

Original Research Article

Evaluation of Antidiabetic Activity of Combination of Trace Elements

ABSTRSCT

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 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.

key words: Trace elements, Lipid peroxidation, antihyperlipidemic, Triglycerides

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 diabetes¹. 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 complications². 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 diabetes³. 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 diabetes⁴. 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 disease⁵.

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 functions⁶. Essentials trace elements are required by man in amounts ranging from 50 micrograms to 18 milligrams per day⁷. 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 mediate carbohydrates metabolism, also takes part in glycemia homeostasis⁸. Zinc and chromium have been well known to be important trace elements in diabetes as a cofactor for insulin although in glycemic homeostasis⁹. 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 clear¹⁰. Vanadium and selenium have been observed to have several physiological insulin-like effects by post-insulin receptor mechanisms¹¹. Furthermore vanadium, selenium, chromium, and magnesium are known for their

free radical scavenging activity¹². It is known that several of the complications of diabetes may be related to increase in intracellular oxidants and free radicals¹³.

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 itself¹⁴. 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 generated¹⁵. 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 necrosis¹⁶.

Streptozotocin administered rats become hyperglycemic in a short period of time, followed by hepatic glucose overproduction. Intraperitoneal 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 rats¹⁷.

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.

MATERIALS AND METHODS

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.

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 (100mg/kg) and selenium (18µg/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 (0.46gm/kg) and selenium (18µg/kg). Diabetic control rats (Group V) were administered the vehicle. Diabetic rats (Group VI) were administered combination of vanadium (100mg/kg) and selenium (18µg/kg), Diabetic rats (Group VII) were administered combination of Chromium picolinate (0.2 mg/kg) and selenium (18µg/kg). Diabetic rats (Group VIII) were administered magnesium (0.46gm/kg) and selenium (18µg/kg) and Diabetic rats (Group IX) were administered 10mg/kg of glibenclamide 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.

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.

TABLE NO-1

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 µg/kg)
Group V	Administered a glibenclamide (10 mg/kg)

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

ESTIMATION OF BIOCHEMICAL PARAMETERS:

1. Estimation of total and HDL cholesterol

Serum total and HDL cholesterol 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

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. 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

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 nm 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

5. Catalase

Catalase was estimated according to the method of Aebi Method. To 2ml 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

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

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

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

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 $1000 \times g$ 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

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

190 carry out lipid peroxidation and glutathione activity, phosphate buffer to carry out
191 catalase activity, citrate buffer pH 6.5 to carry out glucose-6-phosphatase activity and for
192 the determination of glycogen content in liver. The pancreas tissue was fixed in 10 % v/v
193 formalin solution for histopathological evaluation.

194 **Statistical analysis**

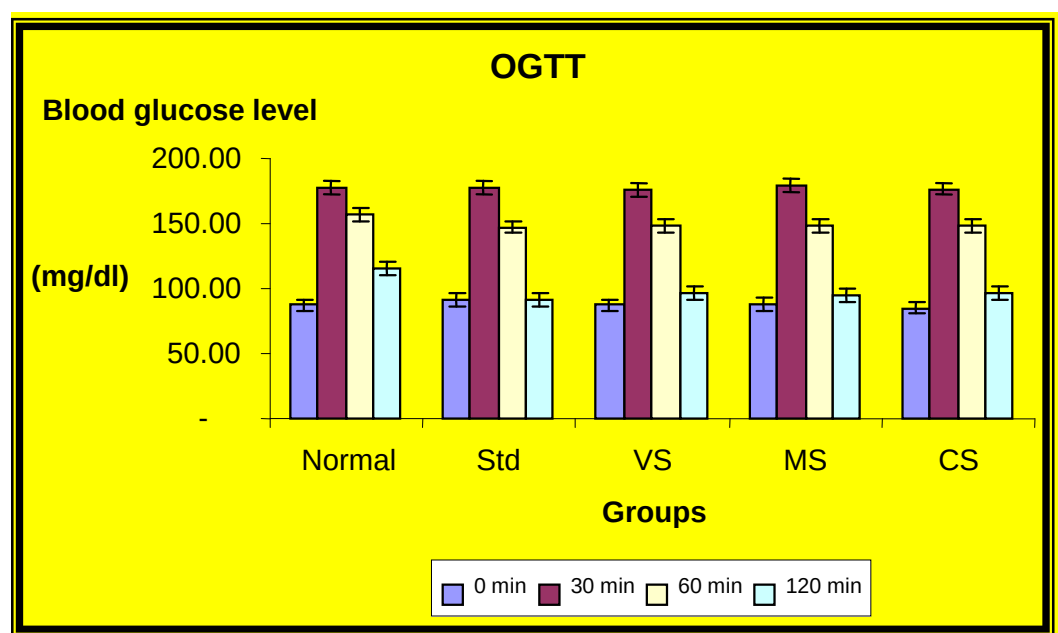
195 Statistical analyses were performed using SPSS15.0 (SPSS, Chicago, IL). All the
196 experimental data were expressed as **mean $\pm$ SEM**. For significance verification by
197 groups, one-way or two-way ANOVA was performed, followed by Tukey's test. Two-
198 way repeated measures ANOVA was used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

201 **RESULT**

202 **1. Effect of 30 min. Pretreatment of Combined trace elements on Oral Glucose** 203 **Tolerance Test in Nondiabetic rats**

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205 Among the 5 groups, Vehicle Control, Vanadium (100mg/kg) and selenium (18 μ g/kg),
206 Magnesium (0.46gm/kg) and selenium (18 μ g/kg), Chromium picolinate (0.2mg/kg) and
207 selenium (18 μ g/kg) and Glibenclamide 10 mg/kg. BG reached the peak at 30 minutes
208 after glucose administration, and then returned to normal at 120 minutes. BG values of
209 Vanadium (100mg/kg) + selenium (18 μ g/kg), Magnesium (0.46gm/kg) + selenium
210 (18 μ g/kg), Chromium picolinate (0.2mg/kg) + selenium (18 μ g/kg) and Glibenclamide
211 10mg/kg treated rats were showed statistically significant difference ($p < 0.05$) at
212 30minutes after glucose administration as compared to control group. (Table no - 1)

Fig No - 1
Effect of 30 min. of pretreatment of Combination of Trace elements on Oral Glucose Tolerance Test in Nondiabetic rats



Values are expressed as mean $\pm$ S.E.M; n = 6.

All groups were compared with the corresponding values of the control.

NORMAL: Vehicle treated non diabetic rats.

STD: 10mg/kg Glibenclamide treated non diabetic rats.

VS: Vanadium (100mg/kg) and selenium (18 μ g/kg) treated non diabetic rats.

MS: Magnesium (0.46gm/kg) and selenium (18 μ g/kg) treated non diabetic rats.

CS: Chromium picolinate (0.2mg/kg) + selenium (18 μ g/kg) treated non diabetic rats.

2. Effect of combined trace elements on blood glucose levels of streptozotocin induced diabetic rats

The antihyperglycemic effect of Vanadium (100mg/kg) and selenium (18µg/kg), Magnesium (0.46gm/kg) and selenium (18µg/kg), on the fasting blood glucose level of diabetic rats is shown in **table no - 02**. Administration of streptozotocin leads to elevation of blood glucose level as compared to normal control. After 7, 14, 21 and 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 (0.2mg/kg) and selenium (18µg/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 28th day while compared with corresponding BG values of vehicle control diabetic group. (Table No 2).

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TABLE NO-2

259 **Effect of combined trace elements on blood glucose levels of on nondiabetic and**
 260 **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*

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262 Values are expressed as mean ± S.E.M; n=6.

263 All groups were compared with the corresponding values of diabetic control

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

- Combined treatment of Vanadium (100mg/kg) and selenium (18µg/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 (0.46gm/kg) and selenium (18µg/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 (0.2mg/kg) + selenium (18µg/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.

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Table-3

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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 $\pm$ 0.60	109.09 $\pm$ 0.74	93.36 $\pm$ 0.29*	97.05 $\pm$ 0.38*	94.66 $\pm$ 0.47*	105.06 $\pm$ 0.45
Cholesterol (mg/dl)	91.33 $\pm$ 0.27	115.17 $\pm$ 0.41	98.11 $\pm$ 0.36*	105.32 $\pm$ 0.32*	103.47 $\pm$ 0.30*	114.04 $\pm$ 0.39
HDL Cholesterol (mg/dl)	37.80 $\pm$ 2.07	27.25 $\pm$ 1.45	44.60 $\pm$ 3.09*	36.10 $\pm$ 1.73*	42.18 $\pm$ 0.88*	35.92 $\pm$ 0.61*
LDL Cholesterol (mg/dl)	26.87 $\pm$ 1.50	104.94 $\pm$ 5.36	52.64 $\pm$ 6.16*	64.60 $\pm$ 6.13*	60.83 $\pm$ 5.27*	52.28 $\pm$ 6.38*

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Values are expressed as mean $\pm$ S.E.M; n=6.

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All groups were compared with the corresponding values of control.

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* Represents statistical significance Vs control (P<0.05)

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4: Effect of Combined trace elements on lipid Peroxidation of diabetic and nondiabetic rats.

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

Table No-4

Effect of Combined trace elements on lipid Peroxidation 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
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)

5: Effect of Combined trace elements on Glycogen of diabetic and nondiabetic rats.

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

Table No-5

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)

6: Effect of Combined trace elements on Glutathione of diabetic and nondiabetic rats.

Combined effect of Vanadium (100mg/kg) and selenium (18µg/kg), Magnesium (0.46gm/kg) and selenium (18µg/kg), Chromium picolinate (0.2mg/kg) and selenium (18µg/kg) showed increased in glutathione by 38.09%, 47.92% and 6.39% respectively. Treatment of Glibenclamide 10mg/kg showed 54.65% increased in glutathione.

Table No-6 Effect of Combined trace elements on Glutathione 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
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)

7: Effect of Combined trace elements on Glucose-6-phosphatase activity of diabetic and nondiabetic rats.

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

Table No-7

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)

8: Effect of Combined trace elements on Catalase of diabetic and nondiabetic rats.

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

Table No-8

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.

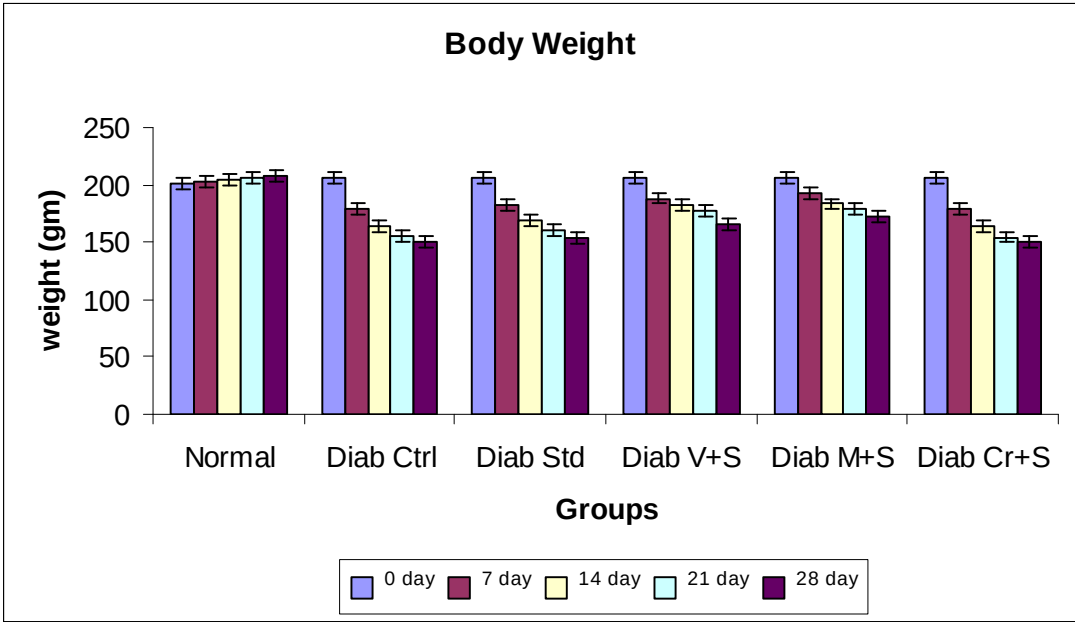
* Represents statistical significance vs control (P<0.05)

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 (fig no 1). 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. Glibenclamide showed less effect on body weight.

Figure No -2

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



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 Glibenclamide 10 mg/kg (E). Also there is extensive damage to the islets of Langerhans in pancreas of Chromium + Selenium treated diabetic rats (G).

Figure No-03 Pancreas tissue of Normal Vehicle Control rat. (H & E 10 X)

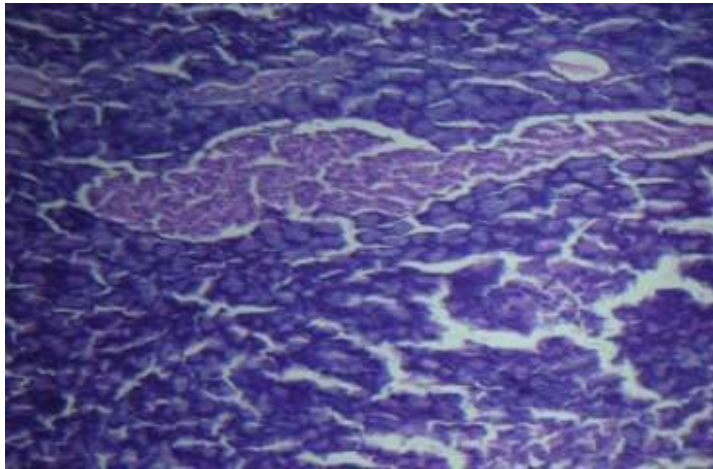


Figure No-04 Pancreas tissue of Diabetic Vehicle Control rat. (H & E 10 X)

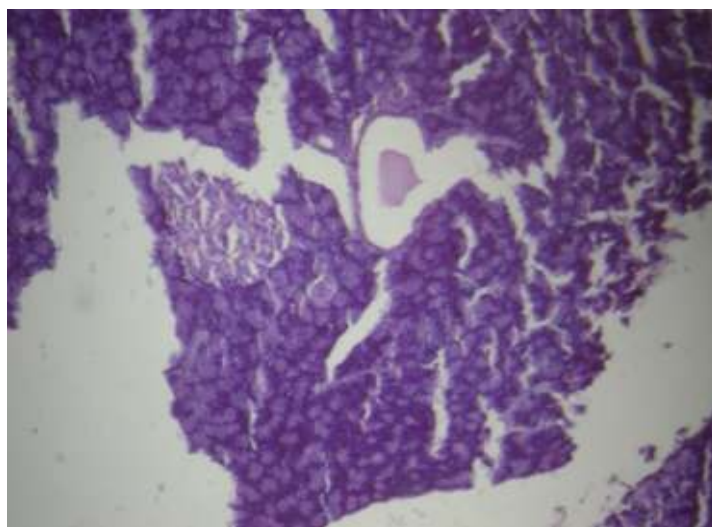


Figure No-05 Pancreas tissue of Combined Vanadium + Selenium Treated Diabetic rat. (H & E 10 X)

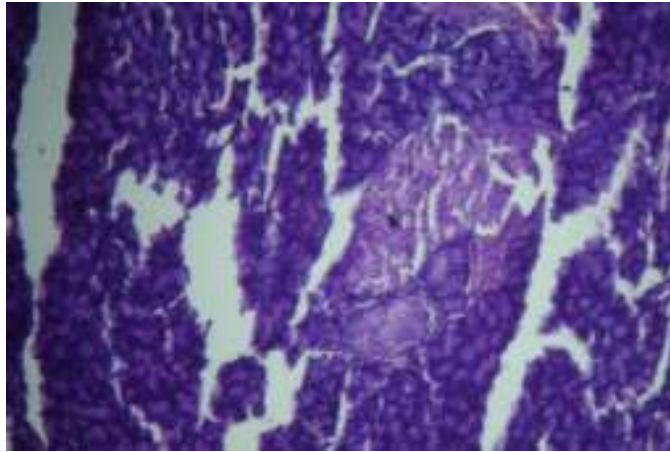


Figure No-06 Pancreas tissue of Combined Magnesium + Selenium Treated Diabetic rat. (H & E 10 X)

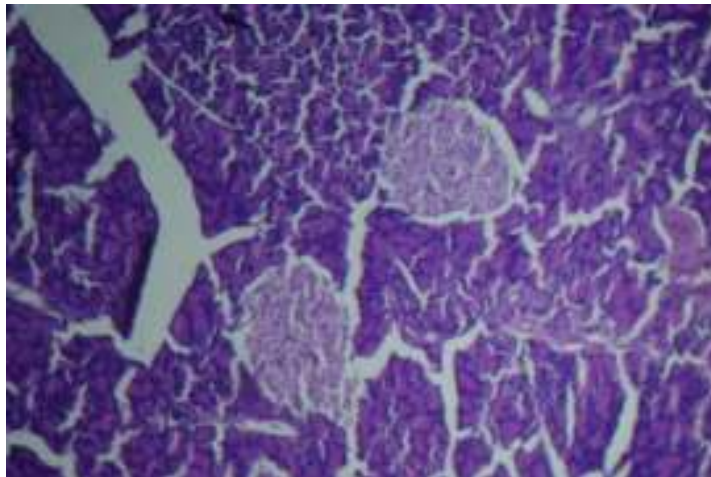


Figure No-07 Pancreas tissue of Combined Chromium + Selenium Treated Diabetic rat. (H & E 10 X)

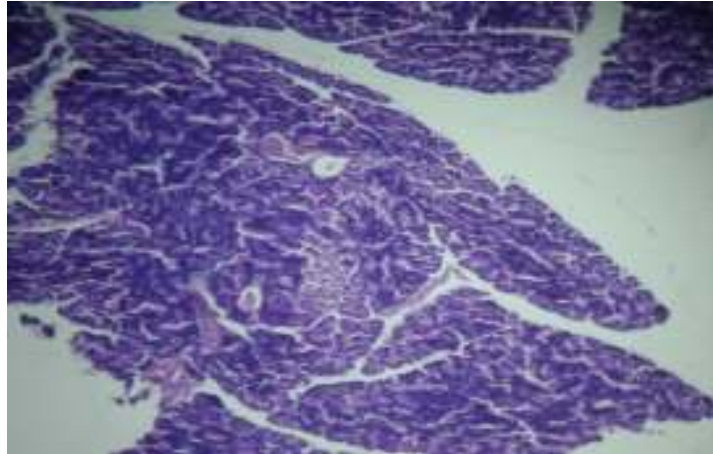
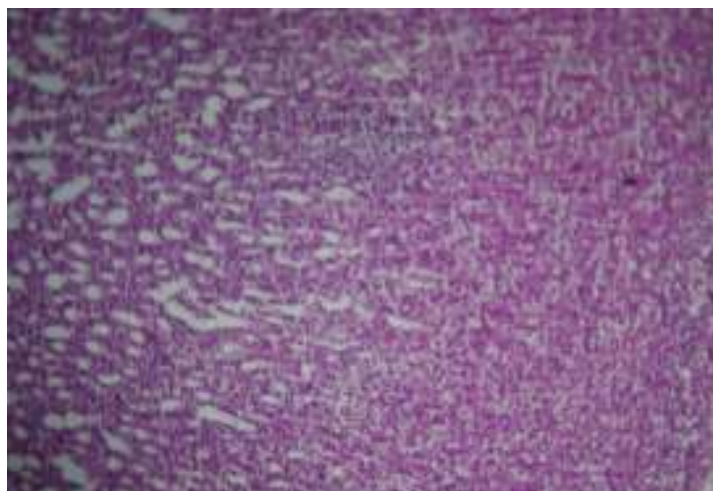


Figure No-08 Pancreas tissue of Glibenclamide (10 mg/kg) Treated Diabetic rat. (H & E 10 X)



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 - 2). 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 significantly ($p < 0.05$) in diabetic animal. Vanadium + selenium and magnesium + selenium have antidiabetic activity without causing hypoglycemia was observed (fig no -1). 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 (figure - 2). 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 blood²⁸. 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(table no - 3).

Liver is the main organ responsible for the maintenance of blood glucose homeostasis where glucose-6-phosphatase plays an important role²⁰. 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 (table no - 6, 7, and 8).

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 peroxides²⁴. Elevation in lipid peroxidation in diabetic state has been supported here from the elevation in TBARS, important indicator of oxidative stress condition in liver²⁷. 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 hyperglycemia²⁹. 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 μ g/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 models³⁰. 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 STZ³¹. After treatment schedule histopathology study (fig no – 2to7) 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.

Reference

1. Huizinga MM, Rothman RL. Addressing the diabetic pandemic: A comprehensive approach. *Indian J Med Res* 2006; 124: 481-4.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes care* 2004; 27:1047-53.
3. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance, In: Gan D, editor. *Diabetes Atlas*. International Diabetes Federation. 3rd ed. Belgium: 2006 p.15-103.
4. Azriel S, Casal F, et al (2014). Glycemic control parameters in insulin-naïve patients with uncontrolled type 2 diabetes referred to endocrinologists, and degree of implementation of the national spanish consensus for the management of hyperglycemia. *Endocrinol Nutr*. pii: S1575-0922(14)00173-9.
5. Nauck MA. (2014). Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. *Drug Des Devel Ther*. 11;8:1335-1380
6. Akerblom, H.K., Vaarala, O., Hyoty, H., et al (2002). Environmental factors in the etiology of type 1 diabetes. *American journal of medical genetics*, 115, 18-29.
7. Atkison MA; Eisenbarth GS, Type 1 diabetes: New Perspective on disease pathogenesis and treatment. *Lancet* 2001; 358: 221-229.
8. Racek J, Sindberg CD, et al. (2013). Effect of chromium-enriched yeast on fasting plasma glucose, glycated haemoglobin and serum lipid levels in patients with type 2 diabetes mellitus treated with insulin. *Biol Trace Elem Res*. 155(1):1-4.
9. Harland BF, Harden-Williams BA. (1994). Vanadium of nutritional importance. *J Am Diet Assoc* 94: 891-894.

10. Poucheret P, Verma S, Gryn timer MD, MC Neil JH. (1998). Vanadium and diabetes. *Mol Cell Biol* 188:73-80.
11. Halberstam M, Cohen N, Shlimovich P, Rossetti L, Shamoon H. (1966). Oral vanadyl sulfate improve insulin sensitivity in NIDDM but not obese non diabetic subject. *Diabetes* 45: 659-666.
12. De Valk H (1999). Magnesium in diabetes Mellitus. *J Med* 54:139-146.
13. Dejian Huang et al, (2005) The Chemistry behind Antioxidant Capacity Assays, *J. Agric. Food Chem.* 53: 1841-1856.
14. Roger Walker, Clive Edwards, *Clinical Pharmacy and Therapeutics*, 3rd International Edition, An imprint of Elsevier science, page no: 657-658.
15. Indu Khurana, *Textbook of Medical Physiology*, 1st Edition (2006); Elsevier, A division of Reed Elsevier India Pvt Ltd. Page no: 783-786.
16. Tripathi, D.K (2004). *Essential of Medicinal Pharmacology*, 5th Edition, Jaypee Brother Medical Publication, New Delhi. 235-253.
17. T. Szkudelski, (2001) The Mechanism of Alloxan and Strptozotocin Action in β Cell of rat Pancreas. *Physiological Research*. 50,536-546.
18. Wybenga DR, et al (1970) Direct manual determination of serum total cholesterol with a single stable reagent. *Clin Chem*. 16(12):980-4.
19. Gloria C B, et al (1966) Serum Triglyceride: Method of Estimation and Levels in Normal Humans. *Can Med Assoc J*. 94(17): 886–888.
20. Romero F J, et al (1998) Lipid peroxidation products and antioxidants in human disease. *Environ Health Perspect*. 106(5): 1229–1234.

21. E. Beutler, (1975). Glutathione in red blood cell metabolism, A manual of biochemical methods (2nd Ed), Grune and Stratton, New York. Pp. 112-114.
22. Beers, R.F. and I.W. Sizer, (1952). Spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. J. Biol. Chem, 195: 133-140.
23. Sadasivam, S, S. and A. Manickam, (1996). Carbohydrates. In: Sadasivam, S, S and A. Manickam (Eds), Methods in Biochemistry. New Age International Pvt. Ltd, pp: 11-12.
24. Swansons, M.A., (1955). Glucose-6-phosphatase from liver. In: Colowick, S.P. and N.O. Kaplan, (Eds.), Methods Enzymol, Academic Press, New York, 2: 541-543.
25. Varley, H. (1999) Practical Clinical Biochemistry, V Edition, 713-729.
26. Vogel, G. H. (2002). Drug discovery and Evaluation: Pharmacological assay, 2nd edition, Springer publication, Germany, p 948-964.
27. Kakkar, R., J. Kalra, S.V. Mantha and K. Prasad, (1995). Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol. Cell. Biochem., 151: 113-119.
28. Okhawa, H., N. Ohishi and K. Yagi, (1979). Assay for lipid peroxidation in animal tissues thiobarbituric acid reaction. Anal. Biochem., 95:351-358.
29. Pillai, A. and S. Gupta, (2005). Antioxidant enzyme activity and lipid peroxidation in liver of female rats co-exposed to lead and cadmium: Effects of vitamin E and Mn²⁺. Free. Rad. Res, 39: 707-712.
30. Panigrahy D, Singer S, Shen LQ et al (2002). "PPAR gamma ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis". J. Clin Invest. 110 (7): 923-32.
31. Huawei Zeng, (2009). Selenium as an Essential Micronutrient: Roles in Cell Cycle and Apoptosis. Molecules 2009, 14, 1263-1278.