

## Insulin-mimetic activity of vanadium and zinc in diabetic experimental rats

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**Abstract:** Forty-two adult male albino rats Sprague –Dawley strain were classified into normal control group and five diabetic rat groups which were control (+ve), drug, zinc , vanadium and zinc with vanadium. The diabetic control (+ve) group showed a significant increase in the values of glucose ,glucosylated hemoglobin ,serum alanine and aspartate amino transferase (ALT & AST), alkaline phosphatase (Alk-phos) enzymes, creatinine , urea ,cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant decrease in final weight, weight gain, FER, insulin, hemoglobin (HB) , packed cell volume ,HDL-c ,liver glycogen, liver glutathione peroxidase (GPX) compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant decrease in the values of serum glucose , glucosylated hemoglobin, ALT ,AST ,urea, serum cholesterol, triglyceride (TG), LDL-c, VLDL-c level , cholesterol/ HDL-c ,liver cholesterol, liver total lipid and liver malondialdehyde (MDA) but a significant increase in the values of final weight, weight gain percent , FER ,insulin ,packed cell volume (PCV) ,HDL-c ,liver glycogen and glutathione peroxidase (GPX) compared to control (+ve) group.

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### 1. Introduction

Diabetes mellitus is one of the most widespread diseases in the world and classified as either type 1 insulin-dependent which can be controlled only by daily injections of insulin or type 2 non-insulin dependent which treated by several types of synthetic therapeutics. Diabetes significantly increases risk of developing multiple micro-vascular and cardiovascular complications. The cardiovascular events associated with type 2 diabetes and the high incidence of other macro-vascular complications, such as stroke and amputation (Huang et al., 2007). Diabetes mellitus needs safe and efficient treatment. Because of the failure and secondary effects of promising treatments, many people resort to using alternative therapies for illnesses (Ryan et al., 2001).

Historically, vanadium was used to treat diabetes mellitus dates backed to 1899, before the discovery of insulin in 1921 and since then numerous studies have described the in vivo and in vitro anti-diabetic effects of vanadium salts and compounds (Mukherjee et al., 2004). Vanadium with atomic number 23, atomic weight 50.9415 and oxidation states from III to V has a wide variety of biochemical and physiological functions. Among them, an insulin-mimetic antidiabetic effect is the most striking, the effect being provided by the oxidation states of

vanadic V (III), vanadyl V (IV) and vanadate V (V) (Tracey and Crans, 1998). Vanadium influences the behavior of enzymes, regulates the activities of second messengers, signal transduction cascades and carbohydrate metabolism, mimics insulin and growth factor activities, stimulates protein tyrosine kinase and inhibits phosphotyrosine phosphatases and modulates gene expression (Srivastava and Mehdi, 2005 and Antonio et al., 2009).

Zinc with atomic number 30, atomic weight 65.39 and oxidation state II is an essential element in all living systems and plays a structural role in many proteins and enzymes. Many proteins have been found to have a zinc-containing motif that serves to bind DNA embedded in their structure (Wolfgang and Harold 2006). Zinc is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis. Zinc requires in immunity, intermediary metabolism, DNA metabolism and repair, reproduction, vision, taste, and cognition/behavior. Zinc was found to have important physiological and pharmaceutical functions involving insulin-mimetic activity (Song et al., 2001 and Yutaka et al., 2004).

The purpose of this study was to investigate the effect of vanadium and zinc or both on diabetic rats.

## 2- Materials and methods

### A – Materials:

#### 1-Chemicals and drugs

Streptozotocin was procured from Sigma, St. Louis, MO, USA. Octozinic capsules produced by October pharma S.A.E and contain 110 zinc sulphate heptahydrate. The therapeutic human dose was 220 mg daily. Vanadyl sulfate-3-hydrate was obtained from Hanawa Extra Pure Reagent China. The human insulin mimics dose of vanadium for treatment of diabetes mellitus was 100 mg daily (Boden et al., 1996). Amaryl drug is antidiabetic drug, produced by Saofi –Avents Egypt under licence of Saofi –Avents Germany. It is antidiabetic sulfonylurea. Each tablet contains 2mg glimepiride. The human therapeutic dose is 4 mg daily. The human therapeutic dose of zinc sulphate heptahydrate, vanadyl sulfate-3-hydrate and amaryl drug were converted to rat dose according to Paget and Barnes, (1964) that were 20, 9 and 0.36 mg/Kg body weight, respectively and dissolved in distilled water and given to rats by oral intubations. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodiagnostics, Dokki, Egypt.

#### 2-Experimental animals:

A total of forty-two Sprague –Dawley adult male rats were purchased from the National Research Center, Giza, Egypt. The average weight was 130±8 g.

#### 3- The standard diet:

The standard diet was performed according to NRC (1995) which comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamin mixture (20g/kg) and DL-methionine (3g/kg).

### B- Methods

#### 1-Experimental design:

Rats were housed in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into six groups (7 rats each). The first group kept as normal control fed basal diet only. The other five groups were injected with a single intraperitoneal dose of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection in order to prevent hypoglycemia (Peschke et al., 2000). After four days, blood samples were taken from orbital plexus for estimation of glucose. The rats having persistent hyperglycemia were considered as diabetic rats and used for the

experiment. The diabetic rats were classified into control (+ve) and treated four groups that were drug, zinc, vanadium and zinc with vanadium.

The food intake was calculated daily and the body weight gain was recorded weekly. Feed efficiency ratio (FER) was determined by Chapman et al., (1950) as following: FER = weight gain (g)/ feed intake (g).

#### 2-Collection of blood, liver samples and Pancreas:

At the end of experiment (ten weeks), rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. The liver of sacrificing rats was removed by careful dissection, blotted free of adhering blood, washed with cold saline solution, and dried between two filter papers. Livers perfuse with 50 to 100 of ice cold 0.9% NaCl solution. Pancreas was dissected out and immediately washed in ice-cold saline and fixed in 10% neutral buffered formaldehyde solution at pH 7.5, cleared in xylol, and embedded in paraffin. 4-5 µm thick section were prepared and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination according to (Bancroft et al., 1996).

#### 3- Blood analysis:

First part of blood samples was heparinized for estimation of hemoglobin and packed cell volume (PCV) (Drabkin, 1949 and Mc Inory 1954). Second part of blood was collected in tubes containing potassium oxalate and sodium fluoride for the estimation of glucose by *O*-toluidine method (Sasaki et al., 1972). Third part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum insulin and glucosylated hemoglobin (Hb A1c %) were estimated according to Wilson and Miles (1977) and Abraham et al., (1978). Serum alanine and aspartate aminotransferase (ALT&AST), and alkaline (AP) activity enzymes were estimated according to Reitman and Frankel (1957) and Kind and King (1954), respectively. In addition, creatinine and urea were estimated according to Bonsens and Taussky, (1984), and Patton and Crouch, (1977), respectively.

Serum cholesterol, triglycerides, high density lipoprotein cholesterol, low and very low density lipoprotein cholesterol were determined according to Trinder and Ann (1969), Young and Pestaner, D.L. (1975), Richmond (1973), Fruchart, (1982) and Friedwald, et al., (1972), respectively. Atherogenic index (cholesterol /HDL-c) was calculated according to Castelli and Levitar, (1977).

#### 4- Liver analysis:

Livers samples were analyzed for estimation of glycogen, cholesterol and total lipids according to Rerup and Lundquist, (1967), Abell et al., (1952), and Folch et al., (1957), respectively. Liver glutathione peroxidase (GPX) and malondialdehyde (MDA) were estimated according to Weiss et al., (1980) and Draper and Hadley,(1990), respectively.

### 8-Statistic analysis:

Collected data were presented as mean  $\pm$ SD and statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance according to Artimage and Berry (1987).

### 3. Results and Discussion

The diabetic control (+ve) group showed a significant decrease in final weight, weight gain and FER at  $p < 0.01$  compared to normal control group. The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed the values of final weight, weight gain percent, food intake and FER around the values of normal control group. The values of final weight, weight gain percent and FER were increased in all treated groups compared to control (+ve) group as shown in table (1).

Nutritionally, vanadium is thought to be a cofactor in various enzymatic reactions. Data from animal and human studies suggest that vanadium mimics the action of insulin. Consequently; it may serve a beneficial role in promoting healthy glucose metabolism in individuals with diabetes. Vanadium deficiency in animals manifests as diminished growth, reproductive impairment, and disruptions of metabolic and cellular function in the kidney, heart and brain. Vanadium has been included in the list of 40 essential micronutrients that are required in small quantities for normal metabolism as well as proper growth and development of mammals (French and Jones, 1993 and Verma et al., 1998). On the other hand, Zn (II) ion, an essential trace element with relatively low toxic profile in animals and humans and has been proven to have a pronounced insulinomimetic activity. Zinc occurs in hundreds of enzymes and in thousands of protein domains. Zinc is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis (Wolfgang and Harold 2006).

The diabetic control (+ve) group showed a significant increase in the values of glucose and glucosylated hemoglobin at  $p < 0.001$  &  $0.01$ , respectively and a significant decrease insulin, hemoglobin (HB) and packed cell volume (PCV) at  $p < 0.001$ ,  $0.05$  &  $0.01$ , respectively compared to normal control group. The diabetic rat groups which

treated with drug, zinc, vanadium and zinc with vanadium showed a significant increase in the values of glucose ( $p < 0.05$  &  $0.01$ ) and a significant decrease insulin at  $p < 0.05$  compared to normal control group while showed a significant decrease in the values of both glucose and glucosylated hemoglobin and a significant increase in the values of insulin and packed cell volume (PCV) compared to control (+ve) group as shown in table (2).

A beneficial effect of vanadyl sulfate at a dose of 100 mg/day for three weeks appeared in improving insulin sensitivity. Measurement of fasting plasma glucose and insulin-mediated glucose disposal during pre- and post-treatment periods showed a beneficial effect of vanadyl sulfate on improving both hepatic and peripheral insulin sensitivity (Cohen et al., 1995). The reductions in fasting blood glucose and hemoglobin A1c (HbA1c), and improved responses to oral glucose tolerance testing, in vanadium treatment of type 2 diabetes compared to exacerbated diabetic symptoms in the two placebo controls (Thompson et al., 2009). Vanadium increase insulin sensitivity due to inhibit protein tyrosine phosphatase, reduce gluconeogenesis and increase glycogen deposition (Sakurai et al., 2006). The elevation in the glucose levels of STZ-rats treated with Vanadyl complex was significantly lower than those of the control, indicating that vanadyl complex improved the diabetic state of animals. Similarly, zinc (II) complex substantially lowered the blood glucose levels in mice (Akira et al., 2009). The effects of Zn (II) ion on both glucose oxidation and lipolysis stimulation are inhibited by extracellular catalase, largely resulting from  $H_2O_2$  generation. Zn (II) ion increase of specific insulin binding and stimulates both lipogenesis and glucose transport in the adipocytes. Zn (II) ion was indicated to relate to the carbohydrate metabolism through insulin or insulin receptor (Wolfgang and Harold 2006).  $Zn^{2+}$  is an essential element for normal exocrine and endocrine function of the pancreas. Serum glucose concentration is another parameter associated with liver and pancreatic function. Elevated glucose levels in serum can be associated with a decreased pancreatic endocrine function. Additionally, since glucose is regulated by the liver, elevated serum glucose concentration may also be related to exposure to hepatotoxins (Fugono et al., 2002 and Liu et al., 2009).

The diabetic control (+ve) group showed a significant increase in the values of serum alanine and aspartate amino transferase (ALT & AST), alkaline phosphatase (Alk-phos) enzymes, creatinine and urea at  $p < 0.001$  compared to normal control group. The diabetic rat groups which treated with

drug, zinc and vanadium showed a significant increase in the values of serum amino transferase (ALT & AST), alkaline phosphatase (Alk-phos) enzymes, creatinine and urea at  $p < 0.05$  &  $0.01$  while the diabetic rat group which treated with zinc with vanadium showed a significant increase in the above mentioned parameters except the value of AST compared to normal control group. The diabetic rat groups which treated with drug showed a significant decrease in the values of serum ALT, AST and urea while the diabetic rat group which treated with zinc showed a significant decrease in the values of serum ALT, AST, alkaline phosphatase enzymes and urea compared to control (+ve) group. The diabetic rat groups which treated with vanadium or zinc with vanadium showed a significant decrease in the above mentioned parameters compared to control (+ve) group as shown in table (3).

Vanadium possesses a regulatory role in the biological system, influences a number of enzymes, regulates the functions of several second messengers and modulates a battery of genes. The discovery of several pharmacological properties, such as the insulin-mimetic action, antihyperlipidemia, antihypertension, antiobesity, enhancement of oxygen affinity of hemoglobin and myoglobin, and diuretic action, opens up a number of therapeutic avenues of this trace element (Mukherjee et al., 2004; Abd El-Ghanny 2007). ZnCl<sub>2</sub> supplementation was reported to increase liver enzyme levels. Serum creatinine is a marker of liver and kidney function. The creatinine precursor, creatine phosphate is biosynthesized in the liver and its muscle byproduct; creatinine is maintained by the kidney. Zinc stabilizes membranes structures and cellular components, is involved in the synthesis of growth hormone, alkaline phosphatase and collagen (Johnson et al., 2010).

The diabetic control (+ve) group showed a significant increase in the values of cholesterol, triglyceride (TG), LDL-c, VLDL-c level and cholesterol/ HDL-c at  $p < 0.001$  but a significant decrease in HDL-c at  $p < 0.001$  compared to normal control group. The diabetic rat group which treated with drug showed a significant increase in the values of triglyceride (TG), LDL-c and VLDL-c level at  $p < 0.05$  &  $0.01$  but a significant decrease in HDL-c at  $p < 0.05$  compared to normal control group. The diabetic rat groups which treated with zinc and vanadium showed a significant decrease in HDL-c at  $p < 0.05$  but a significant increase in serum LDL-c and VLDL-c at  $p < 0.01$  &  $0.05$ , respectively while the diabetic rat group which treated with zinc with vanadium showed a significant increase in serum LDL-c and VLDL-c at  $p < 0.01$  &  $0.05$ , respectively compared to normal control group.

The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant decrease in the values of cholesterol, triglyceride (TG), LDL-c, VLDL-c level and cholesterol/ HDL-c but a significant increase in HDL-c compared to control (+ve) group as shown in table (4).

Vanadium acts by improving the sensitivity of insulin in type 1 and type 2 diabetes. It has also been shown to reduce cholesterol levels and blood pressure (Beliaeva et al., 2000). Adipocytes play a crucial role in energy storage and homeostasis by converting free fatty acids into triglycerides and by producing a variety of adipokines, and therefore provide a very useful model for addressing questions related to insulin signaling and the interface between sugar and fat metabolism (Andre et al., 2006). Zn<sup>2+</sup> supplementation at high levels increases serum Zn<sup>2+</sup> concentration, serum LDL-c, HDL-c, serum total cholesterol and serum triglycerides. Therefore, ZnCl<sub>2</sub> supplementation might be beneficial in obesity situation (Roosbeh et al., 2009).

The diabetic control (+ve) group showed a significant increase in the values of liver cholesterol, total lipid and malondialdehyde (MDA) at  $p < 0.001$  but a significant decrease in the values of liver glycogen and liver glutathione peroxidase (GPX) compared to normal control group. The diabetic rat groups which treated with drug and zinc showed a significant increase in the values of liver total lipid and malondialdehyde (MDA) at  $p < 0.05$  &  $0.001$ , respectively while the diabetic rat groups which treated with vanadium and zinc with vanadium showed a significant increase in the value of liver malondialdehyde (MDA) at  $p < 0.01$  compared to normal control group.

The diabetic rat groups which treated with drug, zinc, vanadium and zinc with vanadium showed a significant increase in the values of liver glycogen and glutathione peroxidase (GPX) but a significant decrease in the values of liver cholesterol, liver total lipid and malondialdehyde (MDA) compared to control (+ve) group as shown in table (5).

Vanadium has insulin-like effects and is currently being considered for oral therapy. It also reduces gluconeogenesis and increases glycogen deposition. Vanadium salts induced sustained falls in blood glucose in diabetic rodents (Ray et al., 2004). Vanadium inhibits hepatic lipid peroxidation and superoxide dismutase (SOD) and elevation of glutathione (GSH) status as well as cytochrome P450 (CYP) and glutathione S-transferase (GST), indicating modulation of the hepatic antioxidant as well as phase I and II xenobiotic metabolizing

enzymes (Chakraborty et al., 2007 and Anupam et al., 2010).  $Zn^{2+}$  is a major requirement for all life forms and promotes physiological and biochemical functions. Biochemically,  $Zn^{2+}$  is very important in many cellular and biochemical functions such as acting as a cofactor and serving as an integral constituent of many antioxidant enzymes such as superoxide dismutase and several other metalloenzymes (Yutaka et al., 2004 and Franklin et al., 2005). Zinc is an essential component of a great number of zinc dependent enzymes, which participate in antioxidant processes and in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids (Koury and Donangelo, 2003).

The histopathological examination showed that control (-ve) rat group showed normal islets with clusters of purple stained  $\beta$ -cells with a normal architecture of pancreas (Pict 1) while the section of control (+ve) rat showed atrophy of  $\beta$ -cells and vacuolar degenerative changes in islets (Pict 2). The diabetic rat group which treated with drug showed normal histological structure (Pict 3). Diabetic rat group which treated with vanadium showed  $\beta$ -cell

degeneration (Pict 4) while diabetic rat group which treated with zinc showed reduction in  $\beta$ -cell numbers with increase in islets (Pict 5). The diabetic rat group which treated with zinc with vanadium showed less marked cellular degeneration of pancreatic  $\beta$ -cell (Pict 6).

The obtained results were in parallel with the histopathological examination of the pancreas. Streptozotocin molecules might increase the production of oxygen free radicals that play a crucial role in determining tissue injury as exert direct or indirect effects on islet endothelium and mediate fragmentation of nuclear DNA in beta cells leading to histological changes as well as functional abnormalities (Kakkar et al., 1998). Vanadium can partially preserve  $\beta$ -cells and increase in the number of surviving  $\beta$ -cells through amelioration of hyperglycemia but cannot completely prevent  $\beta$ -cells cytotoxicity from streptozotocin. (Ramachandran et al., 2003)

**Table (1): Mean values  $\pm$  SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups.**

| Variables<br>Groups | Initial<br>Weight<br>(g)          | Final<br>Weight<br>(g)               | Weight<br>Gain<br>(g)               | Food<br>Intake<br>(g/d)          | FER                                  |
|---------------------|-----------------------------------|--------------------------------------|-------------------------------------|----------------------------------|--------------------------------------|
| Control<br>(-ve)    | 130.56 $\pm$<br>3.55 <sup>a</sup> | 210.71 $\pm$<br>5.14 <sup>a</sup>    | 61.36 $\pm$<br>3.22 <sup>a</sup>    | 18.32 $\pm$<br>1.14 <sup>a</sup> | 0.072 $\pm$<br>0.002 <sup>a</sup>    |
| Control<br>(+ve)    | 132.17 $\pm$<br>3.45 <sup>a</sup> | 168.32 $\pm$<br>4.13 <sup>b***</sup> | 27.35 $\pm$<br>2.17 <sup>b***</sup> | 16.88 $\pm$<br>1.20 <sup>a</sup> | 0.035 $\pm$<br>0.001 <sup>b***</sup> |
| Drug                | 135.31 $\pm$<br>3.71 <sup>a</sup> | 211.15 $\pm$<br>5.61 <sup>a</sup>    | 56.04 $\pm$<br>2.69 <sup>a</sup>    | 18.41 $\pm$<br>1.35 <sup>a</sup> | 0.068 $\pm$<br>0.003 <sup>a</sup>    |
| Zinc                | 131.45 $\pm$<br>3.25 <sup>a</sup> | 199.89 $\pm$<br>4.98 <sup>a</sup>    | 52.06 $\pm$<br>2.61 <sup>a</sup>    | 17.55 $\pm$<br>1.12 <sup>a</sup> | 0.064 $\pm$<br>0.001 <sup>a</sup>    |
| Vanadium            | 133.65 $\pm$<br>2.99 <sup>a</sup> | 205.11 $\pm$<br>5.47 <sup>a</sup>    | 53.46 $\pm$<br>2.68 <sup>a</sup>    | 17.90 $\pm$<br>1.31 <sup>a</sup> | 0.066 $\pm$<br>0.004 <sup>a</sup>    |
| Zinc +<br>Vanadium  | 134.40 $\pm$<br>2.49 <sup>a</sup> | 213.61 $\pm$<br>5.66 <sup>a</sup>    | 58.93 $\pm$<br>3.11 <sup>a</sup>    | 18.35 $\pm$<br>1.11 <sup>a</sup> | 0.071 $\pm$<br>0.003 <sup>a</sup>    |

Significant with control (-ve) group \*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

Mean values in each column having different superscript (a, b, c) denote significant difference.

**Table (2): Mean values  $\pm$  SD of glucose, insuline, hemoglobin (HB), packed cell volume (PCV) and glucosalated hemoglobin HbA<sub>1C</sub> of the experimental rat groups.**

| Variables<br>Groups | Glucose<br>(mg/dl)                   | Insulin<br>( $\mu$ l)              | HB<br>(g/dl)                      | PCV<br>%                           | HbA <sub>1C</sub><br>%            |
|---------------------|--------------------------------------|------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|
| Control (-ve)       | 90.78 $\pm$<br>4.31 <sup>a</sup>     | 19.70 $\pm$<br>1.51 <sup>a</sup>   | 12.30 $\pm$<br>2.11 <sup>a</sup>  | 35.18 $\pm$<br>29.71 <sup>a</sup>  | 4.85 $\pm$<br>0.58 <sup>b</sup>   |
| Control (+ve)       | 210.77 $\pm$<br>6.22 <sup>a***</sup> | 7.88 $\pm$<br>1.11 <sup>c***</sup> | 9.11 $\pm$<br>1.82 <sup>bc*</sup> | 29.71 $\pm$<br>2.14 <sup>b**</sup> | 7.11 $\pm$<br>0.66 <sup>a**</sup> |
| Drug                | 115.33 $\pm$<br>5.17 <sup>c*</sup>   | 16.01 $\pm$<br>1.25 <sup>b*</sup>  | 10.88 $\pm$<br>1.22 <sup>ac</sup> | 33.25 $\pm$<br>2.99 <sup>a</sup>   | 5.99 $\pm$<br>0.85 <sup>b</sup>   |
| Zinc                | 129.41 $\pm$<br>6.01 <sup>b**</sup>  | 15.91 $\pm$<br>1.25 <sup>b*</sup>  | 11.11 $\pm$<br>1.03 <sup>a</sup>  | 34.11 $\pm$<br>3.15                | 5.44 $\pm$<br>0.90 <sup>b</sup>   |
| Vanadium            | 130.71 $\pm$<br>5.10 <sup>b**</sup>  | 15.41 $\pm$<br>1.19 <sup>b*</sup>  | 10.78 $\pm$<br>1.12 <sup>a</sup>  | 33.20 $\pm$<br>3.11 <sup>a</sup>   | 5.60 $\pm$<br>1.01 <sup>b</sup>   |
| Zinc +<br>Vanadium  | 118.35 $\pm$<br>5.01 <sup>c*</sup>   | 16.36 $\pm$<br>0.99 <sup>b*</sup>  | 11.81 $\pm$<br>1.06 <sup>ac</sup> | 34.51 $\pm$<br>2.87 <sup>a</sup>   | 5.01 $\pm$<br>0.68 <sup>b</sup>   |

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each column having different superscript (a, b, c) denote significant difference.

**Table (3) The Mean values  $\pm$  SD of serum amino transferase (ALT & AST), alkaline phophatase enzymes (Alk-phos), creatinine and urea of control and diabetic treated rat groups**

| Variables<br>Groups | ALT<br>( $\mu$ /ml)                 | AST<br>( $\mu$ /ml)                 | Alk- phos<br>(g/dl)                 | Creatinine<br>(mg/dl)              | Urea<br>(mg/dl)                     |
|---------------------|-------------------------------------|-------------------------------------|-------------------------------------|------------------------------------|-------------------------------------|
| Control (-ve)       | 17.15 $\pm$<br>1.66 <sup>c</sup>    | 35.76 $\pm$<br>3.11 <sup>c</sup>    | 30.80 $\pm$<br>2.69 <sup>c</sup>    | 0.30 $\pm$<br>0.001 <sup>c</sup>   | 33.87 $\pm$<br>3.50 <sup>c</sup>    |
| Control (+ve)       | 39.62 $\pm$<br>3.45 <sup>a***</sup> | 59.20 $\pm$<br>6.01 <sup>a***</sup> | 50.61 $\pm$<br>5.16 <sup>a***</sup> | 1.33 $\pm$<br>0.03 <sup>a***</sup> | 60.14 $\pm$<br>1.16 <sup>a***</sup> |
| Drug                | 30.21 $\pm$<br>3.21 <sup>b**</sup>  | 48.18 $\pm$<br>5.11 <sup>b**</sup>  | 45.01 $\pm$<br>5.31 <sup>ab**</sup> | 1.01 $\pm$<br>0.05 <sup>ab**</sup> | 49.15 $\pm$<br>6.11 <sup>b**</sup>  |
| Zinc                | 25.32 $\pm$<br>2.63 <sup>b*</sup>   | 40.17 $\pm$<br>5.16 <sup>b**</sup>  | 41.29 $\pm$<br>4.91 <sup>b**</sup>  | 0.99 $\pm$<br>0.4 <sup>ab**</sup>  | 47.13 $\pm$<br>5.31 <sup>b**</sup>  |
| Vanadium            | 27.11 $\pm$<br>3.61 <sup>b*</sup>   | 43.51 $\pm$<br>5.14 <sup>b**</sup>  | 42.60 $\pm$<br>4.80 <sup>ab**</sup> | 0.95 $\pm$<br>0.05 <sup>b**</sup>  | 48.22 $\pm$<br>5.08 <sup>b**</sup>  |
| Zinc +<br>Vanadium  | 23.14 $\pm$<br>3.11 <sup>b*</sup>   | 38.26 $\pm$<br>4.11 <sup>bc</sup>   | 37.77 $\pm$<br>3.67 <sup>b*</sup>   | 0.65 $\pm$<br>0.01 <sup>b*</sup>   | 45.11 $\pm$<br>4.86 <sup>b**</sup>  |

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each column having different superscript (a, b, c) denote significant difference.

**Table (4) The Mean values  $\pm$  SD of serum lipid patterns of control and treated rat groups**

| Variables<br>Groups | cholesterol<br>(mg/dl)                | TG<br>(mg/dl)                       | HDLc<br>(mg/dl)                     | LDLc<br>(mg/dl)                       | VLDLc<br>(mg/dl)                    | Cholesterol<br>/HDLc               |
|---------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|------------------------------------|
| Control (-ve)       | 91.81 $\pm$<br>5.14 <sup>b</sup>      | 43.35 $\pm$<br>4.71 <sup>c</sup>    | 37.11 $\pm$<br>3.60 <sup>a</sup>    | 46.03 $\pm$<br>5.69 <sup>c</sup>      | 8.67 $\pm$<br>1.12 <sup>c</sup>     | 2.47 $\pm$<br>0.24 <sup>b</sup>    |
| Control (+ve)       | 178.33 $\pm$<br>11.34 <sup>a***</sup> | 70.81 $\pm$<br>8.11 <sup>a***</sup> | 26.31 $\pm$<br>2.67 <sup>b***</sup> | 137.86 $\pm$<br>10.33 <sup>a***</sup> | 14.16 $\pm$<br>1.35 <sup>a***</sup> | 6.77 $\pm$<br>0.22 <sup>a***</sup> |
| Drug                | 111.41 $\pm$<br>10.22 <sup>b</sup>    | 53.50 $\pm$<br>5.61 <sup>b*</sup>   | 29.91 $\pm$<br>2.91 <sup>b*</sup>   | 70.80 $\pm$<br>8.17 <sup>b**</sup>    | 10.70 $\pm$<br>1.41 <sup>b*</sup>   | 3.72 $\pm$<br>0.19 <sup>b</sup>    |
| Zinc                | 105.18 $\pm$<br>9.17 <sup>b</sup>     | 49.14 $\pm$<br>5.55 <sup>bc</sup>   | 31.14 $\pm$<br>3.15 <sup>b*</sup>   | 64.22 $\pm$<br>7.11 <sup>b**</sup>    | 9.82 $\pm$<br>1.01 <sup>b*</sup>    | 3.37 $\pm$<br>0.17 <sup>b</sup>    |
| Vanadium            | 10.23 $\pm$<br>10.41 <sup>b</sup>     | 51.26 $\pm$<br>5.61 <sup>bc</sup>   | 30.25 $\pm$<br>3.61 <sup>b*</sup>   | 69.73 $\pm$<br>7.39 <sup>b**</sup>    | 10.25 $\pm$<br>1.26 <sup>b*</sup>   | 3.64 $\pm$<br>0.15 <sup>b</sup>    |
| Zinc +<br>Vanadium  | 104.71 $\pm$<br>9.99 <sup>b</sup>     | 48.33 $\pm$<br>4.87 <sup>bc</sup>   | 32.71 $\pm$<br>3.51 <sup>ab</sup>   | 62.34 $\pm$<br>7.42 <sup>b**</sup>    | 9.66 $\pm$<br>0.99 <sup>b*</sup>    | 3.20 $\pm$<br>0.11 <sup>b</sup>    |

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

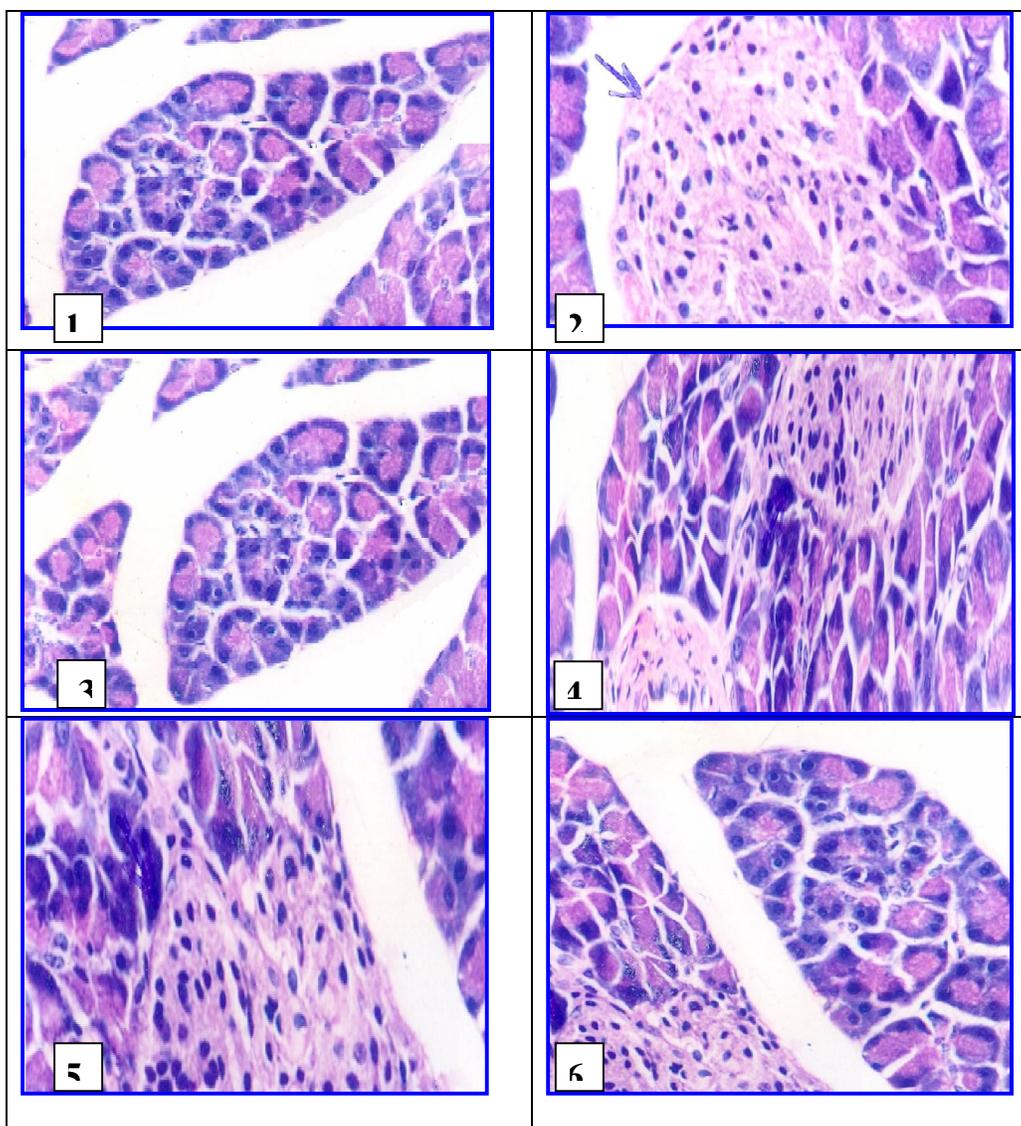
Mean values in each column having different superscript (a, b, c) denote significant difference.

**Table (5): The Mean values  $\pm$  SD of liver glycogen, cholesterol, total lipid glutathione peroxidase (GPX) and malondialdehyde (MDA) in control and diabetic treated rat groups.**

| Variables       | Glycogen (mg/100g)                 | Cholesterol (mg/100g)              | Total lipid (mg/100g)               | GPX ( $\mu$ g/g)                    | MDA (nmol/g)                        |
|-----------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| Control (-ve)   | 11.70 $\pm$<br>1.33 <sup>a</sup>   | 3.11 $\pm$<br>0.33 <sup>b</sup>    | 30.77 $\pm$<br>2.61 <sup>c</sup>    | 39.17 $\pm$<br>2.69 <sup>a</sup>    | 38.41 $\pm$<br>3.47 <sup>c</sup>    |
| Control (+ve)   | 4.61 $\pm$<br>0.48 <sup>b***</sup> | 6.14 $\pm$<br>0.96 <sup>a***</sup> | 47.45 $\pm$<br>4.67 <sup>a***</sup> | 26.11 $\pm$<br>2.71 <sup>c***</sup> | 78.12 $\pm$<br>8.22 <sup>a***</sup> |
| Drug            | 9.14 $\pm$<br>1.41 <sup>a</sup>    | 4.22 $\pm$<br>1.13 <sup>b</sup>    | 39.14 $\pm$<br>3.78 <sup>b*</sup>   | 33.20 $\pm$<br>3.17 <sup>a,b</sup>  | 50.12 $\pm$<br>5.11 <sup>b**</sup>  |
| Zinc            | 8.99 $\pm$<br>1.11 <sup>a</sup>    | 4.11 $\pm$<br>0.88 <sup>b</sup>    | 40.21 $\pm$<br>4.20 <sup>b*</sup>   | 37.11 $\pm$<br>3.21 <sup>a,b</sup>  | 43.60 $\pm$<br>7.36 <sup>b**</sup>  |
| Vanadium        | 9.01 $\pm$<br>1.06 <sup>a</sup>    | 4.31 $\pm$<br>0.77 <sup>b</sup>    | 38.71 $\pm$<br>4.01 <sup>bc</sup>   | 35.21 $\pm$<br>3.16 <sup>a,b</sup>  | 46.51 $\pm$<br>4.61 <sup>b**</sup>  |
| Zinc + Vanadium | 9.89 $\pm$<br>1.05 <sup>a</sup>    | 4.01 $\pm$<br>0.55 <sup>b</sup>    | 35.71 $\pm$<br>3.421 <sup>bc</sup>  | 37.33 $\pm$<br>3.60 <sup>a,b</sup>  | 43.22 $\pm$<br>4.25 <sup>b**</sup>  |

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each column having different superscript (a, b, c) denote significant difference.



**Fig (1) : Histopathological examination of the pancreas**

**Reference**

1. Abd El-Ghanny (2007): Effect of vanadium, radish and dill on some nutritional and biochemical parameters in diabetic rats. *Egyptian J of Nutrition and Health* Vol 2 No 1.67-86.
2. Abell, L.L., Levy, B.B., Brodie, B.B and Kendal, R. (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J .Biol .Chem*, 357-366.
3. Abraham, E. C., Huff, T. A., Cope, N. D., Wilson, J .B., Bransome, E.D and Huisman, T.H. (1978): Determination of the glycosalated heamoglobin (Hb<sub>A1</sub>) with a new microcolumn procedure .Suitability of the technique for assessing the clinical management of diabetes mellitus. *Diaetes*, 27(9):931 -7.
4. Akira, K., Yuriko, M., Yutaka, Y., Hiroyuki, Y. and Hiromu, S. (2009): Evaluation of insulin-mimetic activities of vanadyl and zinc (II) complexes from the viewpoint of heterocyclic bidentate ligands.*Journal of Inorganic Biochemistry* 103, 567–574.
5. Andre, P. S., Lucia, A., Min-Chul, P. and Yang-Sun, K. (2006): Vanadium and insulin increase adiponectin production in 3T3-L1 adipocytes *Pharmacological Research* 54, 30–38.
6. Antonio, Z., Manuel, P., Luc, M., Silvia, G (2009): Arylalkylamine vanadium salts as new anti-diabetic compounds *Journal of Inorganic Biochemistry* 103 559–566.
7. Anupam, B., Abhijeet, W., Mehool, A. P and Malay, C. (2010): Vanadium in the detection, prevention and treatment of cancer: The in vivo evidence. *Cancer Letters* 294, 1–12.
8. Artimage, G.Y and Berry, W.G. (1987): *Statistical Methods* 7th Ed. Ames, Iowa Stata University Press, 39-63.
9. Bancroft, J .D, Stevens, A. and Turner, D. R. (1996): *Theory and practice of histological technique* .4<sup>th</sup> Ed, New York, Churchill, Livingstone.
10. Beliaeva, N.F., Gorodetski,V.K., Tochilkin, A.I., Golubev, M,A., Semenova ,N.V and Kovel'man, I.R. (2000):Vanadium compounds a new class of therapeutic agents for the treatment of diabetes mellitus. *Vopr Med Khim* 46: 344-60.
11. Boden, G., Chen, X and Ruiz, J. (1996): Effects of vanadyl sulfate on carbohydrate and lipid metabolism in patients with non-insulin-dependent diabetes mellitus. *Metabolism*;45:1130-1135.
12. Bonsens, K. E. and Taussky, D. H. (1984): Determination of serum creatinine .*J Chem Inv*, 27: 648-660.
13. Castelli ,T. and Levitar, Y. (1977): Atherogenic ,index *Curr Presc* p39.
14. Chakraborty, T. ,Chatterjee, A., Rana., A., Dhachinamoorthi, D.,Kumar, A.P. and Chatterjee, M. (2007): Carcinogen-induced early molecular events and its implication in the initiation of chemical hepatocarcinogenesis in rats: chemopreventive role of vanadium on this process, *Biochim. Biophys. Acta* 1772 ,48–59.
15. Chapman, D.G.; Gastilla, R. and Campbell, T.A. (1950): Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. *Can. J. Biochem. Physio. I* (37) 679-686.
16. Cohen N, Halberstam M and Shlimovich P, (1995): Oral vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with noninsulin- dependent diabetes mellitus. *J Clin Invest* ;95:2501-2509.
17. Drabkin, D.I. (1949): The standardization of hemoglobin measurements. *Am J Med Sci* , 21 (7): 710.
18. Draper, H .H and Hadley, M. (1990): Malondialdehyde determination as index of lipid peroxidation, *Methods Enzymol.*186, 421-431.
19. Folch, J., lees, M., and Stanley, G.H. (1957): A simple method for isolation and purification of total lipid from animal tissue. *J Biol Chem* 266: 497-509.
20. Franklin, R.B., Feng, P., Milon, B., Desouk ,M .M. and Singh, K.K. (2005): hZIP1 zinc uptake transporter down regulation and zinc depletion in prostate cancer. *Mol Cancer* ; 4:32.
22. French, R.J. and Jones, P.J. (1993): Role of vanadium in nutrition: metabolism, essentiality and dietary consideration. *Life Sci.* 52, 339–346.
23. Friedwald, W .T.; Leve, R .I. and Frederickson, D. S (1972): Estimation of the concentration of low-density lipoprotein separated by three different methods. *Clin. Chem.*, 18:499-502.
24. Fruchart, G. C. (1982): LDL-Cholesterol Determination after Separation of Low Density Lipoprotein. *Rev. Fr. Des. Laboratories*, 103: 7-117.
25. Fugono, J., Fujimoto, K., Yasui, H., Kawabe, K., Yoshikawa, Y., Kojima, Y and Sakurai, H.( 2002): Metallokinetic study of zinc in the blood of normal rats given insulinomimetic zinc(II) complexes and improvement of diabetes mellitus in type 2 diabetic GK rats by their oral administration. *Drug Metabol. Pharmacokin.* 17, 340–347.
26. Huang, E.S., Brown, S.E., Ewigman, B.G., Foley, E.C. and Meltzer, D.O. (2007): Patient perceptions of quality of life with diabetes related

- complications and treatments. *Diabetes Care* 30: 2478-2483.
27. Johnson, O.F, Gilbreathb, E .T .Ogdenc, L., Grahamd, T.C and Gorham, S (2010): Reproductive and developmental toxicities of zinc supplemented rats. *Reproductive Toxicology Journal*, xxx (2010) xxx-xxx.
  28. Kakkar, R., Mantha, S.V., Radhi, J., Prasad, K and Kalra, J. (1998): Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin. Sci. (Colch)*, 94: 623-632.
  29. Kind, P.R and King, E.J. (1954): Estimation of alkaline phosphatase activity by determination of hydrolyzed phenol with aminoantipyrene. *J. Clin.Path.*7, 322.
  30. Koury, J. C and Donangelo, C. M. (2003): Zinco, estresse oxidativo e atividade fisica. *Revista de Nutricao*, 16(4), 433-441.
  31. Liu, X. M., Kong, J., Song, W.W., Lu, Y. (2009): Glucose metabolic and gluconeogenic pathways disturbance in the intrauterine growth restricted adult male rats. *Chin Med Sci J*; 24:208-12.
  32. Mc Inory, R.A (1954): A micro hematocrit for determining the packed cell and hemoglobin concentration on capillary blood. *J Clin, Path*, (7): 32.
  33. Mukherjee, B., Patra, B., Mahapatra, S., Banerjee, P., Tiwari, A. and Chatterjee, M. (2004): Vanadium an element of atypical biological significance, *Toxicol. Lett.* 150 135-143.
  34. NRC (1995): National Research council: Nutrient requirements of laboratory animals. fourth revised edition, PP.29-30 National Academy Press. Washington, DC.
  35. Paget, G.E. and Barnes, J .M. (1964): Inter species dosages conversion scheme in evaluation of results and quantitative application in different species toxicity test, 135-165. Academic Press London and NY.
  36. Patton, C.J. and Crouch, S.R. (1977): Enzymatic colorimetric method to determination urea in serum. *Anal. Chem.*, 49: 464.
  37. Peschke, E., Ebelt ,H., Bromme,H.J and Peschke, D. (2000): Classical and new diabetogens :Comparison of their effects on isolated rat pancreatic islets in vitro .*Cell Mol .Life Sci.*57(1):158-164.
  38. Ramachandran, B., Sathish, D.S., Kandasamy, M., Narayanan, V. and Subramanian, S. (2004): Hypoglycemic effect of macrocyclic binuclear oxovanadium(IV) complex on streptozotocin induced diabetic rats, *Exp. Diabetes Res.* 5:, 1-6.
  39. Ray, R.S., Roy, S., Ghosh, S., Kumar, M and Chatterjee, M. (2004): Suppression of cell proliferation, DNA protein cross-links, and induction of apoptosis by vanadium in chemical rat mammary carcinogenesis, *Biochim. Biophys. Acta* 1675 165-173.
  40. Reitman, S. and Frankel, S. (1957): Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. *Amer. J. Clin. Path.*, 28:56-63.
  41. Rerup, E. and Lundquist, S. (1967): Precipitation and purification of liver glycogen in rats .*Acta Pharmmic. Tox.* 25:47-51.
  42. Richmond, N. (1973): Colorimetric method of determination of total cholesterol and high density lipoprotein cholesterol (HDLc). *Clin.Chem.*,19,1350-1356.
  43. Roozbeh, J., Hedayati, P., Sagheb, M. M., Sharifian , M., Jahromi ,A. H., Shaabani, S. (2009): Effect of zinc supplementation on triglyceride, cholesterol, LDL, and HDL levels in zinc-deficient hemodialysis patients. *Ren Fail*; 31:798-801.
  44. Ryan EA, Pick ME and Marceau C (2001): Use of alternative medicines in diabetes mellitus. *Diabet Med* 18: 242-5.
  45. Sakurai, H., Kojima, Y., Yoshikawa, Y., Kawabe, K. and Yasui, H.(2002): Antidiabetic vanadium(IV) and zinc(II) complexes. *Coord. Chem. Rev.* 226, 187-198.
  46. Sakurai, H, Katoh, A and Yoshikawa Y. (2006): Chemistry and biochemistry of insulin-mimetic vanadium and zinc complexes. Trial for treatment of diabetes mellitus. *Bull Chem Soc Jpn*;79:1645-1664.
  47. Sasaki, T., Matsy, S. and Sonae, A. (1972): Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation, *Rinsh. Kagaku.* , 1: 346-353.
  48. Song, M.K., Rosenthal, M.J., Hong, S., Harris, D.M., Hwang, I., Yip, I., Golub, M.S., Ament, M.E. and Go, V.L.W.(2001): Synergistic antidiabetic activities of zinc, cyclo(His-Pro), and arachidonic acid. *Metabolism* 50, 53-59.
  49. Srivastava, A.K. and Mehdi M.Z, (2005): Insulino-mimetic and anti-diabetic effects of vanadium compounds, *Diabet. Med.* 22 2-13.
  50. Thompson KH, Lichter J, LeBel C, (2009): Vanadium treatment of type 2 diabetes: a view to the future. *J Inorg Biochem*;103:554-558.
  51. Tracey, A.S. and Crans, D.C (1998): Vanadium Compounds. Chemistry, Biochemistry and Therapeutic Applications. In: ACS Symposium Series, vol. 711, American Chemical Society, Washington, DC.
  52. Trinder,P and Ann, A.(1969):Colorimetric determination of total cholesterol. *Clin.Biochem.* 6-24.

53. Verma S, Cam MC, McNeill JH. (1998): Nutritional factors that can favorably influence the glucose/insulin system: vanadium. *J Am Coll Nutr* 1998;17:11-18.
54. Weiss, C., Marker, H.S. and Lehrer, G.M. (1980): Sensitive fluorometric assays for glutathione peroxidase and reductase. *Anal Biochem* 106: 512–516.
55. Wilson, M.A. and Miles, L.E.M. (1977): Radioimmunoassay of insulin in *Hand book of Radio immunoassay* G .E ,Abraham .ed M. Inc. New York, p275.
56. Wolfgang, M and Harold, H. S. (2006): Zinc requirements and the risks and benefits of zinc supplementation. *Journal of Trace Elements in Medicine and Biology*, 20 3–18.
57. Young, A. and Pestaner, D.L. (1975): Determination of triglycerides in serum .*Clin.Chem.* 21:5-7.
58. Yutaka, Y., Eriko, Uedaa., Yoshitane, K. and Hiromu, S. (2004): The action mechanism of zinc (II) complexes with insulinomimetic activity in rat adipocytes .*Life Sciences* 75 741–751.

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