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SODIUM TUNGSTATE;

EFFECT ON LIVER OF STREPTOZOTOCIN INDUCED DIABETIC RABBIT

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ABSTRACT… Objectives: To determine the possible morphological effects of sodium tungstate (ST) on liver of streptozotocin (STZ) induced diabetic rabbits. **Study design:** This prospective study was conducted on 30 rabbits of 30 weeks age. Diabetes was induced in 20 animals by a single intraperitoneal injection of streptozotocin (50 mg/kg). 10 animals were used as control. 10 diabetic rabbits were treated with sodium tungstate (ST) orally and 10 animals were left untreated. Histological examination of liver was performed. **Setting: Place:** Post graduate medical institute lahore. **Duration of study:** July 2010 to August 2011. **Methods:** Blood glucose levels of the rabbits were checked before and after treatment. Histological examination of liver sections of 30 rabbits was performed. Sections were stained with Hematoxylin & Eosin (H & E), Trichrome and Reticulin stains separately. Results were analyzed statistically. **Results:** Blood glucose levels of diabetic rabbits without Sodium Tungstate therapy were high as compared to normal rabbits and diabetic rabbits treated with sodium tungstate. Histological examination of liver of diabetic rabbits with Sodium Tungstate therapy did not reveal any irregular chromatin pattern or fibrosis. **Conclusion:** On the basis of histological examination and blood glucose level it is concluded that sodium tungstate is a powerful anti-diabetic agent when administered orally with no morphological changes in liver.

Key words: Diabetes mellitus, Sodium tungstate (ST), Streptozotocin (STZ)

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INTRODUCTION

Diabetes mellitus is one of the major health problems throughout the world and it is now the fourth leading cause of death in developed countries and a disease that is rapidly increasing in developing countries.[1] The chronic hyperglycemia and metabolic deregulation may be associated with secondary damage to multiple organ systems e.g. kidneys, eyes, heart, limbs, nerves, blood vessels and can endanger pregnancy.[2] With adequate treatment, good control of blood sugar level may be maintained within normal or near normal range. This can be achieved by the use of insulin injections or oral hypoglycemic agents, appropriate diet control, weight reduction and regular exercise. Insulin is available only in the injectable form and requires continuous monitoring. Insulin has many side effects due to overdose, some of them may be life threatening.[3] Recently several inorganic compounds have been shown to have insulin-like effects but their toxicity at effective doses limited their use in clinical practice.[4] Oral administration of tungsten (VI) compounds is as effective as the administration of inorganic vanadium in normalizing blood glucose levels in diabetics. It is virtually innocuous at effective doses and do not produce hypoglycemia because of overdose like insulin.[5] Drug induced liver injury is relatively uncommon but unless recognized early may cause death. So this study was carried out to assess the possible effects of Sodium Tungstate in rabbits in which diabetes was induced by using Streptozotocin.

MATERIALS AND METHODS

Present study was an experimental, interventional study on rabbits. Diabetes was induced in Group B and Group C of experimental rabbits by using Streptozotocin. The study was carried out four

weeks after inducing the diabetes in rabbits.

Study Animals

Thirty rabbits of 30 weeks age were obtained from Veterinary Research Institute, Lahore. They were divided into three groups having 10 rabbits in each group with equal number of male and female rabbits.

Animal groups

Group A (Control): It included 10 healthy rabbits receiving normal diet and distilled water.
Group B: It included 10 diabetic rabbits receiving normal diet and distilled water.
Group C: It included 10 diabetic rabbits with normal diet and solution of 2mg/ml of Sodium Tungstate in distilled water orally.[6] The treatment was carried out for 4 weeks. Blood glucose was measured every third day.

Experimental schedule

Group B and C animals were given single intraperitoneal injection of streptozotocin (STZ), (50mg/kg body weight) in 0.9% NaCl with 10 mmol/L sodium citrate.[7] The blood glucose levels of all these animals were checked by Glucometer before and after (0 and 4th week) Streptozotocin injection. Weights of the rabbits were also recorded at 0 and 4th week. The diabetic animals were divided into groups B and C and these were identified by placing small tattoo marked in the non-vascular part of the left pinna of the animal as follows:

- 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).

Specimen collection, processing and staining

On completion of experiment, specimens were taken after anesthetizing and dissecting the animals according to proper procedure and ethics. The liver was surgically removed, weighed, sliced and kept in labeled jars for fixation containing 10% buffered formalin.[8,9] Tissue processing was done in automatic processor, the paraffin blocks were made, sections were cut by using rotary microtome. Sections were stained with Hematoxylin & Eosin (H&E)[10], Trichrome[11], and Reticulin Silver stains.[12] The prepared stained sections of liver were seen under light microscope (CXZIFS Olympus) by two experts and results were noted down.

Statistical analysis

Results of the study were analyzed statistically by using Student's 't' test.

RESULTS

Comparison of means of maximum dimensions of rabbits' liver, various features of liver cells, chromatin pattern of liver cells and NAS score of liver at 4th week between groups A with B, A with C and B with C showed statistically non-significant ($p>0.05$) differences as shown in Table-I, II, III and IV respectively. Blood glucose levels of group A (control group) were normal whereas of group B were high. The blood glucose levels of group C were high initially reaching up to 291-300 mg/dl and reduced with passage of time. Comparison of blood glucose level as well as weights between group A with group B and group B with group C showed statistically significant difference ($p<0.05$) (Tables V, VI & VII).

Statistical Analysis

A vs B	=	($p>0.05$) (Non-Significant)
A vs C	=	($p>0.05$) (Non-Significant)
B vs C	=	($p>0.05$) (Non Significant)

Key:-

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

Fibrosis staging of liver in groups A, B, C and D at 4thweek

There were no positive changes, regarding fibrosis staging of liver in group B i.e. diabetic rabbits without therapy and group C i.e. diabetic rabbits with Sodium Tungstate therapy when compared with control group (A).

The photographs of microscopy of liver in Groups B and C are shown in Figures1-6.

Maximum Dimension	Group A (Control)	Group B	Group C
Mean ± SD Values	4.3 ± 0.05	4.1 ± 0.08	4.4 ± 0.16
Ranges	6.1	4.5 – 6.3	5.1 – 6.3
Total Subjects	10	10	10

Table-I. Comparison of maximum dimension (cm) of rabbit's liver at 4th week in groups A, B & C

Cellular Features	Group A (n=10)	Group B(n=10)	Group C (n=10)	Level of Significance		
				A vs B	A vs C	B vs C
Cell Border Score 0- 2	1.5 ± 0.53	1.6 ± 0.52	1.53 ± 0.48	p>0.05 (NS)	p>0.05 (NS)	p>0.05 (NS)
Cell Size (μm)	25.30 ± 2.75	26.6 ± 2.91	24.4 ± 0.77	p>0.05 (NS)	p>0.05 (NS)	p>0.05 (NS)
Nuclear: Cytoplasm Diameter Ratio	2.81 ± 0.46	2.89 ± 0.57	2.7 ± 0.52	p>0.05 (NS)	p>0.05 (NS)	p>0.05 (NS)
Total Rabbits	**10**	**10**	**10**	**30**		

Table-II. Comparison of different features of liver cells at 4th week 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

% of cells with Regular or Irregular Chromatin Pattern in 100 Cells	Group A (n=10)	Group B (n=10)	Group C (n=10)	Level of Significant		
				A vs B	A vs C	B vs C
% Regular	90.9 ± 4.81	90.5 ± 5.7	90.5 ± 6.47	p>0.05 (NS)	p>0.05 (NS)	p>0.05 (NS)
%Irregular	9.1 ± 4.81	9.5 ± 5.7	9.5 ± 6.47	p>0.05 (NS)	p>0.05 (NS)	p>0.05 (NS)
Total Rabbits	**10**	**10**	**10**	**30**		

Table-III. Comparison of chromatin pattern of liver cells at 4th week 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

Groups	Number of Rabbits	Mean ± SD value
A (Control)	10	1.2 ± 1.03
B	10	1.5 ± 0.97
C	10	1.6 ± 0.97

Table-IV. NAS score of liver at 4th week in groups A, B & C

Statistical Analysis
A vs B = (p>0.05) (Non Significant)
A vs C = (p>0.05) (Non-Significant)
B vs C = (p>0.05) (Non Significant)

Key:-
Group A = Control Group
Group B = Diabetic Group without Sodium Tungstate therapy
Group C = Diabetic group with sodium Tungstate therapy

Weeks		Mean of levels in Group A	Mean of levels in Group B	Mean of levels in Group C
1st week	1st day	99	100	96
	4th day	105	289	300
	7th day	115	291	291
2nd week	1st day	112	300	220
	4th day	120	298	190
	7th day	101	303	120
3rd week	1st day	91	323	116
	4th day	110	296	110
	7th day	120	299	120
4th week	1st day	108	300	89
	4th day	104	334	100
	7th day	129	345	99

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

Groups	Number of rabbits (n)	Mean ± SD Values
A (Control)	10	109.50 ± 10.51
B	10	289.83 ± 62.31
C	10	159.25 ± 76.85

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

Statistical Analysis

A vs B = ($p<0.05$) (Significant)
A vs C = ($p>0.05$) (Non-Significant)
B vs C = ($p<0.05$) (Significant)

Key:-

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

Groups	Number of rabbits (n)	Mean ± SD Values
A (Control)	10	2.07 ± 0.4
B	10	1.22 ± 0.11
C	10	2.1 ± 0.15

Table-IV. Comparisons of weights (kg) of rabbits at 4th week in groups A, B & C

Statistical Analysis

A vs B = ($p\leq0.05$) (Significant)
A vs C = ($p>0.05$) (Non-Significant)
B vs C = ($p<0.05$) (Significant)

Key:-

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

DISCUSSION

In recent years, several inorganic compounds have been shown to have insulin-like effects but they are very toxic at effective doses.[4] Most of the drugs exert their toxic effects on liver due to their metabolism in liver. However oral administration of Tungsten (VI) compounds is very effective just like vanadium in normalizing blood glucose levels in Diabetes mellitus of both types.

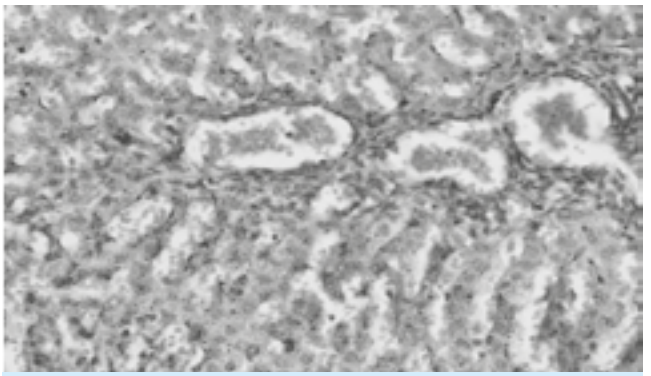

Figure-1. H&E stain: Section of liver showing hepatocytes morphology in group B (diabetic group without treatment) (X500)

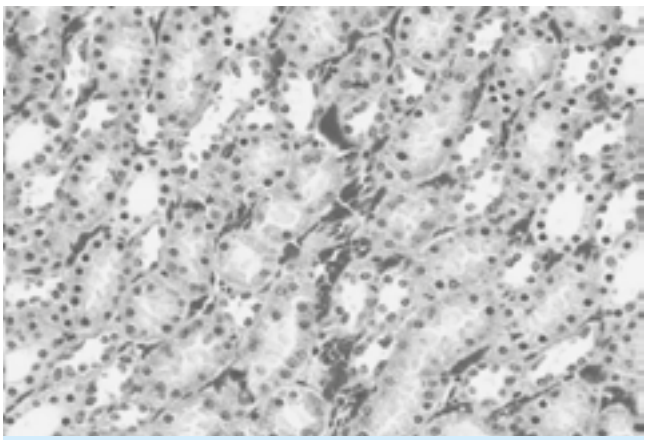

Figure-2. Trichrome stain: section of liver showing no pathology in group B (diabetic group without treatment) (X 500)

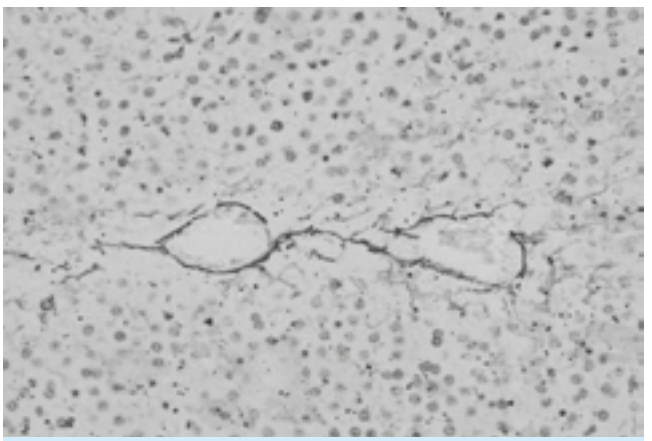

Figure-3. Reticulin silver stain: section of liver showing normal hepatocytes morphology in group B (diabetic group without treatment) (X 500)

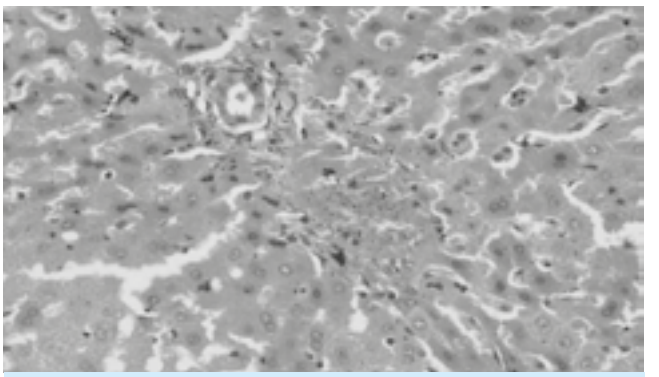

Figure-4. H&E stain: Section of liver showing normal hepatocytes morphology in group C (diabetic group with treatment) (x 500)

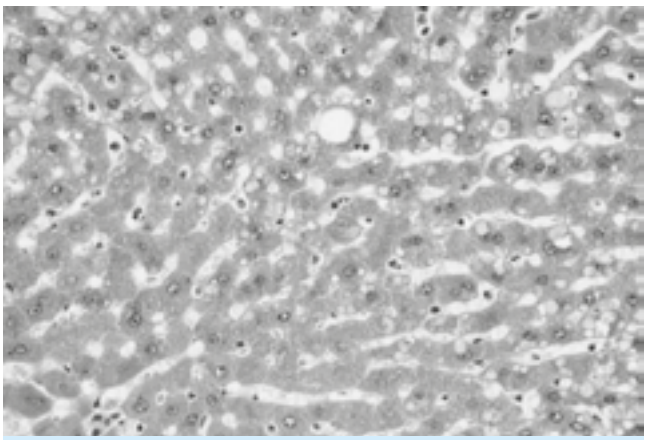

Figure-5. Trichrome stain: section of liver showing no pathology in group C (diabetic group with treatment) (X 500)

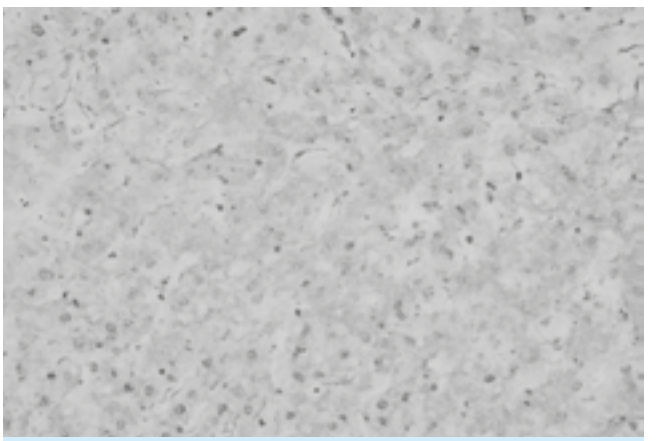

Figure-6. Reticulin silver stain: section of liver showing normal hepatocytes morphology in group C (diabetic group with treatment) (X 500)

This does not produce any side effects and overdose does not cause hypoglycemia.[5] Present study was carried out to demonstrate the effects of Sodium Tungstate therapy on blood glucose levels; overall weight of the animal; on the weight of the liver and histological features of the liver.

Results of the study indicate that the blood glucose

levels of rabbits in group B (Diabetic animals without Sodium Tungstate Therapy) were high as compared to group A (control group) and group C (diabetic rabbits with treatment with sodium tungstate) where the levels decreases with time (Table-5). These findings reveal antidiabetic effect of sodium tungstate. Several studies have shown the anti-diabetic activity of sodium tungstate in many diabetic animal models. This compound increased the effects of insulin in isolated cells and tissues as well as in vivo models. Sodium tungstate also regenerated pancreatic beta-cell population in the neonatal STZ rats in a type 2 diabetes model.[13]

In the present study, comparison between group B & C showed that diabetic rabbits with Sodium Tungstate Therapy (Group C) gained weight and the difference was statistically significant ($p<0.05$). The comparison between group A & C showed that the weight gain was statistically non-significant ($p>0.05$). These findings are same as described by claret et al (2005).[13] Another study by Ignasi et al (2009)[14] revealed reduced weight gain in rats taking sodium tungstate therapy for long period of time.

Gross findings of rabbits liver regarding color, texture, weight, size and cut surface revealed normal morphology in groups A, B and C. Regarding liver weight, there is non-significant difference in Diabetic rabbits without Sodium Tungstate Therapy i.e. group B and with treatment i.e. group C. Fibrosis staging of rabbit's liver at 4th week was done and there was not a single rabbit with positive findings in groups A, B & C. These findings are consistent with the results of Barbera et al (1997)[15] that treatment with sodium tungstate does not change the liver parameters.

There are two types of chromatin patterns in hepatocytes of rabbit. The chromatin pattern was mostly regular in all groups at 4th week duration. Regarding irregular chromatin pattern in hepatocytes only fewer showed irregular pattern in all groups at 4th week duration. This is consistent with studies of Snezana and Maria (2007)[16] who also observed that sodium tungstate has no effect on chromatin.

Lamer et al (2000)[17] showed that ST can be administered orally in water for treatment of diabetes with no damage to liver or muscle cells. Thus it provides a potential treatment for DM. This is also consistent with our results in which there are no gross and histopathological changes in liver of experimental animals.

CONCLUSION

When administered orally, Sodium Tungstate was shown to have good anti-diabetic activity. At therapeutic levels, no toxic effects were observed. Sodium Tungstate also produced no pathological histological changes in the livers of the experimental rabbits.

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3	Dr. Rana Khalid Mahmood	Verification of results, Interpretation, Drafting of research paper	