INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-3, July-Sept-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 24th June-2013 *Revised: 01st July -2013* *Accepted: 03rd July-2013*

Research article

IN-VIVO EVALUATION OF POTENTIAL TOXICITY OF VANADIUM PENTOXIDE IN MALE WISTAR RATS

Deepika Paramanik[a], and Ms.G.Rajalakshmi[b]

MPhil Research Scholar[a], Assistant Professor[b]

[a] and [b] PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore - 641028 Tamil Nadu, INDIA.

E-mail address: deepikaparamanik28@gmail.com[a], rajeragan@gmail.com[b]

ABSTRACT: Diabetes is characterized by derangements in carbohydrates, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion. The decreased oxidative stress in diabetes includes the autoxidation of glucose and non enzymaticglycation and also changes in the antioxidants defense systems. As the diabetes is lowered in human the pharmacological effects of a drug becomes toxic. Our aim was to evaluate the in-vivo effect of the anti diabetic compound Vanadium in non- diabetic rat models. The oral administration of Vanadium peroxide shows significant changes in the Cholesterol and TG with organ morphology and enhancement in clinical and experimental parameters.Vanadium is an important environmental and industrial pollutant. It is a dietary micronutrient and it has been recently considered as a pharmacological agent. Vanadium compounds have a major role in cellular regulation, with profound effects on enzymes of plasma membrane. Vanadium is one of the abundance trace elements widely distributed in the environment. It is used in steel and chemical industries, and is a containment of many ores, coals, and petroleum oils the manufacture of pigments, in photography and in insecticides. It is been used medicinally as anticeptic, spirochetocide, antituberculotic and antianemic agents and general tonic.

Key words: Diabetes, Vanadium, Administration, Pharmacological agent.

INTRODUCTION

Diabetes is a metabolic disorder, in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). It develops many secondary complications such as atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormality, diabetes retinopathy and ocular disorders (Sakurai *et al.*, 2002). Both type 1 and 2 are chronic conditions that usually cannot be cured. As of 2000 at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Type2 diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetes population. It has a considerable impact on both the patient and the society because it typically affects individuals in their most productive years. The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030 [World Health Organization Department of Non-communicable Disease Surveillance (1999)]. Over time, diabetes can damage the heart, blood vessels, eyes, kidneys and nerves; their common feature is impairment of the metabolism of carbohydrates and lipids (Anna Goc, 2006). Vanadium helps in Normalization of the blood-glucose level, Normalization of the lipid metabolism, Normalization of bile acids alteration, Normalization of protein and amino acid metabolism, Normalization of thyroid hormone level. Pharmacological uses of vanadium include lowering of cholesterol, triglycerides and glucose levels, diuretic effects, anti-carcinogenic effect, contraction of blood vessels, enhancement of oxygen-affinity of haemoglobin and myoglobin (Rehder, 1992). Vanadium is widely distributed in human tissues and is toxic when ingested in large doses. The most toxic forms of vanadium to mammals are the pentavalent form compounds (Altamirano-Lozano *et al.*, 1999). Interest in vanadium pharmacology becomes greatly advanced with the discovery of vanadium containing enzymes (Badmaev *et al.*,1999).

MATERIALS AND METHODS

Determination of acute oral toxicity

Table.1. Test substance details

Name of the test substance	Vanadium peroxide
Colour	Yellow
Nature	Crystalline powder

Table.2. Experimental protocol

Name of the study	Acute toxicity
Guideline followed	OECD 423 method. Acute toxic class method
Animals	Healthy young adult Wistar albino rats.
Body weight	150-200
Sex	Male
Administration of dose and volume	70mg/kg body weight, Single dose in 1ml
Number of groups and animals	Two groups each contains 6 mice were used to study LD_{50} value. First group was dosed 70mg/kg and observed for 24hr. Second group was dosed 70mg/kg and observed for 24hr.both the groups were observed for 28days to study the presence of any toxicity signs.
Route of administration	Oral by using rat oral feeding needle
Vehicle	Water

Table.3. Housing and feeding conditions

Room temperature	22˚C+ 3˚C
Humidity	40-60%
Light	12h:12hh (light: dark cycle)
Feed	Standard laboratory animal food pellets with water *ad libitum*

Table.4. Study period and observation parameters

Initial once observation	First 30 minutes and periodically for 24h
Special attention	First 1-4h after drug administration
Long term observation	Upto 28days
Direct observation parameters	Lethargy, sleep, increased appetite
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, etc.

The time of death, if any, is recorded. (Complete observations: annexure 1). After administration of the drug, food is withheld for a further 1-2hrs.

Study procedure:

Acute oral toxicity was performed as per the organization for Economic Co-operation and Development (OECD) guideline 423 methods. The AEALB was administered in a single dose by gavages using specifically designed rat oral needle. Animals are overnight fasted prior to dosing (food was withheld for 3h but not water). Following the period of fasting, animals was weighed and test substance was administered. After the AEALB administration, food was withheld 2h in rats. Animals are observed individually after at least once during the first 30 minutes, periodically during the first 24h, with special attention given during the first 4hrs, and daily thereafter, for total of 28days. Those animals which are found dead or in extreme distress if any are removed and humanely killed for animal welfare reasons (Akbarzadeh *et al*., 2007).

Pharmacological Evaluation

Animals and Management:

Male Albino Wistar rats, 6-8 weeks of age and 150-200g body weight were used in the present study (KMCH College of Pharmacy, Coimbatore, Tamilnadu, India). The rats were acclimatized for 1 week before the experiment. They were maintained under standard laboratory condition with required feed, temperature 24-28˚C, and humidity 60-70% and 12 hour light/dark cycle. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. They were divided into groups and body weight is determined on regular basis. The treated groups were given tap water to drink whereas the control groups received vanadium pentoxide (Fisher Scientific Co., Fair Lawn, NJ, U.S.A.) in drinking water. The experimental procedure was approved by IAEC (Institution of Animal ethical committee).

Preparation and induction of Vanadium pentoxide solution:

The Vanadium pentoxide was purchased from Hi-Media, Coimbatore. Vanadium pentoxide solution was freshly prepared at 70mg/kg. Vanadium pentoxide was dissolved prior to its use in distilled water. It was mixed, after an hour given orally.

EXPERIMENTAL DESIGN

Toxicological study

Rats were divided into 2 groups, six animals in each group. Each rat in treated group received vanadium pentoxide orally and was treated for 4 weeks as follows. The controlled group (six animals) received distilled water only. All the animals were placed in individual metabolism cages. Food and water consumption were measured daily. The average daily food and water consumption was calculated from the difference of the amount supplied to each cage and that left over. Body weights were measured and the protein efficiency coefficients were calculated weekly during the one month. At the end of the experiment three animals of each group were selected to determine possible alterations in the liver and renal functions. These animals were anaesthetized with ether and exsanguinated from the abdominal aorta. All the other animals were killed and the weight of the liver, kidneys, heart, spleen, eyes, brain and lungs measured (Domingo *et al*., 1985).

GROUP	TREATMENT
Group 1	Control rats given only drinking water
Group 2	Rats treated with chemical compound

Morphological studies:

At 4th week after vanadium pentoxide treatment, the animals were sacrificed by an overdose of diethyl ether given by inhalation followed by decapitation. Blood samples were collected from the decapitated site for the determination of vanadium concentration. Organs, including brain, lung, heart, liver, spleen, kidney, and eyes were quickly removed and weighed. The value of organ weight/body weight ratio of each organ was calculated. These organs were examined for any pathological changes. Thereafter, tissue samples were taken from each organ for histological study and for determination of vanadium concentration (Dai *et al*., 1994).

For histopathological studies, the tissues were placed in 10% buffered neutral formalin immediately upon removal and were later processed, embedded in paraffin, and sectioned at 3 microns. Representative sections were stained with haematoxylin and eosin for histological examination.

Body weight ratio:

Starting day of study onwards up to end of the study the animal body weight ratio was measured each day by electrical balance and the changes in the body weight is recorded (Shirwaikar *et al*., 2006).

Organ weight ratio:

The values of organ weight ratio of various organs in Control and vanadyl pentoxide-treated non-diabetic rats are recorded (S.dai *et al*., 1994).

Sample collection:

The rats were anesthetized with diethyl ether and blood was collected from retro orbital method for haematology parameter and serum was separated for biochemical parameter and rats were sacrificed by cervical dislocation. Liver, pancreas, spleen, eyes, lungs, brain and kidney were dissected out, washed in ice cold saline to remove the blood then collected in formalin solution for histopathology (Fröde and Medeiros, 2008).

Histopathology :

The organs were collected, weighed and washed under saline and preserved in 10% buffered neutral formalin (DNF). The trimmed tissue sections are subjected to prepare paraffin blocks. 5 micron thickness sections are cut and stained with Haematoxylin and Eosin (H&E) and observed under compound microscope.

Tissue homogenate:

10% tissue homogenate was prepared with 0.025M Tris –HCl buffer, (pH 7.5). After centrifugation at 10,000rpm for 10mins, the clear supernatant was used to measure Thio Barbituric Acid reactive substances (TBARS). The tissue were minced and homogenized with (10% w/v) in 0.1M Tris-buffer, (pH 7.4) and centrifuged at 10,000rpm for 10 min and the resulting supernatant was used for the further study.

Collection of Blood for Hematological Studies

After the treatment period the animals were anaesthetized by diethyl ether and the blood was collected from Retro-orbital sinus by using capillary into centrifugation tubes which contains EDTA for hematological parameters and without EDTA for serum biochemical parameters. It was allowed to clot at room temperature and serum was centrifuged at 5000rpm for 10min.

The hematological parameters like RBC, WBC, Hb percentage were estimated.

Estimation of Serum Biochemical Parameters

The separated serum was used for estimation of alkaline phosphatas (ALP), Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT), Serum Creatinine, Triglycerides, Albumin, Total protein, Alkaline phosphatise, Total cholesterol, HDL cholesterol was estimated using whole blood. All the above biochemical parameters were estimated using semi-auto analyzer (Photometer 5010 $_{V5+}$, Germany) with enzymatic kits procured from Primal Healthcare limited, Lab Diagnostic Division. Mumbai, India.

In-Vivo Activity

The animals were sacrificed after being treated with diethyl ether. Blood was collected with commercial syringe. Brain, heart, kidney, liver, lung, eyes, spleen were isolated, weighed and washed with bidistilled water. The separated Liver and Kidney samples were cut into pieces (deducted about 10-30~o of the total) and submitted to the same treatment as the other tissues. The tissues were collected in plastic vials and lyophilized under standardized conditions (Edel *et al*., 1984)

Estimation of Proteins

Procedure described by Lowery *et al*., 1951 was used for protein estimation. The method is based on the biuret reaction, formation of protein-copper complex and reduction of phosphomolybdo tungstate reagent (Folin-ciocalteu phenol reagent) by tyrosine and tryptophan residues of protein to form a colored product.

Purification of Protein

Ammonium Sulfate Fractionation

70gm of Ammonium sulfate was added to 100ml of extract supernatant. The medium was mixed thoroughly to bring saturation for 70%. After the provided time the precipitated proteins were regimented by centrifugation at 16000rpm at 4° C for 15minutes. The resulted pellet was dissolved in 20mM Tris-HCl buffer at pH 8.5 (Joshi, 2010). The left supernatant was applied again with ammonium sulfate to achieve 60% and 100% saturation. The protein content was determined for separate fraction. (Kashmiri *et al*., 2006).

Dialysis Against Distilled Water and Buffer

The obtained ammonium sulfate precipitate was introduced into a plastic bag for dialysis against distilled water for 3 hours, followed by dialysis against phosphate buffer at pH 7.0. The obtained protein was concentrated against sucrose and kept in the refrigerator at 5 ° C for further purification. (S.Kumar *et al*., 2005)

Isolation of Protein

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis

To separate protein by Sodium dodecyl Sulphate Polyacrylamide gel electrophoresis. SDS is an ionic detergent which binds strongly and denatures protein. The number of SDS molecule bound to a polypeptide chain is approximately half the number of aminoacid residues. The protein SDS complex carries net negative charge hence moves towards anode and the separation is based on the protein.

Statistical Analysis

The data were analyzed by using GRAPH PAD PRISM, VERSION 5.04, analysis of variance (ANOVA) followed by one-way ANOVA, followed by Dunnett test. Values were considered significant, when $P<0.05$.

RESULTS

Determination of Acute Oral Toxicity in Animal Models

All fractions did not show toxicity at 70mg/kg. Vanadium increased body weight and reduced cholesterol and bilirubin in the scheduled days. The SGOT, SGPT, CREATININE, UREA. URIC ACID, ALBUMIN, TC, TG, HDL, showed significant changes than the control.

Evaluation of Body Weight

Table.5. Shows result of the change in the body weight

GROUP	TREATMENT	CHANGE IN BODY WEIGHT	
		Day 0	Day 28
1	Control	206.67± 3.342	223.56 ± 3.342
2	Treated	154.68 ± 4.564	176.56 ± 2.012

The body weight of both Treated and Control rats increased 23% in 28 days ($P<0.001$). $P<0.001$, ns- significant

Data is expressed as mean ± sem. (n=6, animals in a group). Values are statistically significant at $p<0.001$.

Statistical comparison:

One way ANOVA Dunett test, followed by comparison of all columns V/S control column was performed.

Evaluation of Hematological Parameters

Table.6. Shows result of the change in the hematological parameters

Parameters	Mean ± SD
Total Haemoglobin (Hb) (g/dl)	14.82 ± 1.22
Packed Cell Volume (PCV) %	50.83 ± 4.54
Total WBC Count ($x10^3$)	12.17 ± 1.31
Polymorphs %	32.33 ± 7.06
Lymphocytes %	50.83 ± 8.37
Monocytes %	11.33 ± 2.66
Eosinophils %	5.50 ± 1.05
Total RBC Count ($x10^6$)	7.99 ± 0.62
MCV (fL)	63.67 ± 1.03
MCH (pg)	18.33 ± 0.52
MCHC (g/dl)	28.83 ± 0.41
RDW %	15.83 ± 0.75
Platelet Count ($x10^3$)	452.17 ± 50.38
MPV (fL)	8.67 ± 0.52

Hematological parameters such as Haemoglobin, WBC, RBC, Eosinophils, Lymphocytes, Monocytes, and Polymorphs were evaluated based on the significant value of P<0.001. All these parameters showed LD_{50} toxicity.

P<0.001, ns- significant

Data is expressed as mean ± sem. (n=6, animals in a group). Values are statistically significant at p<0.001.

Statistical comparison:

One way ANOVA Dunett test, followed by comparison of all columns V/S control column was performed.

Evaluation of Serum Biochemical Parameters

Table.7. Shows result of the change in the Serum biochemical parameters

Parameters	Control	Vanadium	't'value
SGOT	118.333 ± 18.896	151.833 ± 24.03	2.450
SGPT	56.167 ± 5.947	68.667 ± 9.75	2.447*
ALP	278.500 ± 19.614	319.667 ± 33.83	2.354*
Creatinine	0.233 ± 0.082	0.933 ± 0.197	7.355**
Urea	30.600 ± 1.655	36.767 ± 10.13	1.342ns
Uric acid	2.867 ± 0.472	12.233 ± 14.15	1.478ns
Cholesterol	83.483 ± 10.795	65.450 ± 19.98	1.776*
TG	85.250 ± 21.48	48.283 ± 7.650	3.624*
HDL	50.017 ± 7.908	61.233 ± 7.27	2.334*
Bilirubin	0.783 ± 0.29	0.400 ± 0.322	1.968*

Serum biochemical parameters such as SGOT, SGPT, ALP, CREATININE, UREA, URIC ACID, HDL, showed increase value of toxicity whereas CHOLESTROL, TG, BILIRUBIN showed decrease value of toxicity.

P<0.001, ns- significant

Data is expressed as mean ± sem. (n=6, animals in a group). Values are statistically significant at p<0.001.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Statistical comparison:

One way ANOVA Dunett test, followed by comparison of all columns V/S control column was performed.

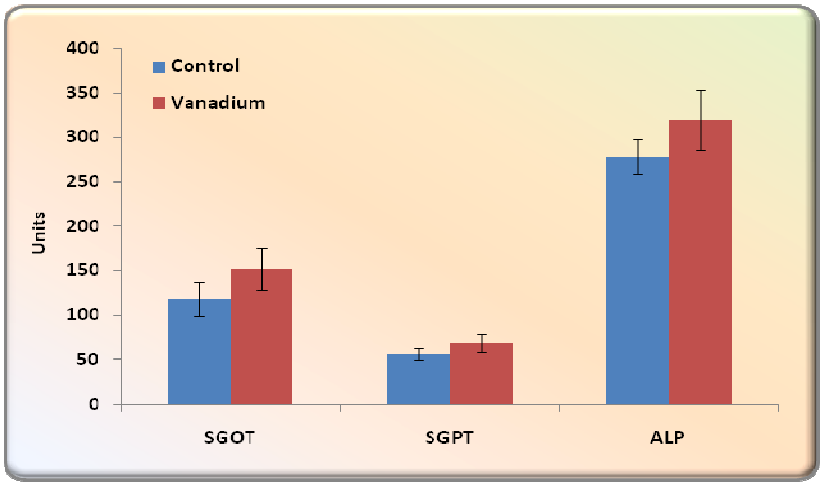

Figure.1. Shows graph of SGOT, SGPT & ALP

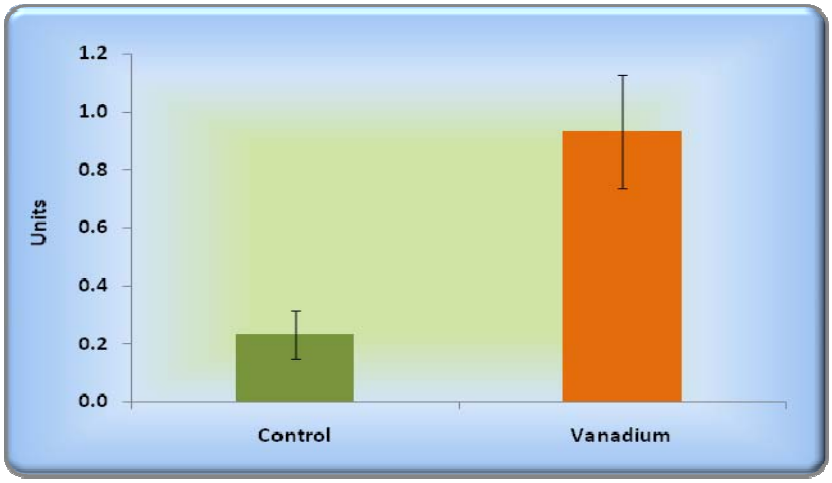

Figure.2. Shows graph of Creatinine

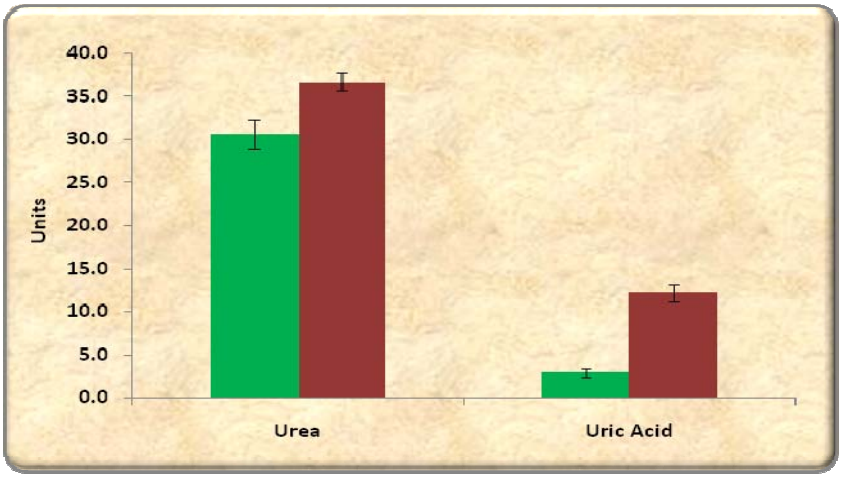

Figure.3. shows the graph of Urea and Uric acid

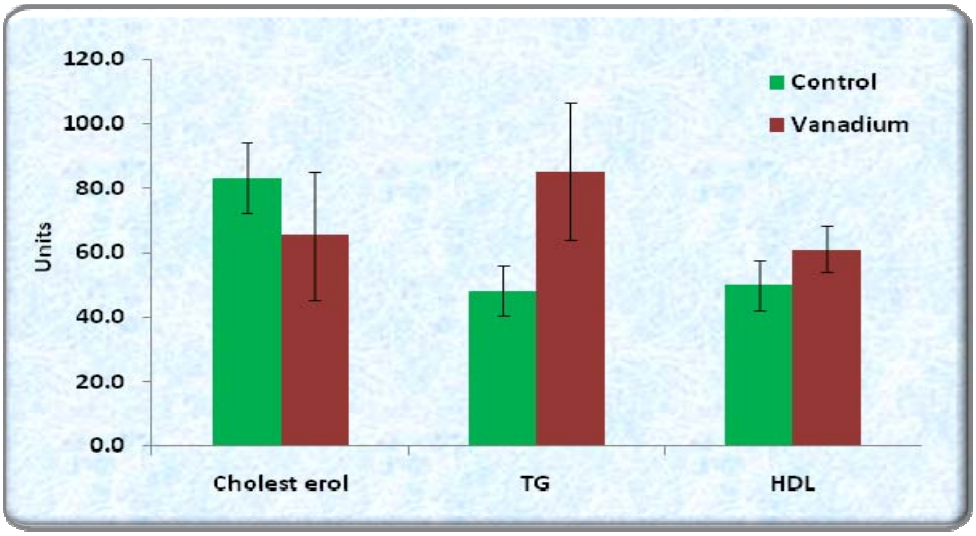

Figure.4. Shows the graph of Cholesterol, TG and HDL

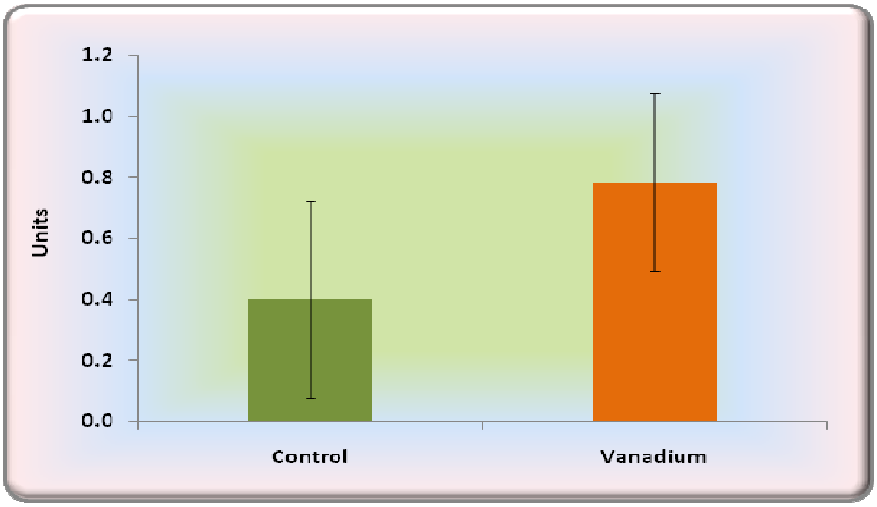

Figure.5. Shows the graph of Bilirubin

Histopathological evaluation of Control and Treated rats' organ

EYE

CONTROL **TREATED**

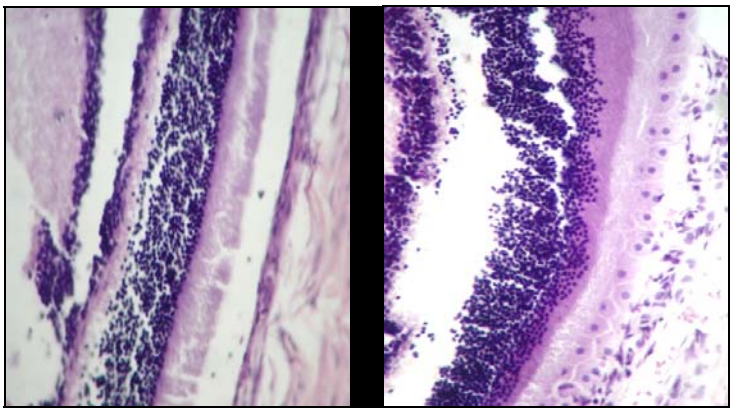

Figure.6. 40x Normal retinal layer of the eye

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

BRAIN

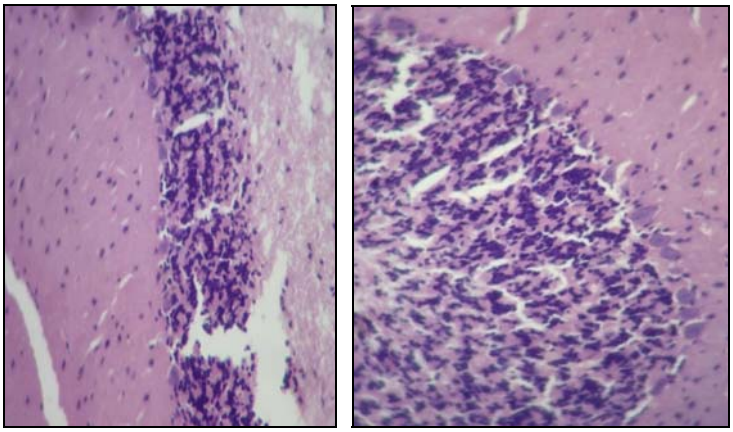

Figure.7. 40x Normal cerebellum with unremarkable purkinjie cells

HEART

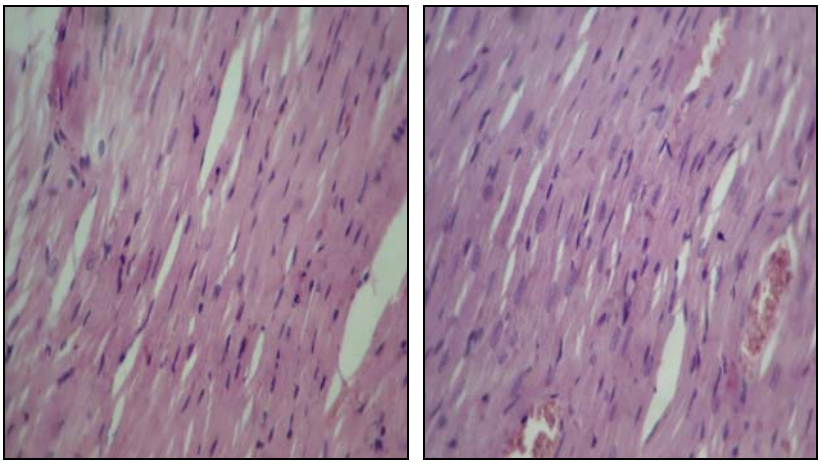

Figure.8. 40x Normal heart muscle

LIVER

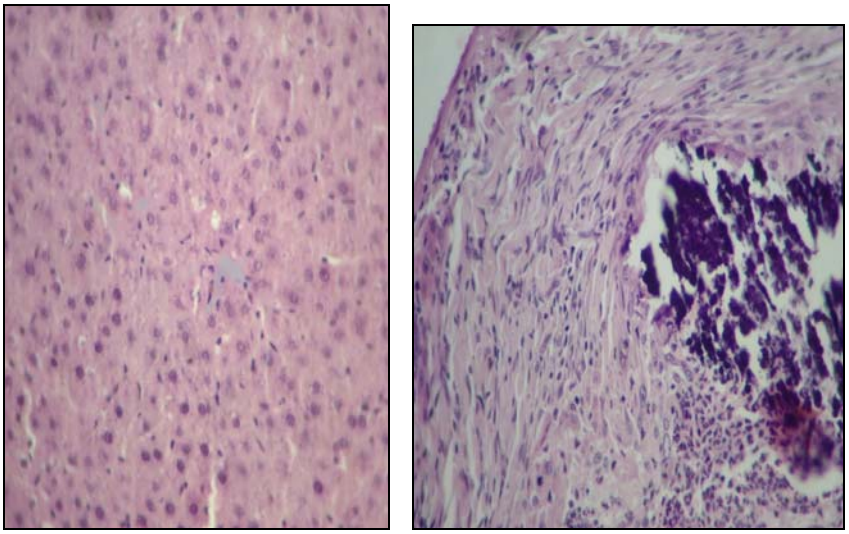

Figure.9. 40x normal central vein and sinusoids 40x Liver showing fibrosis with area of necrosis and acute inflammation

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

LUNGS

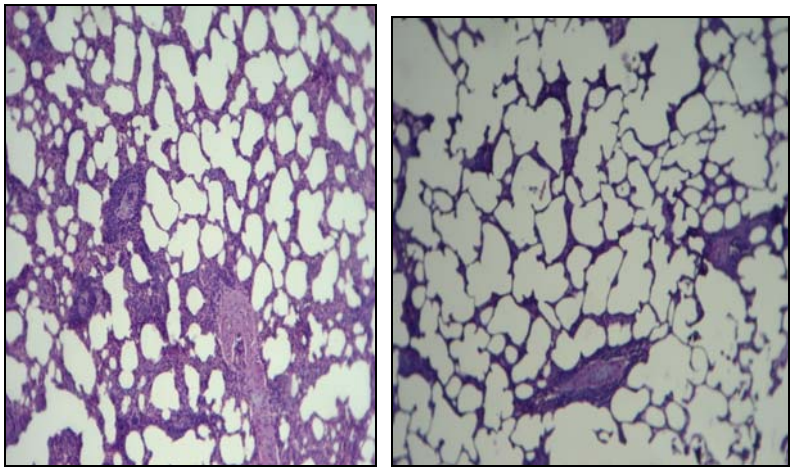

Figure.10. 10x Normal lung parenchyma with unremarkable alveoli

SPLEEN

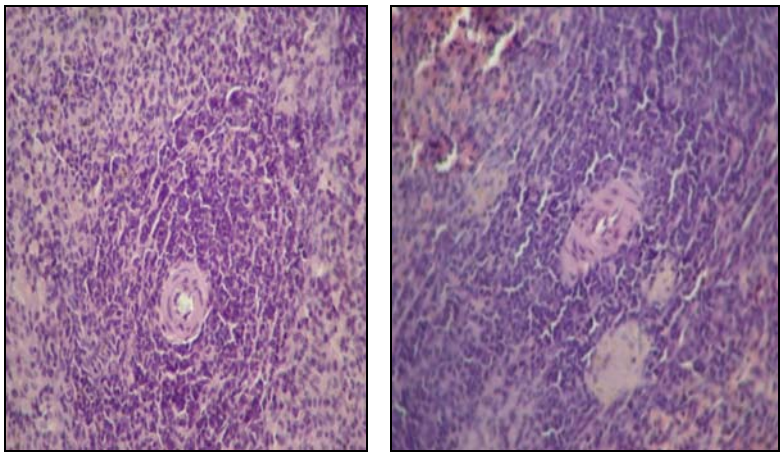

Figure.11. 40x Normal pencillar artery and white pulp

KIDNEY

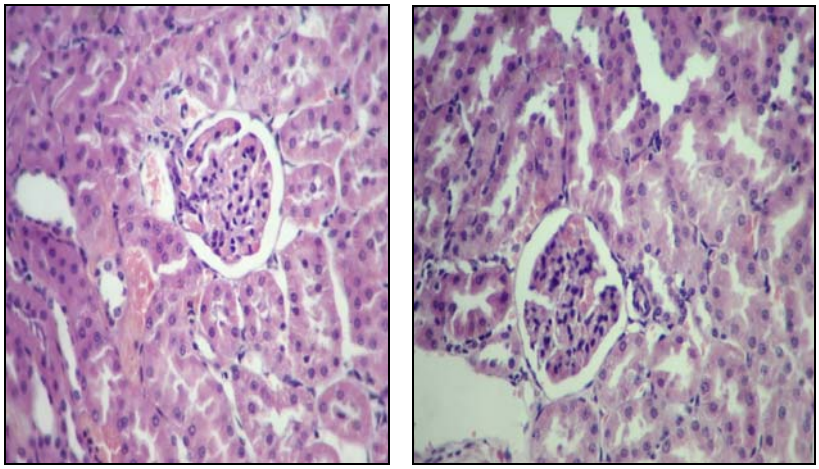

Figure.12. 40x Normal glomeruli and proximal convoluted tubules

Evaluation of DNA Bands from Animal Tissue

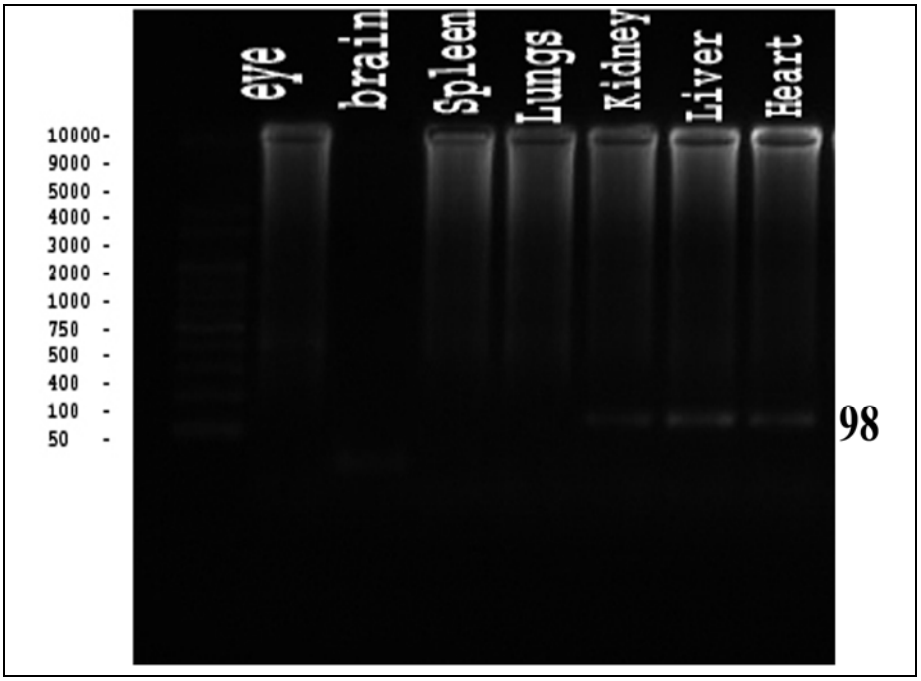

Figure.13. DNA bands formation through Agarose Gel electrophoresis

Table.8. shows the values of the toxicity through gene expression

Organs	Eye	Brain	Spleen	Lungs	Kidney	Liver	Heart
Fragment length	234.33 ± 13.199	199.40 ± 6.641	188.45 ± 5.345	163.50 ± 6.439	322.00 ± 21.993	389.56 ± 18.459	361.50 ± 14.950

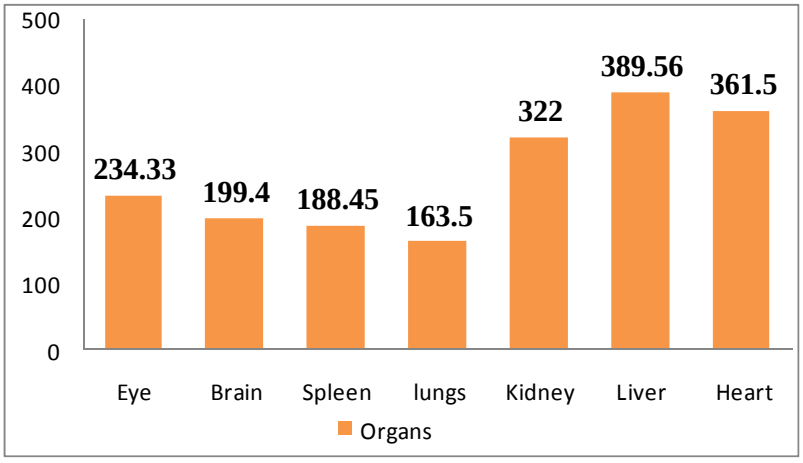

Figure.14. shows the graph of Vanadium toxicity in DNA.

P<0.001, ns- significant

Data is expressed as mean ± sem. (n=6, animals in a group). Values are statistically significant at p<0.001.

Statistical comparison:

One way ANOVA Dunett test, followed by comparison of all columns V/S control column was performed.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Evaluation of Protein Bands from Animal Tissue

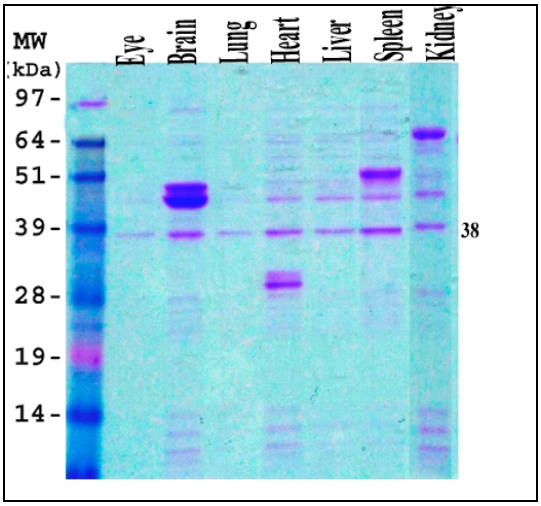

Figure.15. Protein bands (SDS-PAGE)

DISCUSSION

In present study, acute toxicity was tested up to high concentration of 100mg/kg. The rats died of this high concentration of chemical. Since the main purpose of the preliminary acute toxicity study is to get some idea on conspicuous behavioural changes and death, if any. The administration of vanadium pentoxide to decrease and increase the blood glucose level to normal blood glucose level is an essential trigger for the liver and kidney to revert its normal homeostasis during experimental diabetes. The characteristic loss of body weight was due to the abnormal increase and decrease in appetite as the Vanadium concentration was checked for the acute toxicity. These resulted in increased sleep and lethargic behaviour.

Estimation of Hematological Parameters

During this toxic study the hematological parameters showed increase in the Haemoglobin (Hb), Packed Cell Volume (PCV), Polymorphs, Monocytes, Red Blood Cells (RBC) and decrease in Total WBC Count, Lymphocytes and Eosinophils.

Serum biochemical Parameters

Several studies have shown increased in clinical and experimental biochemical parameters. Liver metastasis can be determined by changes in serum ALT (SGOT and SGPT) levels. The result showed the administration of chemical Vanadium Pentoxide significantly increased several parameters, SGOT, SGPT, ALP, CREATININE, UREA, URIC ACID, HDL, BILIRUBIN and decrease in CHOLESTROL and TG level in treated group in dose dependant manner. Significantly lowering of TG and raise in HDL is a very desirable biochemical status for prevention of Arthrosclerosis and Ischemic conditions.

Histopathological Examination

The histopathological examination revealed extensive alteration in the liver and kidney. The liver and kidney of control rats showed normal islets. From the result, we conclude that Vanadium pentoxide lowered complications of TG and HDL Cholesterol. However it seems promising for future trials.

Estimation of Toxicity by Gene Expression

The results obtained in this study showed that vanadium pentoxide caused a significant increase in the length of DNA migration. It showed 38kbps of the fragment length that occurred mostly in both the cases. Treatment of vanadium pentoxide produced DNA damage in liver, kidney, heart, lung, spleen and brain. However, some organs were more susceptible to this kind of damage than others. Liver, heart, and kidney showed the highest response, 322.00 $\pm$21.993, 389.56 $\pm$18.459, 361.50 $\pm$14.950 respectively and all of them have been reported previously as target organs for the action of vanadium. Although the lung has also been reported as a target organ for vanadium, the low sensitivity that was observed in this organ could be due to the administration route used in this study (Altamirano-Lozano *et al*. 1999).

CONCLUSION

As diabetes is a multifactorial disease leading to several complications, and therefore demands a multiple therapeutic approach. Vanadium compounds have been extremely useful as probes of enzyme structure and function and of the role of tyrosine phosphorylation in cellular signaling. Several lines of evidence now indicate that early neurophysiological and neurodegenerative changes should be considered as targets for therapy to supplement existing treatments for diabetic retinopathy. Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Our aim was to evaluate the in-vivo consequences of the Vanadium an Anti-Diabetic agent on the Non-Diabetic rat model. From the results, we concluded that the Vanadium compound lowered the TG and Cholesterol and also without affecting much of the appropriate organs. However, it seems promising that if these data will be validated in the future clinical trials with this chemical compound, it will establish a potent medicine for many disease based on the diet of today's world.

REFERENCES

Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi SH, Farhangi A, Verdi AA, Mofidian SM, RAD BL. (2007). Induction of diabetes by Streptozotocin in rats. Indian Journal of Clinical Biochemistry: Vol.22, 2, 60-4

Altamirano.-Lozano. M., Mahara Valverde, Lucia alvarez-Barrera, Bertha Molina and Emilio Rojas (1999). Genotoxic studies of Vanadium Pentoxide (V_2O_5) in Male Mice. II. Effects in Several mouse tissues. Teratogenesis, Carcinogenesis, and Mutagenesis :Vol.19, 243–255

Anna Goc (2006). Biological activity of vanadium compounds. Central European Journal of Biology: Vol. 1, 3, 314–332

Badmaev, V., Prakash, S., Majeed, M. (1999). Vanadium: A review of its potential role in the fight against diabetes. The Journal of Alternative and Complementary Medicine: Vol. 5, 3, 273–291

Domingo J. L., Llobet J. M. and Tomas J. M., Corbella J. (1985). Short-Term Toxicity Studies of Vanadium in Rats. Journal of Applied Toxicology, VOL. 5, 6, 418-421

Edel.J, Pietra. R., Sabbioni. E., Marafante. E., Springer A. and Ubertalli L. (1984). Disposition of vanadium in rat tissues at different age. Chemosphere, Vol.13, 1, 87 – 93.

Fröde. T S, Medeiros. Y S. (2008). Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology: Vol.115, 2, 173-183

Hiromu Sakurai, Yoshitane Kojima, Yutaka Yoshikawa, Kenji kawabe, Hiroyuki Yasui (2002). Antidiabetic Vanadium (IV) and Zinc (II) complexes. Elsevier. Coordination Chemistry Reviews: Vol.226, 187–198

Kashmiri.M.A, Ahmad Adnan and Beenish Waseem Butt (2006). Production, purification and partial characterization of lipase from *Trichoderma viride*. African journal of Biotechnology: Vol.5, 10, 878-882

Satyendra Kumar, Khyodano Kikon, Ashutosh Upadhyay, Shamsher S. Kanwar, Reena GUPTA (2005). Production, purification, and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. Elsevier. Protein Expression and Purification: Vol. 41, 1, 38–44.

Shirwaikar A, Rajendran K, Barik R. (2006). Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. Journal of Ethnopharmacology: Vol.107, 2, 285-290

S. Dai, K. H. Thompson, E. Vera and J. H. Mcneill (1994). Toxicity Studies on One-Year Treatment of Non-Diabetic and Streptozotocin-Diabetic Rats with Vanadyl Sulphate. Pharmacology & Toxicology: Vol.15, 265-273

Oliver H.Lowry, Nira J.Rosenbrough, A.Lewis Farr and Rose J.Randall. (1951). Protein measurement with the Folin phenol reagent. Journal of Biochemical chemistry: Vol.193, 265-275

World Health Organization Department of Non-communicable Disease Surveillance, (1999).