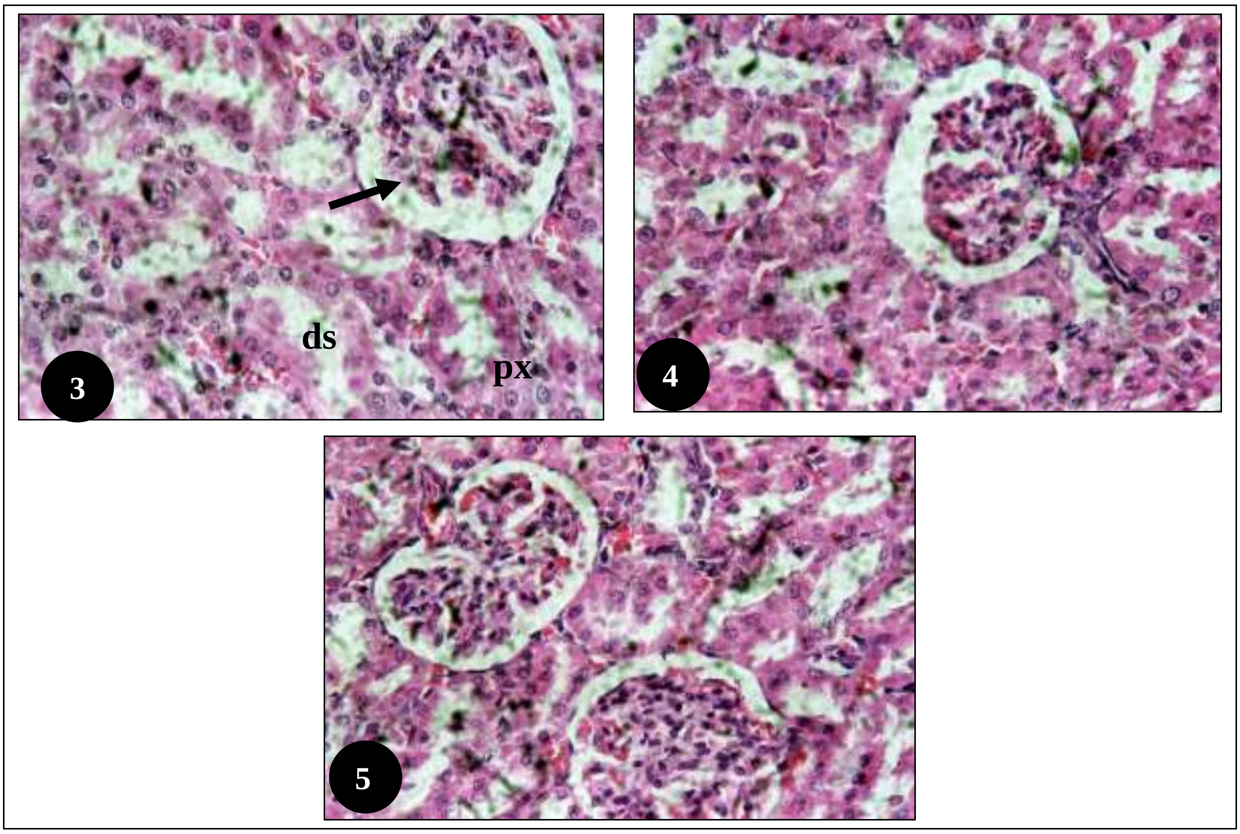

Fig (B): photomicrographs of kidneys sections. 3: STZ diabetic rat treated by GT for 3 weeks. 4: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 3 weeks 5: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 3 weeks. Sections were stained with H&E. Magnifications X400.

Kidney of the diabetic rats gavage treated by GT and exposed to 0.5 Gy/week of γ radiation for 3 or 6 weeks recorded normal observations in tissue sections but small haemorrhagic areas were noted in between the convoluted tubules and the glomeruli appeared slightly congested (Fig. B, 5 and C, 8

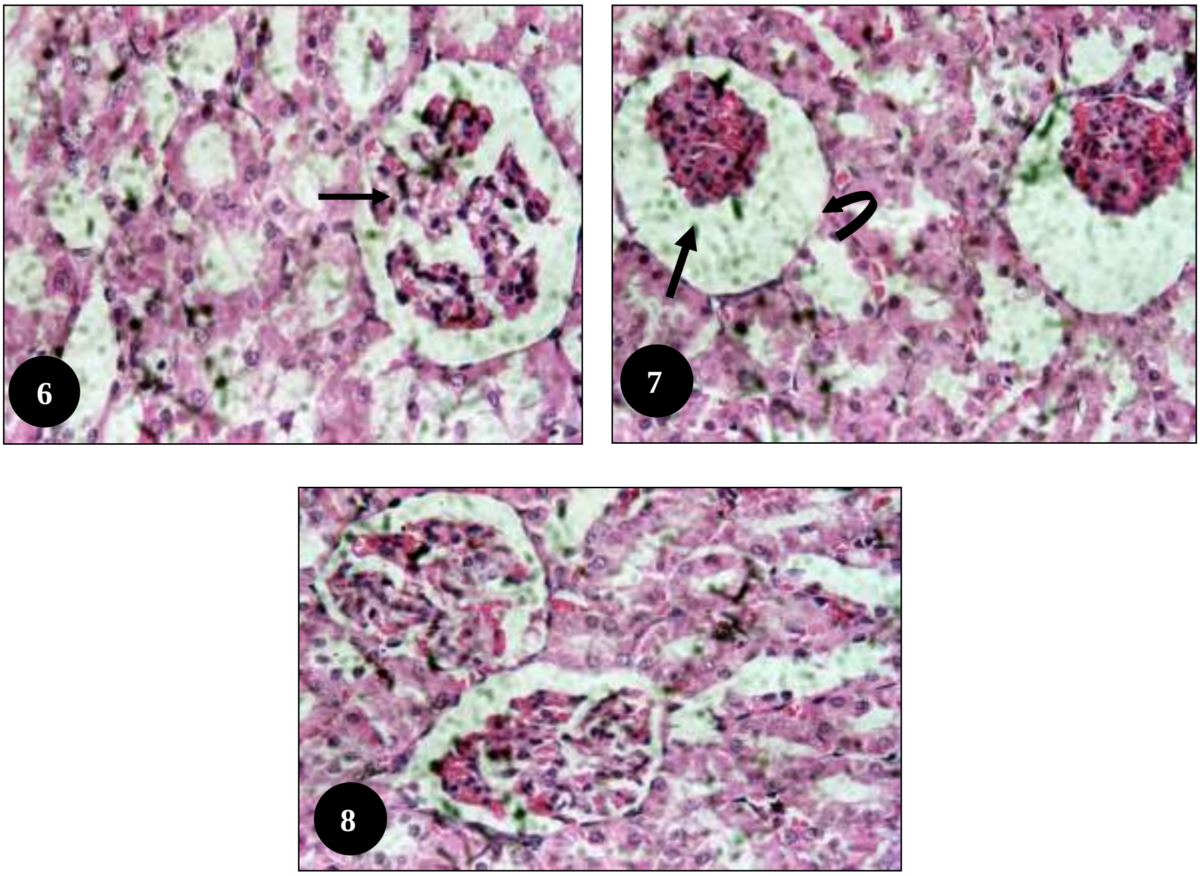

Fig (C): photomicrographs of kidneys sections. 6: STZ diabetic rat treated by GT for 6 weeks. 7: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 6 week. 8: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 6 weeks. Sections were stained with H&E. Magnifications X400.

Histochemical studies

Deoxyribonucleic acid (DNA):

In control animals Kidney sections which stained by Fuelgen method showed that some particles containing DNA material appeared in the nuclei. In some cells of the gluomerulus these particles were abundant, densely stained and scattered in the nucleoplasm, while in the other cells of the distal convoluted tubules they appeared fewer in number, faintly stained, and they were noted mainly in the nuclear periphery (Fig. D, 9). Diabetic group was observed to have a severely decreased coarse chromatin (↑) in the nuclei of cells either in the Glomerulus or in the convoluted tubules (Fig. D, 10 &10a).

Kidneys sections of STZ-diabetic rats treat ed with GT for 3 weeks showed remnants of nuclei (↓) and fragmented chromatin eit her in cells of the bowman's capsule or co nvoluted tubules (Fig. E, 11). In the other hand increase in DNA stainablity either in the convoluted tubules or Bowman's capsu le cells was observed whenever diabetic ra ts treated with GT for 6 weeks (Fig. E, 14).

Exposure of the STZ-diabetic animals to 0.5 Gy/week of γ-radiation for 3 weeks showed normal distribution of DNA particles either in cells of the glomerulus or the convoluted tubules (Fig. E, 12). Also the exposure of the STZ-diabetic animals to 0.5 Gy/week of γ-radiation for 6 weeks represented recurrence of stainable DNA nuclei either in the convoluted tubules (↑) or mesangial Bowman's capsule cells (↓) (Figs. F, 15 & 15a). More recurrence of normally DNA abundant, densely stained particles scattered in the nucleoplasm were detected in the convoluted tubules and glomerulus cells, when STZ-diabetic animals treated with 45mg/1ml/rat/day GT in addition to the exposure to 0.5 Gy/week of γ-radiation either for 3 or 6 weeks (Fig. E, 13 & F16).

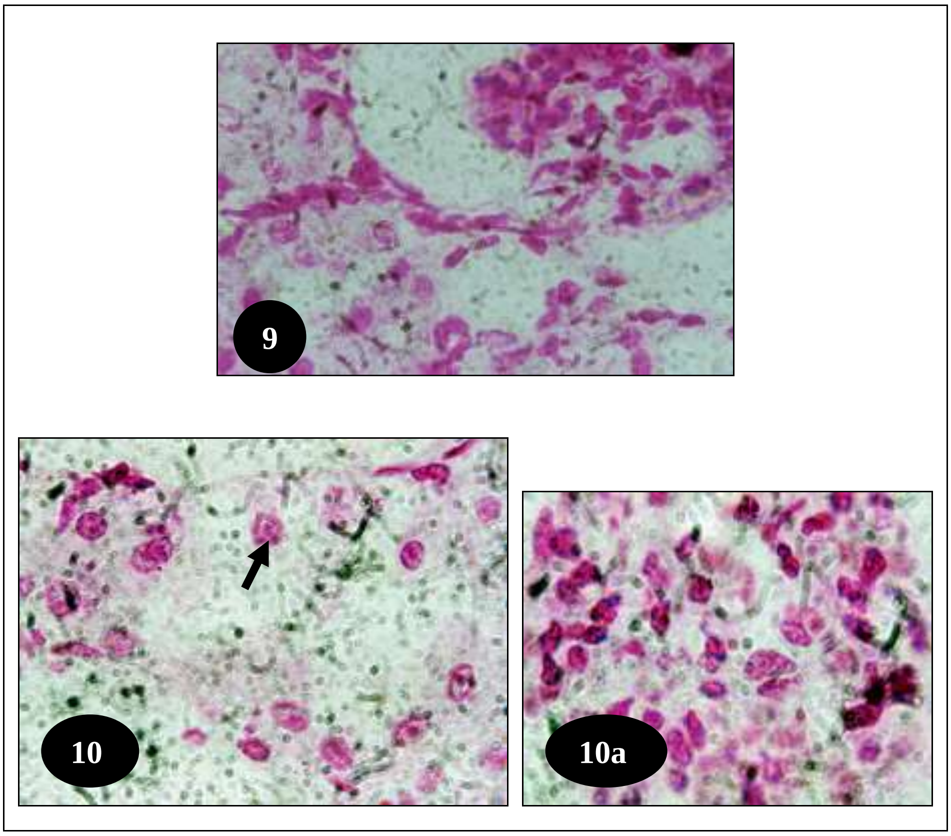

Fig (D): photomicrographs of kidneys sections. Sections showing the DNA content. 9: Control non diabetic rat. 10&10a: STZ diabetic rat. Feulgen method. Magnifications X 1000

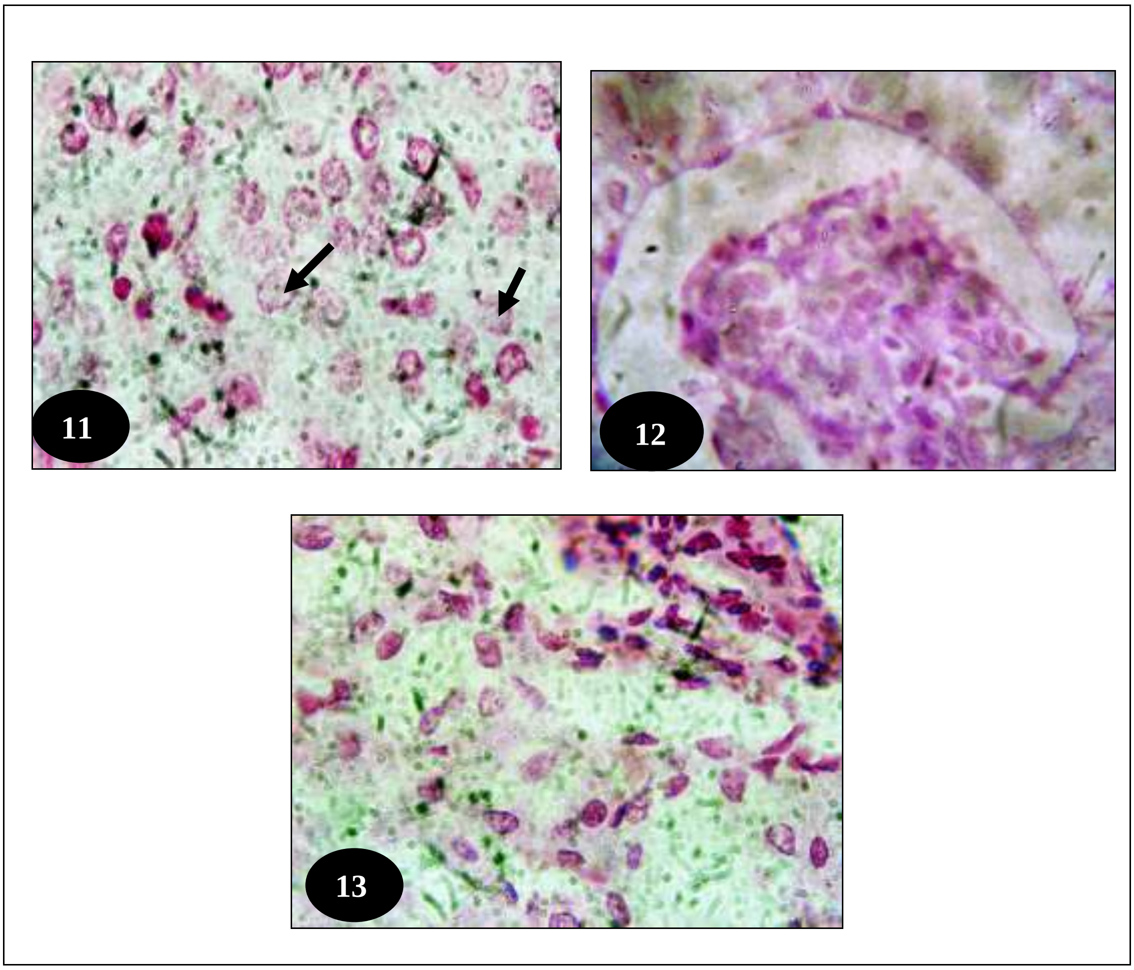

Fig (E): photomicrographs of kidneys sections. Sections showing the DNA content. 11: STZ diabetic rat treated by GT for 3 weeks. 12: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 3 week 13: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 3 weeks. Feulgen method. Magnifications X 1000

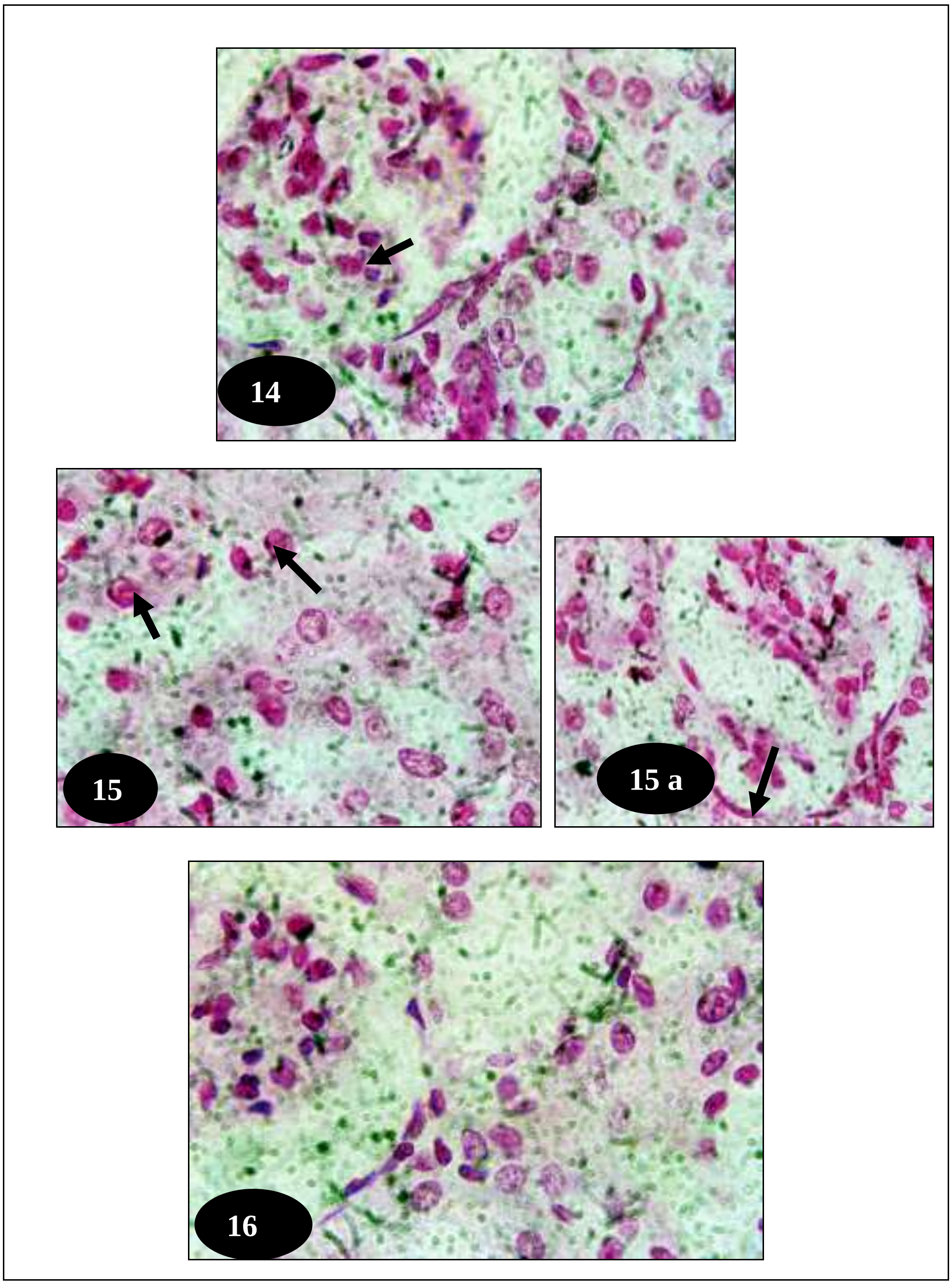

Fig (F): photomicrographs of kidneys sections. Sections showing the DNA content. 14: STZ diabetic rat treated by GT for 6 weeks. 15&15a: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 6 week 16: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 6 weeks. Feulgen method. Magnifications X 1000

Glycogen content:

The use of periodic acid schiff's (PAS) technique was done to demonstrate the presence of polysaccharides in the kidney tissues. The PAS +ve materials were mainly distributed at the brush border and basement membrane of the tubules. In this study control kidney sections represented moderate PAS positive materials in the cytoplasm and brush borders of the proximal convoluted tubules. Glomerulous was also positive to PAS reaction (fig. G17). The diabetic group kidney section appeared intensely positive to PAS reaction with moderate dense positive materials inside the lumen of the kidney tubules (fig. G18).

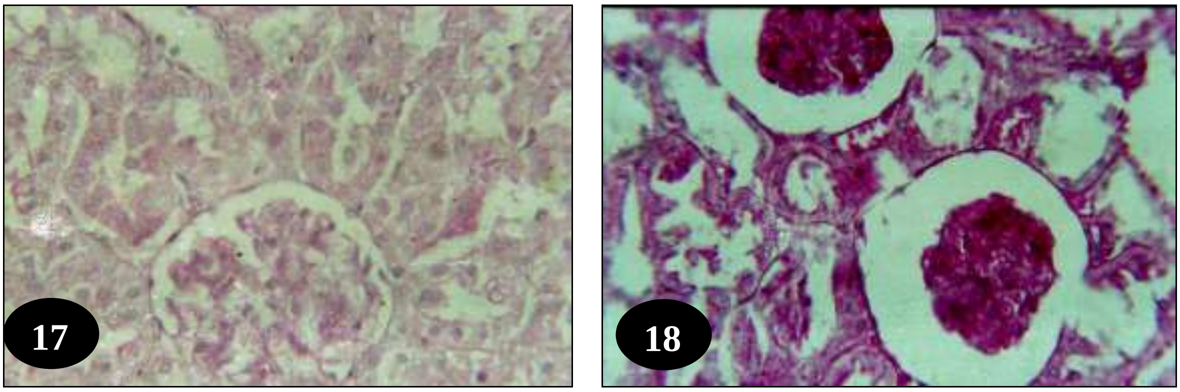

Fig. (G): photomicrographs of kidneys sections. Sections showing the glycogen content.
17: Control non diabetic rat. 18: STZ diabetic rat. PAS technique. Magnifications X 400

Exposure of the diabetic group to 0.5 Gy/ week for 3 (fig. H 20) or 6 weeks (fig. I 23), showed a reduced amount of cytoplasmic glycogen, with diminished density in the basement membranes, and brush borders of the proximal convoluted tubules. Also, the glomeruli were less stained than those of the control group.

Kidney of the diabetic rats gavage treated by GT for 3 weeks (fig. H 19) or 6 weeks (fig. 1 22) recorded less stained PAS reaction either in the basement membranes or the brush borders of the proximal convoluted tubules. Also the glomeruli represented some reactivity to PAS reaction compared to the diabetic group.

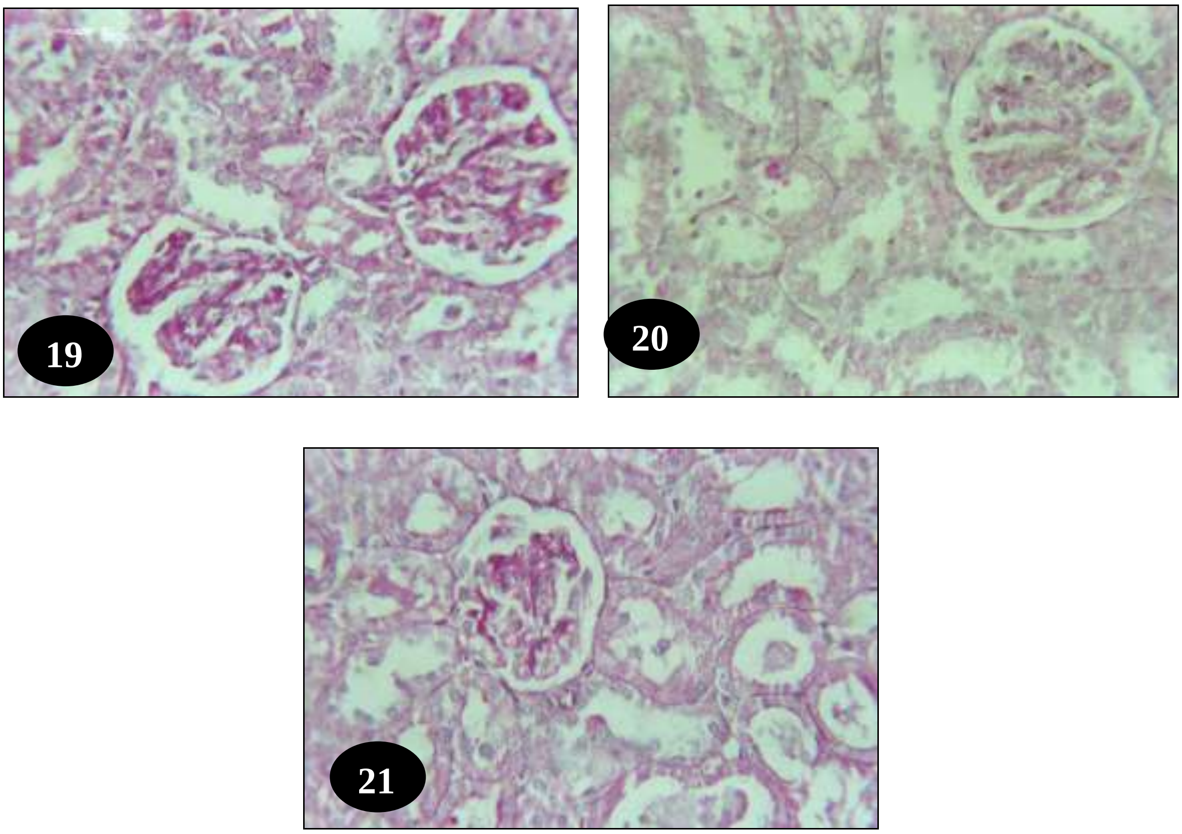

Fig (H): photomicrographs of kidney sections. Sections showing the glycogen content. 19: STZ diabetic rat treated by GT for 3 weeks. 20: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 3 week 21: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 3 weeks. PAS technique. Magnifications X 400

When STZ-diabetic animals gavage treated by 45mg/1ml/rat/day green tea in addition to the exposure to 0.5 Gy/week of γ radiation for 3 weeks (fig. H 21) recorded less stainability of PAS reaction either in the convoluted tubules or the glomeruli. However some reactivity was recorded when STZ-diabetic animals gavage treated by 45mg/1ml/rat/day green tea in addition to the exposure to 0.5 Gy/week of γ radiation for 6 weeks (fig. I 24).

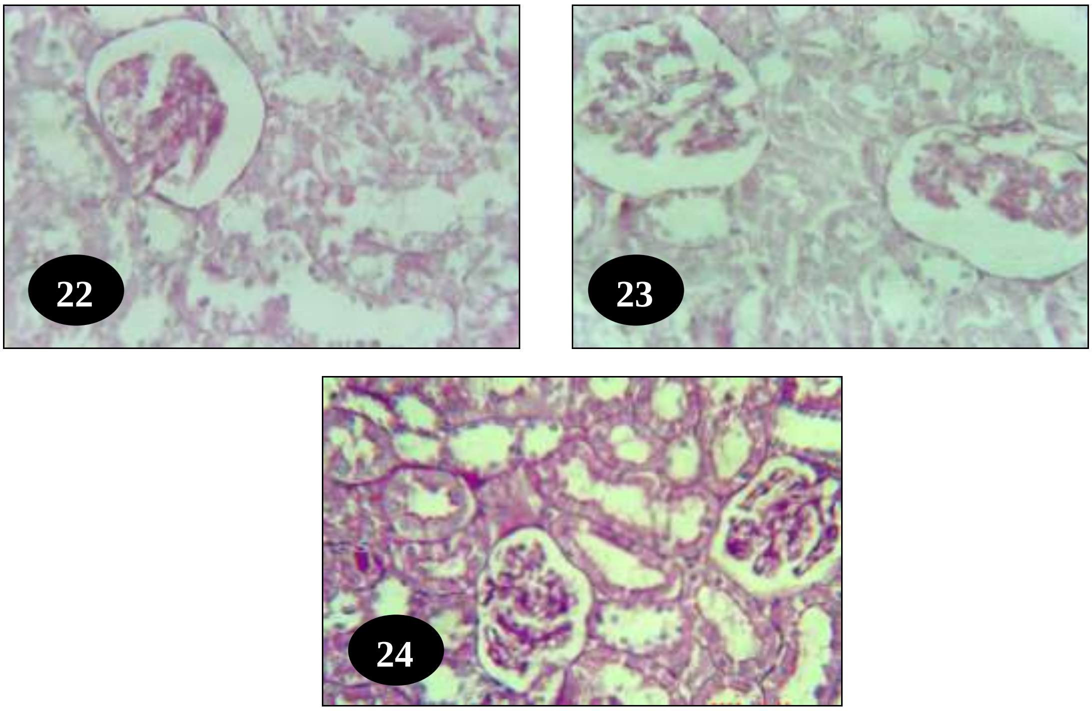

Fig (I): photomicrographs of kidney sections. Sections showing the glycogen content. 22: STZ diabetic rat treated by GT for 6 weeks. 23: STZ diabetic rat exposed to 0.5 Gy/week of γ-radiation for 6 week 24: STZ diabetic rat treated by GT and exposed to 0.5 Gy/week of γ-radiation for 6 weeks. PAS technique. Magnifications X 400.

Discussion

It is known that the increase in oxidative stress may result from over production of precursors to reactive oxygen radicals and/or decreased efficiency of inhibitory and scavenger systems. The stress may be amplified and propagated by an autocatalytic cycle producing tissue damage and cell death (**Brownlee *et al.*, 1998 and Hunt *et al.*, 1993)**. Cell damage will in turn, result in elevated production of reactive oxygen species (ROS). In diabetes increased formation of ROS for reasons may possibly related to an increase in glucose concentrations in plasma and tissues (**Brownlee, 2001; Ha and Kim, 1999**) and may have a role in the pathogenesis of diabetic nephropathy.

Injection of STZ in adult wistar rats induced experimental diabetes mellitus 72 h following STZ treatment **(Akbarzadeh et al., 2007**) and increase in plasma glucose levels was detected. Hyperglycaemia is the principal factor responsible for structural alterations at the renal level and is directly linked to diabetic microvascular complications, particularly in the kidney (**Anonymous, 1993**). DN group in the present study represented a decrease in insulin level, reduction in body weight**,** and increase in urea and creatinine levels for the relationship between diabetic nephropathy and oxidative stress. (**Wagner *et al.* 2001**) and (**Mehrotra *et al.* 2001**), suggesting that oxidative stress affected the progress of diabetic complications.

The severely decreased coarse chromatin in the nuclei either in the Glomeruli or in the convoluted tubules may be due to effect of high glucose on cell proliferation or apoptosis. For DNA synthesis inhibition (**Park *et al.*, 2001 and Allen *et al.*, 2003**). Prevention of nephropathy is a very important concern and this study has been focused on low doses of γ-radiation and herbal medicines such as GT to find novel therapeutic agents for diabetic nephropathy. In this study, we found that either low doses of γ-radiation or GT treatment significantly decreased the blood glucose level and significantly increased insulin level 3 and 6 week of diabetic induction.

Nomura (2002) used the low-dose rate gamma-irradiation to the model mice for Type II diabetes mellitus during their lifetime, measured the level of urine glucose with test slips every week, and investigated the recovery effects of the diabetes by the irradiation. Low doses of radiation, below toxic levels, may modulate various biological responses. Low dose radiation also ameliorates type I diabetes (**Takahashi *et al.,* 2000**), which was caused by dysfunction of pancreatic β cells as a result of self-reactive immune responses (**Yoon *et al.,* 1998**). Such stimulatory effects of radiation, referred to as radiation hormesis (**Liu *et al.,* 1987; Luckey, 1982 and Macklis and Beresford, 1991**).

Green tea (*Camellia sinensis*; GT) is a rich source of polyphenols, particularly flavonoids, which have been shown to have numerous pharmacological effects. Studies using animal models show that GT catechins could be beneficial in suppressing high-fat diet-induced obesity by modulating lipid metabolism and providing some protection against lipid and glucose metabolism disorders implicated in type 2 diabetes (**Matsumoto *et al.*,1993; Nakachi *et al.,* 2000; Murase *et al.,* 2002 and Crespy and Williamson, 2004**).

Administration of GT in streptozotocin (STZ) diabetic animals drastically improved kidney function as a result of its anti-thrombogenic action, which in turn controls the arachidonic acid cascade system (**Yang *et al.,* 1999**). These studies also demonstrated an improvement in the glomerular filtration rate (**Yang *et al.,* 1999 and Rhee *et al.,* 2002 , a,b**). (**Yokozawa *et al* 1999**) examined variables of glomerular filtration in cisplatin (a nephropathy inducer)-treated rats and demonstrated that GT significantly decreased the serum creatinine, kidney excretion of glucose and oxidative stress in the kidney. Another study has shown that GT reduced serum glucose and creatinine levels and increased serum superoxide dismutase, suggesting that catechins influence glucose metabolism and improve kidney function by reducing oxidative stress in alloxan-treated diabetic rats (**Sabu *et al.,* 2002**). Moreover, GT decreased plasma insulin levels but did not affect plasma glucose levels in an oral glucose tolerance test in normal rats (**Wu *et al.,* 2004**). In contrast, (**Mustata *et al.* 2005**) have shown that GT drinking had a marginal effect on nephropathy parameters through improving renal mitochondrial defects; however, neither glycaemia nor urinary albumins were affected in GT-drinking diabetic animals. Also (**Renno *et al.* 2008**) indicate that in STZ diabetes, kidney function appeared to be improved with GT consumption which also prevents glycogen accumulation in the renal tubules, probably by lowering blood levels of glucose. Therefore, GT could be beneficial additional therapy in the management of diabetic nephropathy.

The pervious studies illustrate the normal structure detected in glomerulus's and proximal and distal convoluted tubules, normal distribution of DNA material particles in the kidney tissue and the reduced amount of cytoplasmic glycogen of kidney tissue when STZ-diabetic gavage administrated by GT and/or exposed low doses of γ-radiation

The present results suggested the synergistic effects of combined GT and low doses of γ-radiation treatments are in agreement with the findings of (**Nancy *et al.* 2006**). They significantly lower urea and creatinine excretion rates, significantly decreased fasting glucose level , reaching to nearly of control level in insulin level, recorded normal observations in tissue section , recurrence of normally DNA abundant, densely stained particles scattered in the nucleoplasm and recorded less positive PAS reaction either in the convoluted tubules or the glomeruli.

This may offer potential therapeutic benefit, which warrants clinical study for application in reducing diabetic complications. Further studies are required to examine the clinical use and exact mechanisms behind a possible effect of combined GT and low dose of γ-radiation treatments.

References

1- **Akbarzadeh A. , Norouzian D., Mehrabi MR., Jamshid SH., Farhangi A., Allah Verdi A., Mofidian SMA. and Lame RB. (2007):** induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry, 22 (2):60-64.

2- **Alderson NL., Chachich ME., Frizzell N., Canning P., Metz TO. and Januszewski A.S. (2004):** Effect of antioxidants and ACE inhibition on chemical modification of proteins and progression of nephropathy in streptozotocin diabetic rat. Diabetologia, 47: 1385-1395.

3- **Allen DA., Harwood S., Varagunam M., Raftery MJ., and Yaqoob MM. (2003):** High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases. FASEB J., 7:908–910.

4- **Anonymous (1993):** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N. Engl J Med., 329: 977–986.

5- **Bartels H., Bohmer M. and Heierli C. (1972):** Serum creatinine determination without protein precipitation. Clin. Chem. Acta., 37: 193-197.

6- **Brownlee, M. (2001):** Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813–820.

7- **Brownlee M., Cerami A. and Vlassara H. (1998):** Advanced glycosylation end-products in tissue and the biochemical basis of diabetic complications. N. Engl. J. Med., 318:1315-1321.

8- **Caratero A., Courtade M., Bonnet L., Planel H. and Caratero C. (1998):** Effect of a continuous gamma irradiation at a very low dose on the life span of mice. Gerontology, 44: 272–276.

9- **Chattopadhyay S., Ramanathan M., Das J. and Bhattacharya SK. (1997):** Animal models in experimental diabetes mellitus. Indian J. of Experimental Biol., 35: 1141-1145.

10- **Crespy V. and Williamson G. (2004):** A review of the health effects of green tea catechins in in vivo animal models. J. Nutr., 134: 3431–3440.

11- **Drury RA. and Wallington EA. (1976):** Carlton's Histological Techniques, 6th ed., London: Oxford University Press, Pp. 139–142.

12- **Fawcett JK. and Scott JE.(1960):** A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-15.

13- **Fioretto P. and Mauer M. (2007):** Histopathology of diabetic

14-

nephropathy. Semin. Nephrol., 27:195–207.

15- **Ha H. and Kim KH.(1999):** Pathogenesis of diabetic nephropathy: The role of oxidative stress and protein kinase C. Diabetes Res. Clin. Pract., 45: 147–151.

16- **Hotchkiss RD. (1948):** A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue`preparations. Arch. Biochem., 16: 131.

17- **Hunt JV., Bottoms, MA. and Mitchinson MJ. (1993):** Oxidative alterations in the experimental glycation model of diabetes mellitus are due to protein-glucose adduct oxidation. Biochem. J., 291:529-535.

18- **Lee YJ. and Ducoff HS. (1984):** Radiation-enhanced resistance to oxygen: A possible relationship to radiation-enhanced longevity. Mech. Ageing Dev., **27**: 101–109.

19- **Liu SJ., Liu WH. And Sun JB. (1987):**Radiation hormesis: Its expression in the immune system. Health Phys., 52:579–584.

20- **Luckey TD. (1982):** Physiological benefits from low levels of ionizing radiation. Health Phys., 43: 771–789.

21- **Macklis RM. and Beresford B. (1991):** Radiation hormesis. The Journal of Nuclear Medicine, 32(2): 350-359.

22- **Matsumoto N., Ishigaki F., Ishigaki A., Iwashina H. and Hara Y. (1993):** Reduction of blood glucose levels by tea catechin. Biosci. Biotechnol. Biochem., 57: 525–527.

23- **Mehrotra S., Ling K L. Bekele Y., Gebino E. and Earle, KA. (2001):** Lipid hydroperoxide and markers of renal disease susceptibility in African-Caribbean and Caucasian patients with Type 2 diabetes mellitus. Diabet. Med., 18: 109–115.

24- **Mitra SK., Gopumadhavan S., Muralidhar TS., Antur-likar SD. and Sujatha MB. (1996):** Effect of a herbomineral prepa-ration D-400 in Streptozotocin induced diabetic rats. Journal of Ethnopharmacol., 54: 41-46

25- **Murase T., Nagasawa A., Suzuki J., Hase T. and Tokimitsu I. (2002):** Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int. J. Obes. Relat. Metab. Disord., 26: 1459–1464.

26- **Mustata GT., Rosca M., Biemel KM., Reihl O., Smith MA., Viswanathan A., Strauch C., Du Y. , Tang J., Kern TS. , Lederer MO., Brownlee M., Weiss MF. and Monnier VM. (2005):** Paradoxical effects of green tea (Camellia sinensis)and antioxidant vitamins in diabetic rats: improved retinopathy and

Renal mitochondrial defects but Deterioration of collagen matrix Glycoxidation and crosslinking. Diabetes, 54:517–526.

27- **Nakachi K., Matsuyama S., Miyake S., Suganuma M. and Imai K. (2000):** Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors, 13: 49–54.

28- **Nakagawa T. and Yokozawa T. (2002):** Direct scavenging of nitric oxide and superoxide by green tea. Food Chem., Toxicol., 40:1745–1750.

29- **Nakagawa T., Yokozawa T., Sano M., Takeuchi S., Kim M., and Minamoto S. (2004):** Activity of (-)-epigallocatechin 3-*O*-gallate against oxidative stress in rats with adenine-induced renal failure. J. Agric. Food Chem., 52:2103–2107.

30- **Nakagawa T., Yokozawa T., Terasawa K., Shu S., and Juneja LR. (2002):** Protective activity of green tea against free radical- and glucose-mediated protein damage. J. Agric. Food Chem., 50:2418–2422.

31- **Nancy ML., Borhane A., Marie-Paule L., Kwang SK., Jean-Paul B., Robert M. and Richard B. (2006):** Combined low dose ionizing radiation and green tea-derived epigallocatechin-3-gallate treatment induces human brain endothelial cells death. J. Neuro-Oncology, 80(2):111-121

32- **NomuraT. (2002):** "Effect of low-dose rate irradiation on type ii diabetes model mice" CRIEPI Report Takaharu Nomura, Research Scientist and Kazuo Sakai, Senior Research Scientist, Low Dose Radiation Research Center.Pp. 64-65

33- **Park SH., Choi HJ., Lee, JH., Woo, CH., Kim JH., and Han HJ. (2001):** High glucose inhibits renal proximal tubule cell proliferation and involves PKC, oxidative stress, and TGF-β1. Kidney Int., 59: 1695–1705.

34- **Renno WM., Abdeen S., Alkhalaf M. and Asfa S. (2008):** Effect of green tea on kidney tubules of diabetic rats. Br. J. Nutr., 100(3):652-659.

35- **Rhee SJ., Choi JH. and Park MR. (2002 a):** Green tea catechin improves microsomal phospholipase A2 activity and the arachidonic acid cascade system in the kidney of diabetic rats. Asia. Pac. J. Clin. Nutr., 11: 226–231.

36- **Rhee SJ., Kim MJ. and Kwag OG. (2002 b):** Effects of green tea catechin on prostaglandin synthesis of renal glomerular and renal dysfunction in streptozotocin-induced diabetic rats. Asia. Pac. J. Clin. Nutr., 11: 232–236.

37- **Sabu MC., Smitha K. and Kuttan R. (2002):** Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. J. Ethnopharmacol., 83: 109–116.

38- **Sabu MC., Thachapilly G., Girija K. and Ramadasan K. (2007):** Role of Oxidative Stress, Antioxidant Enzymes, and TNF-α Levels in Diabetes Mellitus. Kuwait Medical Journal, 39 (4): 344-348

39- **Sheehan DC. and Hrapchak BR. (1980):** Theory and Practice of Histotechnology, 2nd Ed, CV Mosby, St. Louis, , Pp. 150.

40- **Takahashi M., Kojima S., Yamaoka Y. and Niki, E. (2000):** Prevention of type I diabetes by low-dose gamma irradiation in NOD mice. Radiat. Res., 154: 680–685.

41- **Temple R.C., Clarck P.M. and Hales C.N. (1992):** Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic Medicine, 9: 503-512.

42- **Wagner Z., Wittmann I., Mazak I., Schinzel R., Heidland A., Kientsh Engel R. and Nagy J. (2001):** N ∈-(carboxymethyl) lysine levels in patients with type 2 diabetes: role of renal function. Am. J. Kidney Dis., 38: 785–791.

43- **Wu LY., Juan CC., Ho, LT., Hsu, YP. and Hwang LS. (2004):** Effect of green tea supplementation on insulin sensitivity in Sprague- Dawley rats. J. Agric. Food Chem., 52: 643–648.

44- **Yang JA., Choi JH. And Rhee SJ. (1999):** Effects of green tea catechin on phospholipase A2 activity and antithro-mbus in streptozotocin diabetic rats. J. Nutr. Sci. Vitaminol., 45: 337–346.

45- **Yokozawa T., Chung HY., He, LQ., and Oura H. (1996):** Effectiveness of green tea tannin on rats with chronic renal failure. Biosci. Biotechnol. Biochem., 60:1000–1005.

46- **Yokozawa T., Dong E., Chung HY., Oura H., and Nakagawa H. (1997):** Inhibitory effect of green tea on injury to a cultured renal epithelial cell line, LLC-PK1. Biosci. Biotechnol. Biochem., 61:204–206.

47- **Yokozawa T., Dong E., Nakagawa T., Kashiwagi H., Nakagawa H., Takeuchi S., and Chung HY. (1998):** In vitro and in vivo studies on the radical-scavenging activity of tea. J. Agric. Food Chem., 46:2143–2150.

48- **Yokozawa T., Nakagawa T., Lee KI Cho EJ. Terasawa K. and Takeuchi S. (1999):** Effects of green tea tannin on cisplatin induced nephropathy in LLC-PK1 cells and rats. J. Pharm. Pharmacol., 51: 1325–1331.

49- **Yokozawa T., Nakagawa T., Oya T., Okubo T., and Juneja LR. (2005):** Green tea polyphenols and dietary fibre protect against kidney damage in rats with diabetic nephropathy. J. Pharm. Pharmacol., 57:773–780.

50- **Yoon JW., Jun HS. and Santamaria P. (1998):** Cellular and molecular mech-anisms for the initiation and progression of β cell destruction resulting from the collaboration between macrop-hages and T cells. Autoimmunity, 27: 109–122.

الإمكانية العلاجية لمستخلص الشاى الأخضروجرعات الإشعاع الصغيرة لعلاج إعتلال الكلي السكرى فى الجرذان البيضاء

نعمات حنفى أحمد و هناء فتحى محمود واعر

يعتبر الإعتلال الكلوى أحد المضاعفات الخطيرة والمترتبة على الإصابة بمرض السكر. صممت هذه الدراسه لتقدير الإمكانية العلاجية لمستخلص الشاى الأخضروجرعات الإشعاع الصغيرة لعلاج إعتلال الكلي السكرى فى الجرذان البيضاء. استعمل في هذه الدراسة ذكورالجرذان البيضاء التى حقنت بالإستربتوسيزوسين لإصابتها بداء السكر. قسمت جرذان التجارب إلى خمسة مجاميع إحداها غير مصابة وغير معالجة وعولجت الجرذان المصابة بمرض السكر بمستخلص الشاى الأخضرأوجرعات الإشعاع الصغيرة منفردة أو متجمعة. خلال مدة التجربة (3 و 6 أسابيع) تم تتبع أوزان الجرذان، مستوى السكر الصائم فى الدم ومستوى الإنسولين , اليوريا والكرياتنين فى السيرم. عينت كذلك التغيرات الهستوباثولوجية ، كثافة السكريات و كثافة حمض الدنا فى النسيج الكلوى. أسفرت النتائج عن نقص أوزان الجرذان المصابة بداء السكر ومستوى الأنسولين في السيرم مع زيادة شديدة فى مستوى سكر الصائم فى الدم وكذلك مستوى اليوريا والكرياتنين فى السيرم .ظهر بالنسيج الكلوى ضرر نسيجى متوسط بالإضافة إلى وجود كثير من البؤرات الفارغة بالجرذان المصابة بداء السكر, زيادة كثافة السكريات كذلك أوضح النسيج الكلوي عن تغير واضح فى كثافة حمض الدنا . من الملاحظ أن علاج الفئران المصابة بمرض السكر بمستخلص الشاى الأخضرأوجرعات الاشعاع الصغيرة منفردة أو متجمعة ادى إلى تحسن التركيب النسيجى و كثافة السكريات و كثافة حمض الدنا بالاضافة إلى التحسن الملحوظ فى مستوى السكر الصائم فى الدم ومستوى الإنسولين, اليوريا والكرياتنين فى السيرم.أو ضحت هذه الدراسه الإمكانية العلاجية لمستخلص الشاى الأخضروجرعات الإشعاع الصغيرة منفردة أو متجمعة على الإقلال من إعتلال الكلي السكرى فى الجرذان البيضاء وإعاقة حدوثه.