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DIABETIC NEPHROPATHY;

EFFECTS OF GINGER EXTRACT ON SERUM CREATININE AND PAIRED KIDNEY WEIGHT IN ALLOXAN INDUCED DIABETIC NEPHROPATHY OF ALBINO RATS

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ABSTRACT… Background: Zingiber Officinale Roscoe (Zingiberaceae family) is known as Ginger. It is famous for its antioxidant properties. **Objectives:** To evaluate the effects of Ginger aqueous extract on the serum creatinine and paired kidney weight in Alloxan induced diabetic nephropathy of albino rats. **Study Design:** Experimental study. **Period:** 06 months 01-01-2013 to 30 June 2013. **Setting:** Anatomy Department, Sheikh Zayed, PGMI Lahore. **Materials and Methods:** Diabetes mellitus was induced with Alloxan intraperitoneally (150 mg/kg body weight) in Experimental groups B & C. Then the rats of experimental group C received 200mg/kg body weight of ginger aqueous extract by gavage daily for five weeks starting from 8th day after Alloxan injection. **Results:** Serum creatinine levels increased more in experimental group B as compared to experimental group C. Group wise comparison of creatinine level revealed that the difference among control (A group) and experimental (B & C Groups) was significant having p-value <0.001. We observed that Paired kidney weight in experimental group B increased as compared to control group A. Less increase in the paired kidney weight was observed in experimental group C as compared to experimental group B. The difference of mean paired kidney weight among three groups was significant having p-value <0.001. **Conclusion:** The results of the present study indicated that the co-treatment of Ginger aqueous extract prevented alloxan induced diabetic nephropathy in albino rats. The aqueous extract of Ginger showed amazing results on paired kidney weight.

Key words: Diabetes Mellitus, Kidney, Diabetic Nephropathy, Ginger, Alloxan.

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INTRODUCTION

Diabetes Mellitus (DM) is not a single disease but a group of metabolic disorders having the common feature of hyperglycaemia.[1] It is now 4th main non-communicable disease.[2] The prevalence of diabetes is reported to be high in Pakistan.[3] Commonest form of diabetes diagnosed in childhood is diabetes mellitus Type 1. Diabetes mellitus Type 2 have strong association with obesity.[4] Diabetes induced nephropathy is one the known cause of end stage renal disease.[5] Diabetic patients with ESRD are left with the options of haemodialysis, peritoneal dialysis or kidney transplantation.[6] One of the abnormality which occurs in renal structure is the enlargement of glomerulus[7] which is caused by certain factors like glycogen accumulation, lipogenesis and protein synthesis in diabetic kidney.[8] Alloxan is used to induce diabetes in experimental rats.[9] Alloxan accumulates in beta cells of pancreas and results in breakdown of DNA strands.[10] As it selectively destroys insulin-producing pancreatic beta cells, it results in an insulin-dependent diabetes mellitus.[11,12] Ginger is a botanical root[13] which have antioxidant properties.[14] Spicy aroma of ginger is due to ketones, especially gingerols. This is the primary component of ginger.[15] It results in improvement of renal function tests.[16] Ginger effects on lipid levels in hyperlipidemic patients had also been studied. Significant reductions were seen in Low Density Lipoproteins and Triglycerides. Increase in High Density Lipoproteins levels were also observed with the treatment of ginger.[17] Ginger decreases plasma glucose concentration in diabetic rats by pancreatic and extra pancreatic mechanisms. Pancreatic mechanisms include increased release of insulin from pancreatic beta cells or release of

bound insulin.[18] Extra pancreatic mechanisms include increasing glucose utilization in liver or other tissues or by reducing intestinal glucose absorption.[19] Diabetes is gradually increasing in our society. A good glycemic control is always required. The study was designed to evaluate the effects of Ginger aqueous extract on the gross appearance of kidneys and their weight in Alloxan induced diabetic nephropathy.

MATERIALS AND METHODS

Animals

This study was approved by the Institutional Review Board, Federal postgraduate Medical Institute Lahore, Shaikh Zayed hospital, National Health Research Complex. IRB No: 1208. Ref No: F.39/NHRC/Admin/IRB/389. Dated: 23/11/2012. Total 45 adult wistar albino rats of male sex having weight between 250-300g were randomly selected for the study. They were brought from Veterinary Research Institute, Lahore. These rats were kept in cages in the animal house of PGMI, Bird wood road Lahore. Free access to water and food were allowed to the rats. Chick feed No.1 (commercial brand) was given to rats. 12 hour dark/light cycle was observed at room temperature 27C.[11] Prior to study, animals were acclimatized to their surroundings for seven days.

Induction of Diabetes

After overnight fasting, diabetes was induced in the experimental animals by injecting Alloxan (150 mg/kg BW)[20] intraperitoneally in single dosage, (Sigma-Aldrich, Lot # BCBD6557V, Cat # A7413-10G, Pcode: 101054491, USA), prepared one hour before administration in distilled water.[11] After injection, water and food were given. To counter hypoglycemic shock, 10% glucose solution was given to drink overnight.[16] The plasma glucose concentration (non fasting) was measured by using One Touch Ultra Two Glucometer (Lifescan, Uk) in rats at day 3 after starting the injection.[18,21] The animals which had plasma glucose level above 250mg/dl were labelled as diabetics and chosen for the experiment.[16] After diabetes confirmation rats were allowed for 4 days to acclimatize to diabetic conditions.

Ginger Aqueous Extract Preparation

Preparation was done in PCSIR, Laboratories Complex, Lahore by the following procedure. Fresh, raw and untreated Ginger was purchased from the market. On crushed ice Ginger roots (500g) were peeled then small pieces were made. These were homogenized in 250ml ice cold water and 750ml cold, sterile 0.9% Normal saline solution to form a total volume of 1000ml. Blender was used for homogenization for 12 minutes. Then cheese cloth was used to filter it for three times. It was centrifuged at 2000rpm for ten min. Supernatant fraction was collected and normal saline was used to make its volume 1000ml. As the weight of ginger in start was 500g so the concentration of the prepared ginger extract was considered to be 500mg/ml. Extract was freeze dried in sample tubes at -20°C till the rats were fed.[11] From Department of Chemistry, Forman Christian College Lahore, active ingredients were quantified of by Gas chromatography–mass spectrometry (GC-MS).

Grouping of Animals

The animals were divided into three groups i.e normal, non diabetic (Group A), diabetic untreated (Group B) and diabetic plus ginger treated (group C). 1. Normal (Group A) The rats of this group received distilled water 20ml/kg body weight by gavage. 2. Diabetic (Group B) Alloxan (150 mg/kg BW)[20] was injected intraperitoneally for induction of diabetes in rats. 3. Diabetic plus Ginger treated (Group C) After diabetes was confirmed, diabetic rats received 200mg/kg body weight of ginger aqueous extract by gavage daily for five weeks starting from eighth day after injection of Alloxan. It was labeled as the 1st day of treatment.[18]

Biochemical Assays

Serum Creatinine level was checked at the end of experiment by taking cardiac blood sample.[16] It was estimated on chemistry auto analyzer; Dimension-RXL, Siemens, USA by Jaffee's method. [22]

DISSECTION

After the completion of experiment, all the animals were euthanized by giving morphine 5-24 mg/kg body weight intraperitoneally as an

analgesic agent [23] and sodium pentabarbitol was intraperitoneally injected in100mg/kg body weight dose.[24] kidneys were dissected out. Ice cold saline was used to wash the kidneys after isolation and then Paired kidney weight was recorded.[18,21]

Statistical Analysis

Analysis of Data was done by SPSS 22.0. Quantitative data was reported as mean + S.D. ANOVA was used for comparison among groups. Turkey test was used for post hoc analysis where ever required. < 0.05 P-value was significant with 95% confidence level.

RESULTS

Serum Creatinine

The Creatinine level for animals in control group A was 0.5±0.1 and for experimental groups B and C were 1.1±0.1 and 0.7±0.1 respectively. (Table-I)

Difference among control (A Group) and experimental (B & C Groups) was significant having p-value <0.001 when comparison was done group wise. Experimental (B Group) had higher serum Creatinine level significantly than control (A Group) and experimental (C Group) having p-value <0.001, and experimental (C Group) had higher than control (A Group) having p-value <0.001 (Table-II&III, Figure-1).

Paired Kidney Weight

The mean paired kidney weight for control group A was 3.59±0.17 gram and for experimental groups B and C was 3.94±0.20 and 3.78±0.20 respectively. (Table-IV, Figure-2)

Three groups had significant difference among them having p-value <0.001. Group wise comparisons showed that the control (A Group) had lower paired kidney weight significantly as compared to experimental (B and C Groups) having p-values <0.001 and 0.024 respectively. Experimental (C Group) had difference for paired kidney weight as compared to experimental (B Group) having p-value 0.048 which was to be considered significant. (Table-V&VI)

Groups	Serum Creatinine level (mg/dl)			
	Mean	Standard Deviation	Minimum	Maximum
A Group	0.5	0.1	0.4	0.6
B Group	1.1	0.1	0.9	1.2
C Group	0.7	0.1	0.6	0.9

Table-I. Serum Creatinine level at the end of experiment for control (A Group) and experimental (B & C Groups)

	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	2.41	2	1.21	152.68	< 0.001**
Within Groups	0.33	42	0.008		
Total	2.75	44			

Table-II. Comparison among control (A Group) and experimental (B & C Groups) for serum creatinine level

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	P-value
A Group	B Group	-0.567*	0.033	< 0.001**
	C Group	-0.260*	0.033	< 0.001**
B Group	C Group	0.307*	0.033	< 0.001**

Table-III. Group wise comparison among control (A Group) and experimental (B & C Groups) for serum creatinine level
****Difference highly significant (P< 0.001)**

Groups	Kidney Weight			
	Mean	Standard Deviation	Minimum	Maximum
A Group	3.59	0.17	3.32	3.89
B Group	3.94	0.20	3.63	4.23
C Group	3.78	0.20	3.45	4.10

Table-IV. Paired kidney weights of animals of control (A Group) and experimental (B & C Groups)

	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	0.96	2	0.48	13.06	< 0.001**
Within Groups	1.54	42	0.04		
Total	2.49	44			

Table-V. Comparison among control (A Group) and experimental (B & C Groups) for paired kidney weight

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	P-value
A Group	B Group	-0.36*	0.07	< 0.001**
	C Group	-0.19*	0.07	0.024**
B Group	C Group	0.17	0.07	0.048**

Table-VI. Group wise comparison of animals of control (A Group) and experimental (B & C Groups) for paired kidney weight
**Difference highly significant d (P< 0.001)

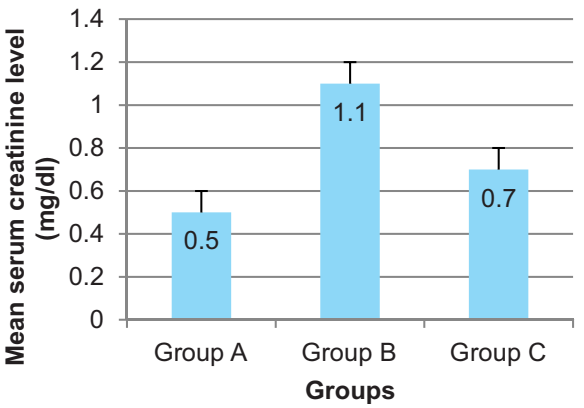

Figure-1. Mean serum creatinine levels of animals of control (A Group) and experimental (B & C Groups) at different reading times

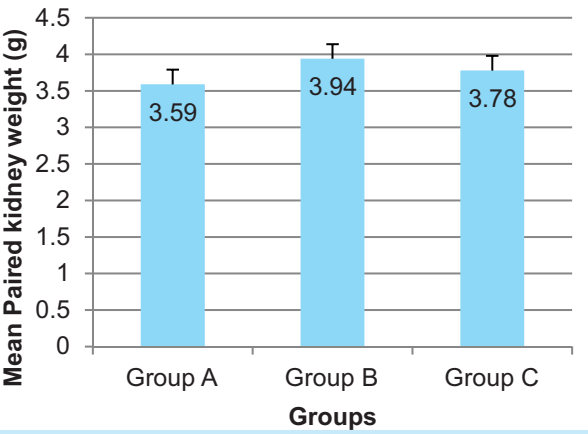

Figure-2. Paired kidney weight of animals of control (A Group) and experimental (B & C Groups)

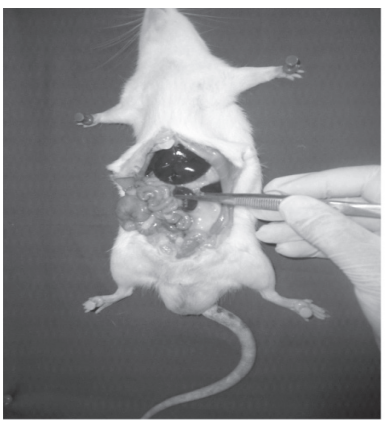

Figure-3. Photograph showing dissection of albino rat and handling of kidney

Figure-4. Photograph showing paired kidney weight of Albino rat

DISCUSSION

Diabetes mellitus (DM) is a syndrome. Its characteristic features include chronic elevated blood glucose levels and relative insulin deficiency, resistance or both.[1] Diabetic complications include heart disease, peripheral vascular disease, nephropathy, retinopathy, neuropathy and renal failure.[25] The kidney is an organ which excretes metabolic waste products.[26] The functions of kidneys are to maintain plasma osmolality, electrolytes concentration and end products excretion.[27] One of the leading cause of end stage renal disease is considered to be diabetic nephropathy.[5] Various pathophysiologiacal mechanisms are considered to be responsible for this. Oxidative stress is one of them. A variety of vasoactive mediators are formed due to oxidative stress. This affects the functioning of kidney either directly by vasoconstriction of renal vasculature or by decreasing the coefficient of glomerular capillary ultra filtration. Then glomerular filtration rate is reduced.[28]

In our research work, Experimental (B Group) had higher serum Creatinine levels than experimental group C treated with ginger aqueous extract. Results of my study coincided with the research done by Swaroopa Maralla et al.[29] This is also consistent with findings of Attalla F. El-Kott et al.[20] Oxidative stress can be prevented by use of Ginger.[30] Ginger has its scavenging effects by inhibition of lipid peroxidation and free radicals. Due to it, the production of vasoactive mediators decreases. It is likely that Ginger by this way plays an important role in improving renal function parameters in diabetes.[28]

Our research work revealed that paired kidney weight was more increased in experimental group B than experimental group C. Results coincided with Abdullah G. al-Kushi findings who mentioned significant increase in paired kidney weight of diabetic rats in his study.[21] Consistent findings were also observed by Shanmugam KR et al.[18] It was noted that Alloxan treated animals of experimental (B group) showed Increase in the weight of kidney (hypertrophy) as compared to control (A Group) animals. The kidney enlargement is caused by certain factors like glycogen accumulation, lipogenesis and protein synthesis in diabetic kidney.[8]

Ginger significantly increases the activities of enzymes Glucose 6 Phosphatase Dehydrogenase, Succinate dehydrogenase, and Glutamate dehydrogenase which reduces the fatty infiltration in kidneys.[31] Ginger improves the hyperglycemia by both pancreatic and extra pancreatic mechanisms and decreases the oxidative stress which in turn reverses the factors resulting in kidney hypertrophy.[18,19] Ginger has shown many potential clinical benefits in different experimental studies. Clinical benefits in rats include cardiotonic, antilipemic, antiemetic, carminative, antiulcer, anti-inflammatory, hypoglycemic, antineoplastic and antioxidant.[32]

CONCLUSION

Results of my study indicated that treatment with Ginger aqueous extract reduced the progression of diabetic nephropathy induced by Alloxan in albino rats. Aqueous extract of Ginger showed amazing results biochemically and histopathologically. The overall reno-protective effect of Gingeris probably due to a counteraction of free radicals by its antioxidant components and improvement of hyperglycemic state by pancreatic and extra pancreatic mechanisms. Further studies regarding higher dosages or longer periods of treatment are needed to see the protective effect of ginger on kidneys against diabetic nephropathy in human beings.

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The man who fears no truth has nothing to fear from lies.

– Thomas Jefferson –

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Faiza Irshad	Ensured the design of work and inclusion interia, helped out in methodology, collected the data for analysis and obtained resutls.	
2	Rabia Sajjad Toor	Worked out in orjanijunt introduction, literature review and discussion of the study. Worked in preparimy manuscript according to TPMJ criteria and in writing learn citation and references of the study.	
3	Madiha Hussain