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SODIUM TUNGSTATE;

EFFECT OF SODIUM TUNGSTATE ON KIDNEY OF STREPTOZOTOCIN INDUCED DIABETIC RABBIT.

Afra Samad[1], Munawar Hussain Shah[2], Rana Khalid Mahmood[3], Muhammad Abubakre Khalid[4], Muhammad Usama[5], Muhammad Sulaiman Saeed[6]

1. MBBS, DCP, M.Phil.
Assistant Professor,
Department of Pathology
Multan Medical and Dental
College Multan.
2. MBBS, M.Phil.
Associate Professor,
Department of Pathology
Nishtar Medical University Multan.
3. MBBS, M.Phil.
Assistant Professor,
Department of Pathology
Nishtar Medical University Multan.
4. MBBS
House Officer
Shaikh Zayed Medical College
Rahim Yar Khan.
5. MBBS
4th Year Student
Shaikh Zayed Medical College
Rahim Yar Khan.
6. B.Sc MLT, M.Sc. Biochemistry,
M.Phil. Biochemistry
Biochemist,
Department of Pathology
Nishtar Medical University Multan.

Correspondence Address:
Dr. Rana Khalid Mahmood,
Assistant Professor,
Department of Pathology
Nishtar Medical University Multan.
drkhalid.rana@yahoo.com

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ABSTRACT… Objectives: The purpose of this study was to reveal the effects of sodium tungstate (ST) on kidneys of diabetic rabbits. **Study Design:** Rabbits of 30 weeks age were taken and divided into three groups A, B and C. Each group contained 10 animals. Group A was selected as control group. Diabetes was induced in groups B and C by injecting streptozotocin (50mg/mL) intraperitoneally. Group C was treated with sodium tungstate orally and group B was left untreated. Histology of the kidney was examined in all of these groups using Hematoxylin & Eosin (H & E), Reticulin and Trichrome stains. Blood glucose estimations of all animals were performed every third day. Blood glucose levels of diabetic rabbits treated with sodium tungstate were checked before and after treatment. Results were statistically analyzed using students t-test. **Place of Work:** Post graduate medical institute Lahore. **Duration of study:** July 2010 to August 2011. **Results:** Treatment of diabetic rabbits with sodium tungstate (Group C) lead to lowering of blood glucose levels as compared to untreated diabetic rabbits (Group B). Microscopic features of kidneys in groups B and C did not reveal any pathological and morphological changes in comparison with control group A. **Conclusion:** Sodium tungstate is a good anti-diabetic agent when administered orally and causes no morphological changes in kidneys.

Key words: Sodium Tungstate (ST), Diabetes Mellitus, Streptozotocin (STZ).

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INTRODUCTION

Diabetes mellitus (DM) is a prominent health problem worldwide and is a major cause of morbidity and mortality which is becoming an epidemic as recognized by World Health Organization (WHO). In most developed countries it is 7th major cause of death and it is increasing rapidly in industrialized countries.[1]

Worldwide prevalence of diabetes mellitus has increased from 108 million in 1980 to more than 422 million in 2014.[2] Prevalence of diabetes mellitus is high in Pakistan as 6.7% people ranging 20-79 years of age, suffer from DM.[3] Carbohydrate metabolism is defective in diabetes mellitus and it is characterized by abnormally large amount of sugar in blood and urine. The chronic hyperglycemia and metabolic deregulation associated with diabetes mellitus may lead to secondary damage to multiple organ systems e.g. kidney, eye, heart, limbs, nerves, blood vessels.[4] A large proportion of diabetics suffer from diabetic complications. To avoid these complications blood glucose level should be maintained within normal or near normal level by taking adequate treatment. This can be achieved by the use of insulin injections or oral hypoglycemic, appropriate diet control, weight reduction and regular exercise. Insulin is administered through injections and continuous monitoring is essential. Overdose may lead to many side effects, some of which may be life threatening.[5] Many inorganic compounds have insulin-like effects but their toxicity at effective doses limits their clinical use.[6] Tungsten (VI) compounds can be administered orally for normalization of blood glucose level in diabetics and do not produce hypoglycemia in overdose. Kidney is major organ for drug excretion[7] hence this study was designed to monitor the effects of

sodium tungstate on kidney as well as its capability to lower blood glucose level in diabetics.

METHODOLOGY

This was an interventional study on rabbits. Thirty rabbits of 30 weeks age were obtained from Veterinary Research Institute, Lahore. The animals were maintained under optimal atmospheric and hygienic conditions, with food and water available all the time according to the animals ethics described in Helsinki announced principles. After a week of acclimatization, the experiment was started. Ethical approval was taken from Ethical Review Committee of Postgraduate Medical Institute Lahore. Three groups were made each having 10 rabbits with equal number of males and females, as described below:

Control Group A

It included rabbits receiving normal diet and distilled water.

Group B

It included rabbits with normal diet and distilled water. Streptozotocin was used to induce diabetes in these animals. These animals were left untreated.

Group C

It included rabbits receiving normal diet and distilled water. Streptozotocin was used to induce diabetes in these animals. These animals were daily orally treated with solution of 2mg/ml Sodium Tungstate in distilled water. Sodium Tungstate treatment continued for 4 weeks.

Rabbits of Group B and C were given single intraperitoneal injection of streptozotocin (STZ) (50mg/kg body weight) in 0.9% NaCl with 10mmol/L sodium citrate to induce diabetes. These animals were monitored for hyperglycemia, 24-48 hours after injection of STZ.

Study was carried out for 04-weeks in all groups, after inducing the DM in rabbits with STZ in groups B and C. A small tattoo marked in the non-vascular part of the left pinna of the animal was used to identify different groups of rabbits as given below:

- No mark Group A (normal rabbits).
- One mark for Group B (diabetic rabbits without Sodium Tungstate treatment).
- Two marks for Group C (diabetic rabbits with Sodium Tungstate treatment).

Measurement of weight and blood glucose level

Weights and blood glucose levels were measured before and after injection of streptozotocin. Blood glucose level of each animal was measured every third day after induction of diabetes and measurement continued for 4 weeks. Glucometer (Accu Check Performa Meter) was used for measurement of blood glucose.

Specimen collection, processing and staining

Animals were anaesthetized and dissected on the completion of experiment and specimens were collected. Protocol of American Veterinary Medical Association (AVMA) was used for Euthanasia. Kidneys were surgically removed, weighed; dimensions of the kidneys were recorded, sliced and kept in labeled jars containing 10% buffered formalin. Automatic tissue processor (Thermofischer scientific) was used for tissue processing. Paraffin blocks were made and sections were cut using rotary microtome (Thermo Shandon Finesse 325). Prepared sections were stained with Hematoxylin & Eosin (H&E)[8], Trichrome[9], and Reticulin Silver stains[10] according to established protocols. Stained sections were observed under light microscope by pathologists and results recorded.

Statistical analysis

Students' 't' test was used for statistical analysis of results. Mean, standard deviation and P value was calculated using Statistical Package for the Social Science (SPSS).

RESULTS

A) Comparison of weights and maximum dimensions of the kidneys

Comparison of weights and maximum dimensions of kidneys between Groups A vs B, B vs C and between A vs C showed statistically non-significant change as shown in Table-II.

B) Microscopic examination of kidneys

- Glomerular Changes

Microscopic examination for glomerular changes

i.e. lobulation, necrosis, adhesions and sclerosis showed no difference between groups A vs B, A vs C and B vs C.

- Renal Basement Membrane

There was no change in renal basement membrane i.e. regularity, thickening and mesangial widening between groups A vs B, A vs C and B vs C.

- Interstitial Changes

Microscopic examination for interstitial changes i.e. oedema, fibrosis, necrosis and inflammation showed no change between groups A vs B, A vs C and B vs C.

- Renal Tubular Changes

No renal tubular change i.e. architecture and necrosis was observed between groups A vs B, A vs C and B vs C.

- Renal Blood Vessels

No difference was found in renal blood vessels i.e. hyalinization, thickening and necrosis between groups A vs B, A vs C and B vs C.

The microscopic findings in kidney of all groups are shown in Figures-1 to 6.

C) Blood glucose levels

Blood glucose levels of group A (control group) were normal while group B (diabetic rabbits without treatment) showed high levels of blood glucose. The blood glucose levels of group C (diabetic rabbits with sodium tungstate treatment) were initially high reaching up to 301 mg/dL but later on reduced with the passage of time (Table-I). Comparison of blood glucose levels between group A vs B and group B vs C showed statistically significant difference ($p<0.05$) while comparison of blood glucose levels between group A vs C indicated statistically non-significant results (Table-II).

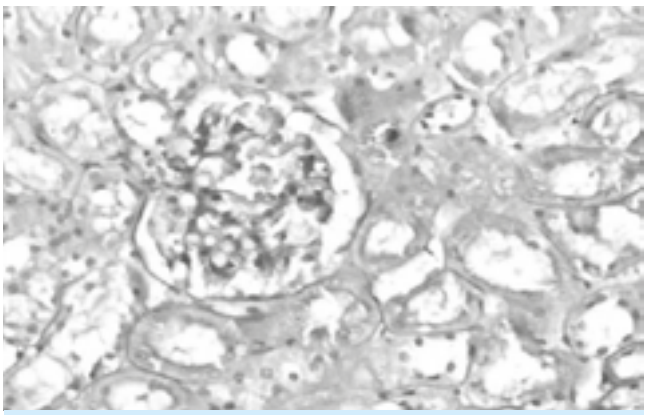

Figure-1. H&E stain: Section of kidney showing normal histology in control group (A) (x 500)

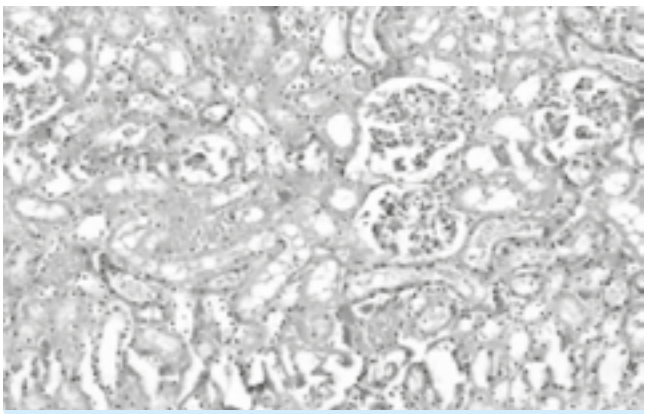

Figure-2. H&E stain: Section of kidney showing normal renal glomeruli and tubules in group B

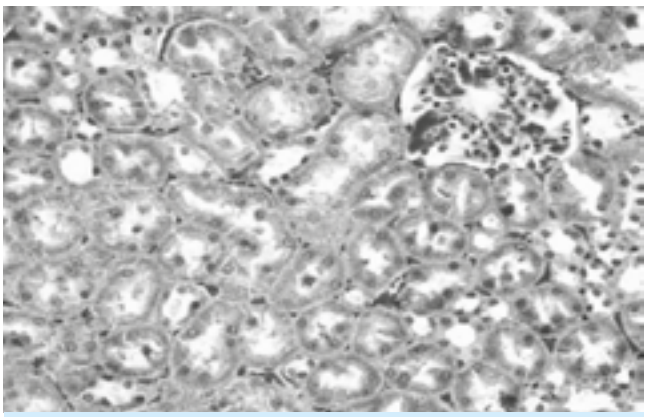

Figure-3. TRICHROME stain: Section of kidney showing normal renal glomeruli and tubules in group B (diabetic group without treatment) (x 500)

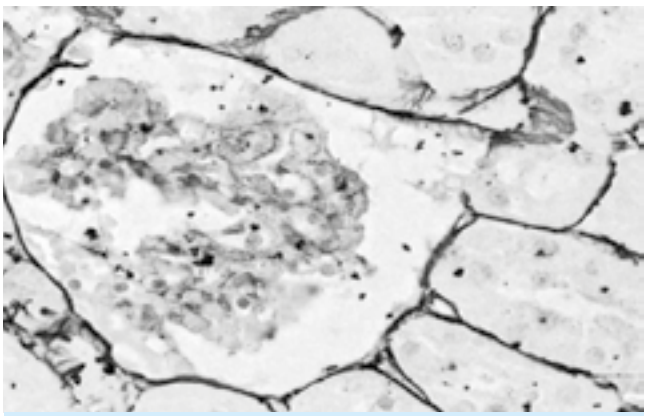

Figure-5. Trichrome stain: Section of kidney showing normal renal tubules and glomeruli in group C (diabetic group with treatment) (x 500)

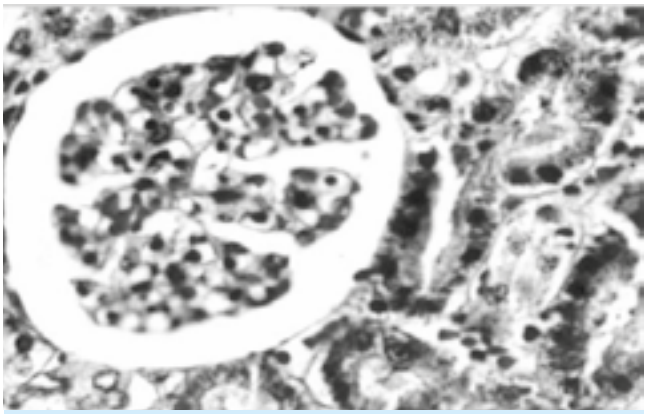

Figure-5. Trichrome stain: Section of kidney showing normal renal tubules and glomeruli in group C (diabetic group with treatment) (x 500)

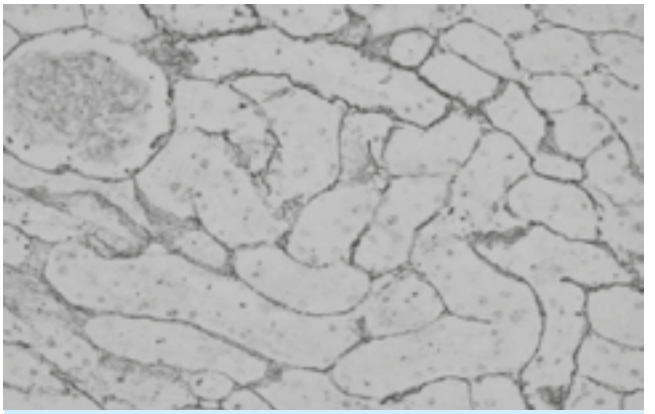

Figure-6. Reticulin silver stain: Section of kidney showing renal glomeruli and tubules in group C (diabetic group with treatment) (x 500)

Weeks		Mean of levels in Group A	Mean of levels in Group B	Mean of levels in Group C
1st week	1st day	98	200	95
	4th day	106	287	301
	7th day	110	293	290
2nd week	1st day	113	295	222
	4th day	118	299	191
	7th day	103	304	120
3rd week	1st day	95	320	116
	4th day	106	298	110
	7th day	119	291	120
4th week	1st day	109	301	88
	4th day	108	330	101
	7th day	125	343	99

Table-I. Comparison of blood glucose levels in groups A, B & C

Groups	Number of rabbits (n)	Mean ± SD Values	Statistical Analysis
Comparison of Blood glucose levels			
A (Control)	10	109.17 ± 8.664	A vs B =(p<0.05)
B	10	297.0 ± 34.557	A vs C =(p>0.05)
C	10	154.42 ± 77.141	B vs C =(p<0.05)
Comparison of weights of rabbits at 4th week			
A (Control)	10	2.07 ± 0.4	A vs B =(p<0.05)
B	10	1.22 ± 0.11	A vs C =(p>0.05)
C	10	2.1 ± 0.15	B vs C =(p<0.05)
Comparison of weights of rabbits kidney			
A (Control)	10	3.82 ± 0.59	A vs B =(p>0.05)
B	10	3.53 ± 0.49	A vs C =(p>0.05)
C	10	3.72 ± 0.65	B vs C =(p>0.05)
Comparison of maximum dimensions of rabbits kidney			
A (Control)	10	3.23 ± 0.41	A vs B =(p>0.05)
B	10	2.96 ± 0.52	A vs C =(p>0.05)
C	10	2.91 ± 0.43	B vs C =(p>0.05)

Table-II. Comparison of means of blood glucose levels, weight of rabbits, weights of rabbits kidney and maximum dimension of rabbits kidney in groups A, B & C

Key:-

Group A	=	Control Group
Group B	=	Diabetic Group without Sodium Tungstate therapy
Group C	=	Diabetic group with Sodium Tungstate therapy

$p<0.05$ = Significant difference
$p>0.05$ = Non-Significant difference

D) Comparison of weights of the rabbits

Comparison of weights between group A vs B, group B vs C showed statistically significant difference (p value <0.05) while comparison of weights between group A vs C indicated non-significant difference (p value > 0.05) (Table-II).

DISCUSSION

Effective treatment of diabetes mellitus requires control of blood glucose at normal or near normal levels to prevent the long term effects of poorly controlled blood glucose i.e. diabetic complications (reference addition needed). Any compound which is not toxic at effective dose and do not produce hypoglycemia on overdose will be highly beneficial for treatment of diabetes mellitus. Tungsten (VI) compounds have been shown to be effective in the treatment of diabetes mellitus and do not show any side effect on liver during their metabolism.[11] Moreover their overdoses do not produce hypoglycemia. So these compounds need further evaluation for their toxicity on kidney as drugs are mainly excreted through kidneys.[7] In this study effects of sodium tungstate on blood glucose level and various morphological and histological features of the kidney were evaluated to check any toxic effect on renal micro texture.

In this study gross findings of rabbits kidneys regarding colour, texture, weight, size and cut surface revealed normal morphology in all the groups A, B & C. Regarding kidney weight, it was seen that mean kidney weight of group B and C showed no statistical difference ($p>0.05$) (Table-II). The mean dimension of rabbit's kidney in groups B & C also showed non-significant difference as in comparison with control group A (Table-II).

No positive change was detected regarding glomerular changes i.e. lobulation, necrosis, adhesion and sclerosis in any of the rabbits in groups A, B & C. No positive change in basement membrane (i.e. regularity, thickening and mesangial widening) was detected in kidneys of any rabbit in Groups A, B & C at 4th week. Regarding interstitial changes i.e. edema, fibrosis & necrosis, not a single animal showed positive changes. However mild inflammation was present in all the three groups. Moreover no positive change was observed regarding renal tubular changes (i.e. architecture and necrosis) and vascular changes of kidney at 4th week (i.e. hyalinization, thickening and necrosis) in diabetic rabbits without and with Sodium Tungstate therapy (Group B and C respectively) as well as in control group A. Regarding vascular changes of kidney at 4th week i.e. hyalinization, thickening and necrosis, no positive change was observed in rabbits of Groups A, B & C.

Blood glucose level of ST treated diabetic rabbits (Group C) were lower as compared to untreated diabetic rabbits (Group B). These findings are consistent with studies of Munoz et al (2001)[12] who observed that tungstate is effective as an antidiabetic agent in diabetic rats and reduces the hyperglycemia observed in the untreated animals to values ~200 mg/dl without damaging kidney. Moreover these finding are also consistent with the study of Barbra et al (2001)[13] who observed that Sodium Tungstate (ST) treatment did not change the renal parameters as well as morphology and prevented diabetes-induced morphological complications in the kidney and ocular lens and resulted in reduction of mortality. No hypoglycemic episodes or undesirable side effects were observed in treated diabetic or healthy rats. In addition, there is no evidence of intolerance development after prolonged use of Sodium Tungstae (ST). Hence ST could play a helpful part in the long term treatment of diabetes.

CONCLUSION

Oral administration of Sodium Tungstate shows good anti-diabetic activity. No toxic effect was observed at therapeutic levels. Moreover it does not produce any morphological and histological

changes in the kidneys of rabbits.

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PREVIOUS RELATED STUDY

Afra Samad, Munawar Hussain Shah, Rana Khalid Mahmood. Sodium tungstate; Effect on liver of streptozotocin induced diabetic rabbit (Original) Professional Med J 2016;23(12): 1566-1572.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Afra Samad	Designing and conduction of research	
2	Munawar Hussain Shah	Assessment and interpretation of results	
3	Rana Khalid Mahmood	Assessment and interpretation of results	
4	M. Abubakre Khalid	Experimentation, Analysis.	
5	Muhammad Usama	Experimentation, Analysis.	
6	M. Sulaiman Saeed	Drafting of research paper, data analysis.	