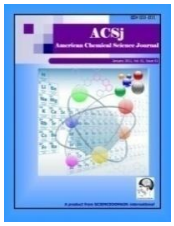

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Evaluation of Antidiabetic Activity of Combination of Trace Elements

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SS and SK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author HSC managed the analyses of the study. Author JM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: In order to compare the three combinations of trace elements for correction of hyperglycemia, hyperlipidaemia, glycogen content in liver and activities of some important carbohydrate metabolic enzymes in diabetes mellitus, the anti diabetic effect and acute oral toxicity of a combination of trace elements were assessed.
Material and Methods: The anti-diabetic activity of the combination of the trace elements was compared with various non diabetic groups and Streptozotocin induced diabetes groups for period of 30 days.
Results and Conclusion: The results of the anti-diabetic activity of the combination of trace elements the aspects of Cholesterol, Triglycerides, Lipid Peroxidation, Glutathione, Catalase, Glycogen, Glucose -6- phosphates, oxidative stress parameters in liver indicated that the increased anti-diabetic activity and the protective efficacy of pancreas injury for diabetes were observed. The

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strong antihyperglycemic and antihyperlipidemic effect observed in streptozotocin-induced diabetic rats justifies the use of these combined trace elements for the treatment of diabetes-related complications.

Keywords: Trace elements; lipid peroxidation; antihyperlipidemic; triglycerides.

1. INTRODUCTION

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus. The treatment goal for patients with type 2 diabetes mellitus is generally agreed to be to maintain near normal levels of glycemic control, both in fasting and postprandial states. Postprandial hyperglycemia is the earliest metabolic abnormality to occur in type 2 diabetes [1]. Postprandial blood glucose levels may be elevated in presence of normal levels of fasting plasma glucose (FPG), constituting an early stage in type 2 diabetes, which some have called “postprandial diabetes”. Early identification of postprandial hyperglycemia and its effective control, therefore, offer the potential for early intervention and prevention of diabetic complications [2]. Although diet and exercise are the first steps towards achieving treatment goals, 90% of patients with type -2 diabetes cannot maintain long term glycemic control with diet and exercise alone. Thus antihyperglycemic drugs are necessary for treatment of type 2 diabetes [3]. A number of new antidiabetic therapies-the miglitinides, α-glucosidase inhibitors and insulin lispro and aspart –target postprandial blood glucose spikes, and these agents should be considered increasingly in the long term management of patients with type 2 diabetes [4]. Use of these agents may lead to achievement of overall target glycemic levels in a greater proportion of patients, thus helping to prevent the excessive morbidity and mortality associated with the disease [5].

Trace elements are involved in almost every biochemical process in body cells, and inadequacy or unbalance of trace elements supply consequently affects a number of physiological functions [6]. Essentials trace elements are required by man in amounts ranging from 50 micrograms to 18 milligrams per day [7]. Vanadium, zinc, chromium, magnesium, molybdenum and selenium have all been proposed as possible adjuncts in the treatment of diabetes mellitus. Magnesium as a cofactor for enzymes that medicate carbohydrates metabolism, also takes part in glycemia homeostasis [8]. Zinc and chromium have been well known to be important trace elements in diabetes as a cofactor for insulin although in glycemic homeostasis [9]. Zinc and chromium have been well known to be important trace elements in diabetes as a cofactor for insulin although their real mechanisms in carbohydrate metabolism are not clear [10]. Vanadium and selenium have been observed to have several physiological insulin-like effects by post-insulin receptor mechanisms [11]. Furthermore vanadium, selenium, chromium, and magnesium are known for their free radical scavenging activity [12]. It is known that several of the complications of diabetes may be related to increase in intracellular oxidants and free radicals [13].

Streptozotocin, induces "chemical diabetes" (Streptozotocin diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release. Streptozotocin enters the B cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself [14]. Poly ADP-ribosylation leads to depletion of cellular NAD + and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated [15]. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, B cells undergo the destruction by necrosis [16].

Streptozotocin administered rats become hyperglycemic in a short period of time, followed by hepatic glucose overproduction. Intraperitonal administration of Streptozotocin (55 mg/kg body weight) effectively induced diabetes mellitus in

normal rats as reflected by glycosuria, hyperglycemia, polyphagia, and polydipsia and bodyweight loss compared with normal rats [17].

The aim for the present work is to compare the three combinations of trace elements for correction of hyperglycemia, hyperlipidaemia, glycogen content in liver and activities of some important carbohydrate metabolic enzymes in diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Animals

Male Sprague Dawley rats (180-200g) were obtained from animal house of Truba Institute of Pharmacy, Bhopal (Reg. no.-1196/a/08/CPCSEA). Animals received human care and had free access to drinking water and feed with standard pellet diet manufactured by Amruth Laboratories, Mumbai. Experiment was carried out following the guidelines set by the institutional animal ethics committee. The trace elements such as vanadium sulphate, sodium selenate, magnesium sulphate and chromium picolinate were obtained from the Sigma Aldrich, Mumbai. The powdered form of drugs are dissolved in distilled water (vehicle) and administered orally through gastric intubation.

2.2 Grouping of Animals

After the induction of diabetes, rats were divided into 9 groups of seven rats per group. Control rats (Group I) were administered only the vehicle. Drug control rats (Group II) were administered combination of vanadium (100 mcg/kg) and selenium (18 µg/kg), Drug control rats (Group III) were administered combination of chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg). Drug control rats (Group IV) were administered magnesium (0.46 gm/kg) and selenium (18 µg/kg). Diabetic control rats (Group V) were administered the vehicle. Diabetic rats (Group VI) were administered combination of vanadium (100 mcg/kg) and selenium (18 µg/kg), Diabetic rats (Group VII) were administered combination of Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg). Diabetic rats (Group VIII) were administered magnesium (0.46 gm/kg) and selenium (18 µg/kg) and Diabetic rats (Group IX) were administered 10mg/kg of metformin once daily for 30 days respectively.

On 28th day animals were sacrificed by decapitation under chloroform anesthesia. Blood was collected and allowed to clot at room temperature for 1 hr. Serum separated was then processed for biochemical parameters, viz; triglycerides, cholesterol, and plasma insulin. A 10% v/v liver homogenate was prepared using phosphate buffer pH 7.4 to carry out lipid peroxidation and glutathione activity, phosphate buffer to carry out catalase activity, citrate buffer pH 6.5 to carry out glucose-6-phosphatase activity and for the determination of glycogen content in liver. The pancreas tissue was fixed in 10% v/v formalin solution for histopathological evaluation.

2.3 Oral Glucose Tolerance Test in Nondiabetic Rats

The oral glucose tolerance test was performed on the 5 groups mentioned below. These animals were fasted overnight.

At 0 hour, all groups were given a glucose load of 2 mg/kg body weight. Thereafter glucose was estimated at 0, 30, 60, and 120 minutes.

3. ESTIMATION OF BIOCHEMICAL PARAMETERS

3.1 Estimation of Total and HDL Cholesterol

Serum total and HDL cholesterols levels were estimated according to Wybenga and pileggi method. Cholesterol reacts with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives a lavender coloured complex which is measured at 560 nm. High density lipoproteins (HDL) are obtained in the supernatant after centrifugation. The cholesterol in the HDL fraction is also estimated by this method [18].

3.2 Estimation of Triglycerides

Glycerol released from hydrolysis of triglyceride by lipoprotein lipase is converted by glycerol kinase into glycerol-3-phosphate which is oxidized to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red coloured compound [19].

3.3 Lipid Peroxidation (LPO)

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has

been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm [20].

3.4 Glutathione

Glutathione level was estimated according to the method of Beutler and Duron Method. Glutathione (GSH) is the most abundant thiol (SH) compound in animal tissues, plant tissues, bacteria, and yeast. (5, 5'- Dithiobis (2-nitrobenzoic acid) {DTNB}, known as Ellman's Reagent, was developed for the detection of thiol compounds in 1985. The glutathione recycling system by DTNB and glutathione reductase created a highly sensitive glutathione detection method. DTNB and Glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow coloured product, GSH concentration in a sample solution can be determined by the measurement at 412 n m absorbance. GSH is generated from GSSG by glutathione reductase, and reacts with DTNB again to produce 2-nitro-5-thiobenzoic acid. Therefore, this recycling reaction improves the sensitivity of total glutathione detection [21].

3.5 Catalase

Catalase was estimated according to the method of huge Aebi Method. To 2 ml of 30 mM hydrogen peroxide solution in cuvette 1 ml of tissue sample was added and the kinetics of the reaction was monitored for 60 seconds spectrophotometrically at 240 nm [22].

3.6 Glycogen

In currently used methods for the determination of glycogen, tissue is extracted either by boiling with 30% potassium hydroxide solution (KOH) or by homogenization with trichloroacetic acid solution (TCA) [23].

3.7 Glucose -6- phosphates

The G-6-pase activity can be determined by measuring the amount of glucose or inorganic phosphate formed on incubation with G-6-P [24].

3.8 Estimation of Triglycerides

At the end of the experiment, serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were determined using commercially available kits (Zhongsheng Clinical Reagent Co., Ltd, Beijing, China) [25].

3.9 Measurement of Oxidative Stress Parameters in Liver

Liver of rats were removed and placed in 10% KCl (10 ml/g tissue), and homogenized on ice for 120 s with a DY89-II homogenizer (Ningbo Scientz Biotechnology Co. Ltd. China) at 600 rpm. Tissue homogenates were centrifuged at 1000 × g at 4°C for 10 min to remove tissue debris, and clear supernatant was used for further analyses. The superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities, glutathione (GSH) and malondialdehyde (MDA) in liver samples were measured using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China) [26].

3.9.1 Histopathological observation

On 28th day animals were sacrificed by decapitation under ether anesthesia. Blood was collected and allowed to clot at room temperature for 1 hr. Serum separated was then processed for biochemical parameters, viz; triglycerides, cholesterol, and plasma insulin. A 10% v/v liver homogenate was prepared using phosphate buffer pH 7.4 to carry out lipid peroxidation and glutathione activity, phosphate buffer to carry out catalase activity, citrate buffer pH 6.5 to carry out glucose-6-phosphatase activity and for the determination of glycogen content in liver. The pancreas tissue was fixed in 10% v/v formalin solution for histopathological evaluation.

3.9.10 Statistical analysis

Statistical analyses were performed using SPSS15.0 (SPSS, Chicago, IL). All the experimental data were expressed as mean ± SEM. For significance verification by groups, one-way or two-way ANOVA was performed, followed by Tukey's test. Two-way repeated measures ANOVA was used to examine the overall effects of treatment and time on the change in blood glucose and insulin levels. A P value of <0.05 was considered significant.

4. RESULT

4.1 Effect of 30 min. Pretreatment of Combined Trace Elements on Oral Glucose Tolerance Test in Nondiabetic Rats

Among the 5 groups, Vehicle Control, Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) and Metformin 10 mg/kg. BG reached the peak at 30 minutes after glucose administration, and then returned to normal at 120 minutes. BG values of Vanadium (100 mcg/kg) + selenium (18 µg/kg), Magnesium (0.46 gm/kg) +selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) + selenium (18 µg/kg) and Metformin 10 mg/kg treated rats were showed statistically significant difference ($p<0.05$) at 30minutes after glucose administration as compared to control group (Table 1).

4.2 Effect of Combined Trace Elements on Blood Glucose Levels of Streptozotocin Induced Diabetic Rats

The antihyperglycemic effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), on the fasting blood glucose level of diabetic rats is shown in Table 3. Administration of streptozotocin leads to elevation of blood glucose level as compared to normal control. After 7, 14, 21and 28 days repeated administration of combined trace elements once day resulted reduction in blood glucose 8.38%, 13.67%, 17.59% and 19.96% and respectively. But Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) after repeated administration for 28 days does not showed the reduction in blood glucose levels. Statistical significant difference ($p<0.05$) was found at 7, 14, 21 and 28^{th} day while compared with corresponding BG values of vehicle control diabetic group (Table 3).

4.3 Effect of Combined Trace Elements on Lipid Profile of Diabetic and Nondiabetic Rats

- Combined treatment of Vanadium (100 mcg/kg) and selenium (18 µg/kg), brought down the enhanced levels of TG, TC and LDL were significantly after 28 days. It showed 17.64% reduction in TG, 19.60% in TC and 38.44 % in LDL cholesterol were observed in treated diabetic rats. There was 32.47% increase in HDL cholesterol in treated diabetic rats.
- Combined treatment of Magnesium (0.46 gm/kg) and selenium (18 µg/kg), brought down the enhanced levels of TG, TC and LDL were significantly after 28 days. It showed 21.15% reduction in TG, 23.28% in TC and 36.32% in LDL cholesterol were observed in treated diabetic rats. There was 55.73% increase in HDL cholesterol in treated diabetic rats.
- Combined treatment of Chromium picolinate (0.2 mg/kg) + selenium (18 µg/kg), slightly brought down the enhanced levels of TG, TC and LDL were significantly after 28 days. It showed 5.90% reduction in TG, 2.23% in TC and 31.23% in LDL cholesterol were observed in treated diabetic rats. There was 30.23% increase in HDL cholesterol in treated diabetic rat.

4.4 Effect of Combined Trace Elements on Lipid Peroxidation of Diabetic and Nondiabetic Rats

Combined effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) showed decrease in lipid peroxidation by 13.71%, 16.23% and 4.16% respectively. Treatment of Metformin 10mg/kg showed 26.03% reduction in lipid peroxidation.

4.5 Effect of Combined Trace Elements on Glycogen of Diabetic and Nondiabetic Rats

Combined effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) showed increased in glycogen by 19.6%, 24.14% and 3.50% respectively. Treatment of Metformin 10 mg/kg showed 46.86% increased in glycogen.

4.6 Effect of Combined Trace Elements on Glutathione of Diabetic and Nondiabetic Rats

Combined effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) showed increased in glutathione by 38.09%, 47.92% and

Table 1. Oral glucose tolerance test in nondiabetic rats

Group I	Administered a single dose of vehicle.
Group II	Administered a combination of vanadium (100 mg/kg) and selenium (18 µg/kg)
Group III	Administered a combination of magnesium (0.46 gm/kg) and selenium (18 µg/kg)
Group IV	Administered a combination of chromium picolinate (0.2 mg/kg) and selenium (18 mg/kg)
Group V	Administered a metformin (10 mg/kg)

6.39% respectively. Treatment of Metformin 10 mg/kg showed 54.65% increased in glutathione.

4.7 Effect of Combined Trace Elements on Glucose-6-phoshatase Activity of Diabetic and Nondiabetic Rats

Combined effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) showed decreased in glucose-6-phoshatase by 6.90%, 9.87% and 2.04% respectively. Treatment of Metformin 10 mg/kg showed 10.03% reduction in glucose-6-phosphatase.

4.8 Effect of Combined Trace Elements on Catalase of Diabetic and Nondiabetic Rats

Combined effect of Vanadium (100 mcg/kg) and selenium (18 µg/kg), Magnesium (0.46 gm/kg) and selenium (18 µg/kg), Chromium picolinate (0.2 mg/kg) and selenium (18 µg/kg) showed increased in catalase by 5.66%, 8.48% and 2.01% respectively. Treatment of metformin 10 mg/kg showed 15.39% increased in catalase.

4.9 Effect of Combined Trace Elements on Body Weight of Streptozotocin Induced Diabetic Rats

Normal vehicle control animals were found to be stable in their body weight but diabetic control rats showed significant reduction in body weight during 28 days (Table 2). Streptozotocin caused body weight reduction, which is reversed by combined trace elements mainly Vanadium and Selenium, and Magnesium and Selenium. Combination of chromium and selenium showed significant reduction in body weight during 28 days. Metformin showed less effect on body weight.

4.10 Histopathological Study

Photomicrographs showed normal acini, and normal cellular population in the islets of Langerhans in pancreas of vehicle treated nondiabetic rats (A). Extensive damage to the islets of Langerhans in pancreas of vehicle treated diabetic rats (B). The partial restoration of β- cells was shown by Combination of Magnesium + Selenium (C). and Vanadium +Selenium (D), And Metformin 10 mg/kg (E). Also there is extensive damage to the islets of Langerhans in pancreas of Chromium + Selenium treated diabetic rats (G).

5. DISCUSSION

In the present study administration of combination of trace elements such as vanadium and selenium, and magnesium and selenium showed a significant decrease in fasting blood glucose levels, whereas the combination of chromium + selenium dose not showed a significant reduction in fasting blood glucose levels (Table 3). In oral glucose tolerance test in normal as well as diabetic rats revealed that this combination of trace elements such as vanadium + selenium and magnesium + selenium has capacity to lower the blood glucose level. Vanadium + selenium and magnesium + selenium showed reduction in FBG significantally ($p<0.05$) in diabetic animal. Vanadium + selenium and magnesium + selenium have antidiabetic activity without causing hypoglycemia was observed (Table 2). In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. Oral administration of Vanadium + selenium and magnesium + selenium for 28 consecutive days to diabetic rats improved body weight. This could be due to a better control of the hyperglycemic state in the diabetic rats, whereas oral administration of combination of chromium + selenium for 28 days does not improved the body weight (Fig. 1). Decreased FBG could improve body weight in streptozotocin-induced diabetic rats. Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid.

The abnormally high concentration of lipids in diabetes mellitus is mainly due to an increase in

the mobilization of free fatty acids from the peripheral fat depots, Excess of fatty acids in plasma produced by streptozotocin promotes the liver conversion of some fatty acids to phospholipids and cholesterol [27]. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood [28]. As a result serum phospholipid is elevated. Administration of Vanadium + selenium and magnesium + selenium to diabetic rats reversed all the above mentioned changes it not only lowered TC, TG and LDL but also enhanced the cardio protective lipid HDL (Tables 4 and 5).

Liver is the main organ responsible for the maintenance of blood glucose homeostasis where glucose-6-phosphatase plays an important role [20]. To focus the underlying biochemical mechanism of the action of antidiabetogenic activities of these combined trace elements, we measured the enzyme activities in liver in different groups. After the supplementation of these three combined trace elements, out of this two combination such as vanadium + selenium and magnesium + selenium showed a significant recovery of the enzyme activity that may be another possible way for antidiabetogenic potency of these trace elements (Tables 7, 8 and 9).

After the combined trace elements supplementation in diabetic rat, there was a significant recovery in hepatic glycogen level towards the control level. This focuses one of the possible mechanisms of antidiabetogenic action of these trace elements by modulating glycogen metabolism.

Diabetes is also associated with lipid peroxidation as insulin secretion is closely associated with lipooxygenase derived peroxides [24]. Elevation in lipid peroxidation in diabetic state has been supported here from the elevation in TBARS, important indicator of oxidative stress condition in liver [27]. The elevation in lipid peroxidation in above tissue in diabetic state has been supported here by the diminution of Catalase and glutathione, important antioxidant enzymes in liver. Supplementation of combined vanadium and selenium and magnesium and selenium results significant elevation or restoration of the antioxidant enzymes followed by diminution of TBARS levels to the control which proposed the another mechanism of antidiabetic effect of this trace elements.

The combination of chromium and selenium does not show any significant recovery in above mention activities. Thus the present study shows that administration of combination of trace elements such as vanadium and selenium and magnesium and selenium is more effective than combination of chromium and selenium to challenge the diabetic state. This combination of trace elements may recover the disorders in carbohydrate metabolism noted in diabetic state by protecting the oxidative stress induced damage for such disorder and or by stimulating those carbohydrate regulatory enzyme activities in target organ or by stimulating and regenerating β cells in pancreas. The actual mechanism is not clear and further biochemical and pharmacological investigations are needed.

It is widely accepted that the most challenging goal in the management of diabetes is to achieve blood glucose levels as close to normal as possible. α-Amylases, endoglucanases that the catalyze hydrolysis of the internal α-1, 4-glucosidic linkage in starch and other related polysaccharides, have also been targets for suppression of postprandial hyperglycemia [29]. So in this study, the combination of trace elements is used to determine the inhibitory effects on α-amylase. The Significant difference was notice in relation to inhibition of α- amylase of vanadium and selenium, that shows 92.2% inhibition at 12.5 µg/ml.

The number of functionally intact β-cells in the islet organ is of decisive importance in the development course and outcome of DM. The renewal of β-cells in diabetes has been studied in many animal models [30]. Selenium as an antioxidant and a free radical scavenger prevent auto play (ADP-ribosyl)-ation of PARP, thereby stabilizing Rege gene transcriptional complex and resulting in the regeneration of β-cells and protection of pancreatic islets against STZ [31]. After treatment schedule histopathology study (Figs. 1 to 6) of pancreas were done to determine potential of Vanadium + selenium and magnesium + selenium to recover from necrosis which was produced by streptozotocin. The result of the present study revealed that there is a partial restoration of β-cells normal after oral administration of Vanadium + selenium and magnesium + selenium for 28 days.

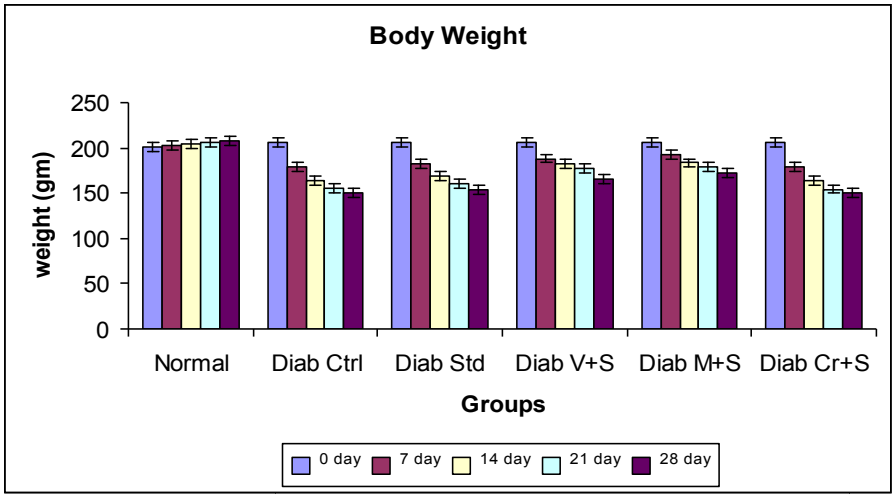

Fig. 1. Effect of Combined trace elements on body weight of streptozotocin (55 mg/i.v) induced diabetic rats

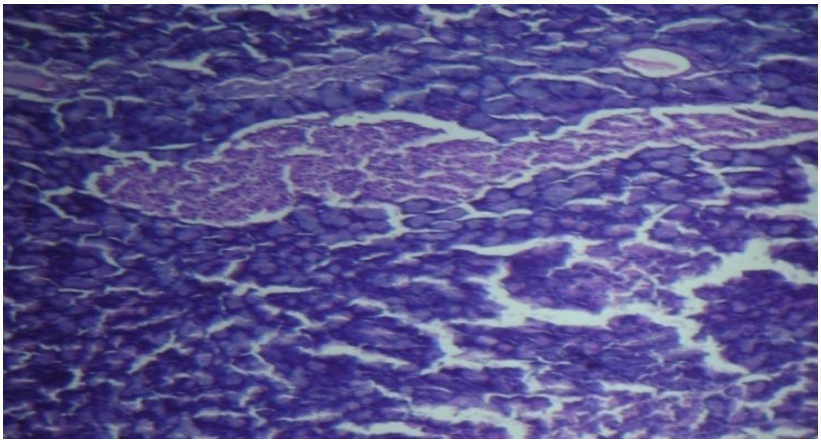

Fig. 2. Pancreas tissue of normal vehicle control rat (H & E 10 X)

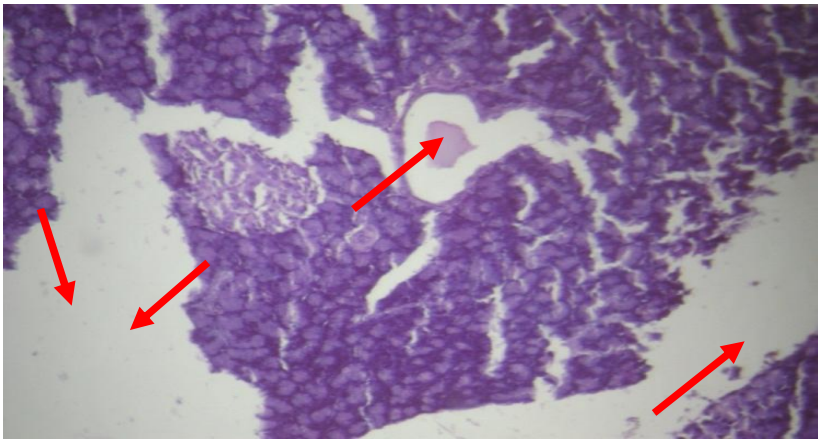

Fig. 3. Pancreas tissue of diabetic vehicle control rat (H & E 10 X)

Table 2. Effect of 30 min. of pretreatment of combination of trace elements on oral glucose tolerance test in nondiabetic rats

Time in minutes	Group I. nondiabetic control	Group II. nondiabetic (vanadium+selenium)	Group III. nondiabetic (magnesium+selenium)	Group IV. nondiabetic (chromium picolinate + selenium)	Group V. nondiabetic standard (10 mg/kg)
0	87.08±1.00	87.08±1.15	87.66±2.75	85.33±2.90	91.16±1.57
30	177.60±2.16	175.75±1.18*	178.83±2.4	176.66±2.47	177.66±1.76*
60	157.00±1.75	148.00±1.48*	147.66±2.6*	148.83±5.05*	147.33±1.14*
120	115.83±1.81	96.33±1.4*	94.84±1.35*	97.00±5.91	91.50±1.25*

Values are expressed as mean ± S.E.M; n = 6. All groups were compared with the corresponding values of the control. Represents statistical significance Vs control (P<0.05).*

Table 3. Effect of combined trace elements on blood glucose levels of on nondiabeticand streptozotocin induced diabetic rats

Days	Nondiabetic control	Nondiabetic test vanadium + selenium	Nondiabetic test magnesium + selenium	Nondiabetic test chromium + selenium	Diabetic control	Diabetic test vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium	Diabetic standard 10 mg/kg.
0	99.16±9.43	95.00±4.46	99.16±9.43	94.66±4.66	457.50±15.30	466.17±7.92	453.17±7.55	453.16±16.04	454.50±18.62
7	96.50±5.72	96.5±5.72	96.66±7.72	93.17±5.23	447.66±10.78	315.00±2.28*	311.83±7.72*	465.16±18.30	369.33±18.11*
14	93.17±5.23	86.66±6.19	94.66±4.66	91.66±4.73	475.33±25.10	302.30±21.92*	296.66±8.6*1	462.16±18.50	303.30±14.41*
21	91.17±5.23	89.66±5.25	91.66±4.73	90.50±2.73	486.50±5.95	271.50±23.42	256.50±9.78*	478.80±5.82	288.16±23.92*
28	90.83±3.31	89.66±5.25	91.16±2.66	86.17±5.30	484.83±8.11	250.83±19.62*	236.33±10.65*	495.60±16.37	274.33±3.61*

Values are expressed as mean ± S.E.M; n=6.All groups were compared with the corresponding values of diabetic control

Table 4. Effect of Combined trace elements on lipid profile of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic standard 10mg/kg	Diabetic test vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Triglyceride(mg/dl)	77.32±0.60	109.09±0.74	93.36±0.29*	97.05±0.38*	94.66±0.47*	105.06±0.45
Cholesterol (mg/dl)	91.33±0.27	115.17±0.41	98.11±0.36*	105.32±0.32*	103.47±0.30*	114.04±0.39
HDL Cholesterol (mg/dl)	37.80±2.07	27.25±1.45	44.60±3.09*	36.10±1.73*	42.18±0.88*	35.92±0.61*
LDL Cholesterol (mg/dl)	26.87±1.50	104.94±5.36	52.64±6.16*	64.60±6.13*	60.83+5.27*	52.28+6.38*

Values are expressed as mean ± S.E.M; n=6.All groups were compared with the corresponding values of control. Represents statistical significance Vs control (P<0.05)*

Table 5. Effect of combined trace elements on lipid peroxidation of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic Standard 10 mg/kg	Diabetic test vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Lipid Peroxidation (n moles/g)	7.33± 0.06	18.29± 0.17	12.27± 0.13*	15.12± 0.28*	14.54±0.20*	17.33± 0.10

*Values are expressed as mean ± S.E.M; n=6. All groups were compared with the corresponding values of control. * Represents statistical significance Vs control (P<0.05)*

Table 6. Effect of Combined trace elements on Glycogen of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic standard 10mg/kg	Diabetic test vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Glycogen (gm/100 g)	5.20±0.01	1.33±0.02	2.50±0.02*	1.82±0.01*	1.93±0.01*	1.42±0.01*

*Values are expressed as mean ± S.E.M; n=6. All groups were compared with the corresponding values of control. * Represents statistical significance Vs control (P<0.05)*

Table 7. Effect of combined trace elements on glutathione of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic Standard 10mg/kg	Diabetictest vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Glutathione (mg/ml)	64.30±0.17	36.14± 0.36	58.72±0.21*	51.88±0.31*	55.94±0.32*	38.78±0.18

Values are expressed as mean ± S.E.M; n=6. All groups were compared with the corresponding values of control. Represents statistical significance Vs control (P<0.05)*

Table 8. Effect of combined trace elements on glucose-6-phosphatase activity of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic standard 10mg/kg	Diabetic test V+S	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Glucose-6-Phosphatase (micro moles/min/g)	16.94±0.12	23.22±0.21	20.12±0.15*	21.09±0.23*	20.17±0.20*	22.59±0.36

*Values are expressed as mean ± S.E.M; n=6. All groups were compared with the corresponding values of control. * Represents statistical significance Vs control (P<0.05)*

Table 9. Effect of combined trace elements on catalase of diabetic and nondiabetic rats

Parameter	Nondiabetic control	Diabetic control	Diabetic standard 10mg/kg	Diabetic test vanadium + selenium	Diabetic test magnesium + selenium	Diabetic test chromium + selenium
Catalase (units/mg)	81.20±1.34	45.84±0.52	66.54±0.66*	53.47±0.65*	57.25±0.75*	48.55±0.84

*Values are expressed as mean ± S.E.M; n=6. All groups were compared with the corresponding values of control. * Represents statistical significance vs control (P<0.05)*

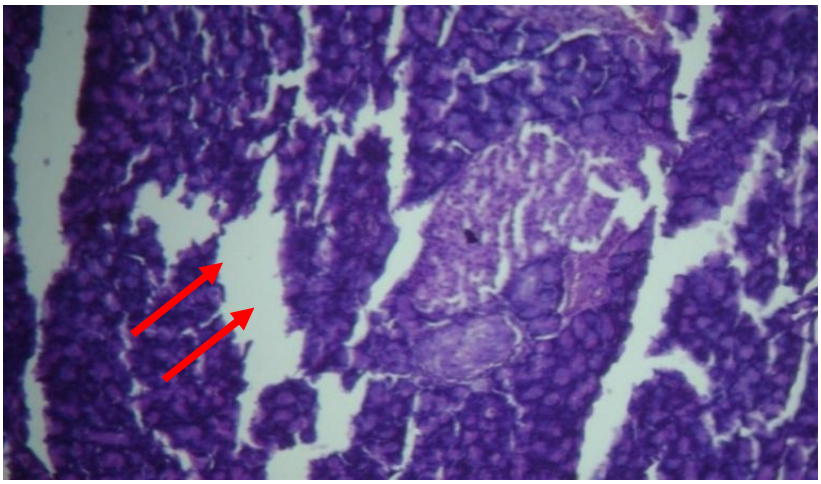

Fig. 4. Pancreas tissue of combined vanadium + selenium treated diabetic rat (H & E 10 X)

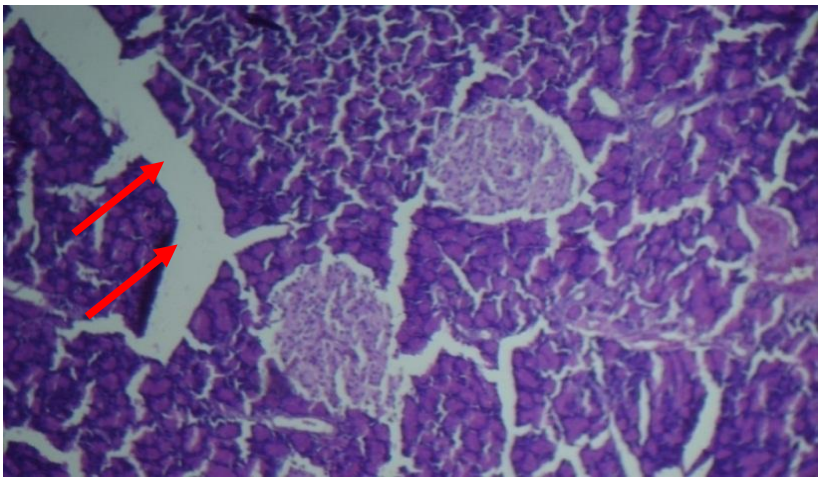

Fig. 5. Pancreas tissue of combined magnesium + selenium treated diabetic rat (H & E 10 X)

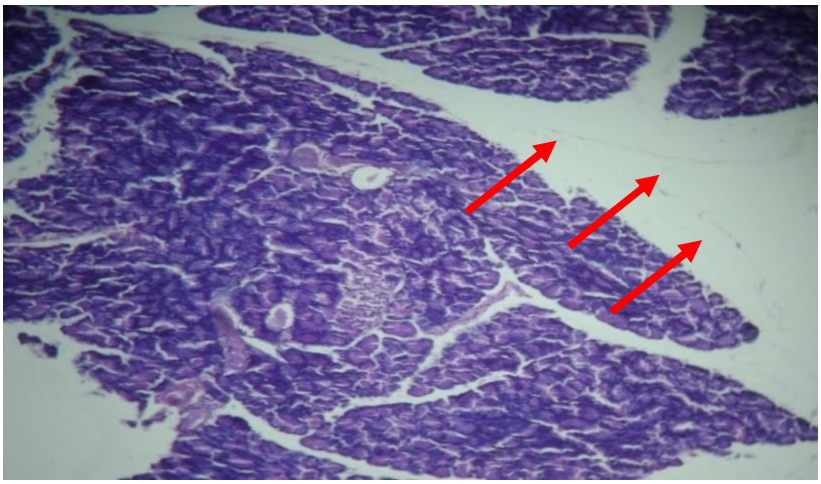

Fig. 6. Pancreas tissue of combined chromium + selenium treated diabetic rat (H & E 10 X)

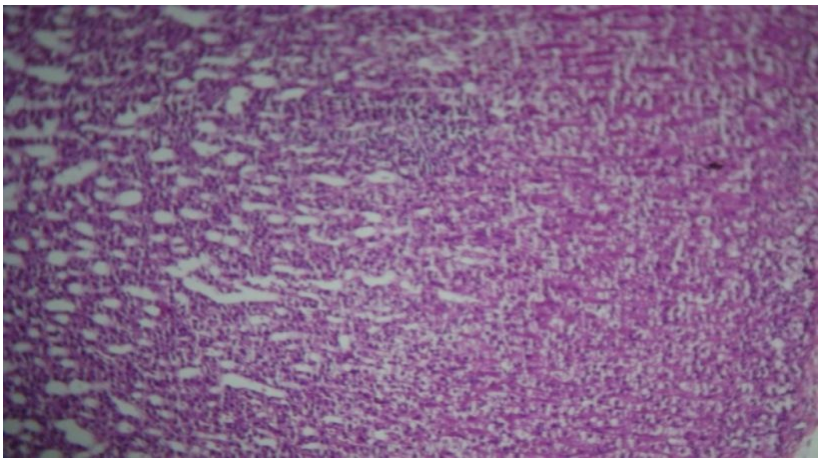

Fig. 7. Pancreas tissue of Metformin (250 mg/kg) treated diabetic rat (H & E 10 X)

4. CONCLUSION

The combination of trace elements the aspects of Cholesterol, Triglycerides, Lipid Peroxidation, Glutathione, Catalase, Glycogen, Glucose -6-phosphates, oxidative stress parameters in liver indicated that the increased anti-diabetic activity and the protective efficacy of pancreas injury for diabetes. The strong antihyperglycemic and antihyperlipidemic effect observed in streptozotocin-induced diabetic rats justifies the use of these combined trace elements for the treatment of diabetes-related complications

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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