

Evaluation of Antidiabetic Activity of Combination of Trace Elements

ABSTRSCT

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. 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. 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.

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”. This state initiates the development of early microvascular and macrovascular complications of 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. However most of the currently available antidiabetic therapies reduce FPG but have little impact on postprandial glycemic excursions; therefore they do not normalize postprandial hyperglycemia. 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.

During the last three decades the insulin-mimetics effects of certain trace elements have been studied in detail. 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. Acting as catalytic or structural components of larger molecules, they have specific functions and are indispensable for life. 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 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

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 huge 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

Statistical analysis

Statistical analyses were performed using SPSS15.0 (SPSS, Chicago, IL). All the experimental data were expressed as mean $\pm$ standard deviation (SD). 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.

RESULT

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 (100mg/kg) and selenium (18 μ g/kg), Magnesium (0.46gm/kg) and selenium (18 μ g/kg), Chromium picolinate (0.2mg/kg) and selenium (18 μ g/kg) and Glibenclamide 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 (100mg/kg) + selenium (18 μ g/kg), Magnesium (0.46gm/kg) +selenium (18 μ g/kg), Chromium picolinate (0.2mg/kg) + selenium (18 μ g/kg) and Glibenclamide 10mg/kg treated rats were showed statistically significant difference ($p < 0.05$) at 30minutes after glucose administration as compared to control group. (Table no - 1)

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TABLE NO- 1

200 **Effect of 30 min. of pretreatment of Combination of Trace elements on Oral Glucose**
 201 **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 (10mg/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*

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

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

204 * Represents statistical significance Vs control (P<0.05)

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

212 The antihyperglycemic effect of Vanadium (100mg/kg) and selenium (18µg/kg), Magnesium
213 (0.46gm/kg) and selenium (18µg/kg), on the fasting blood glucose level of diabetic rats is shown
214 in table 02. Administration of streptozotocin leads to elevation of blood glucose level as
215 compared to normal control. After 7, 14, 21 and 28 days repeated administration of combined
216 trace elements once day resulted reduction in blood glucose 8.38%, 13.67%, 17.59% and 19.96%
217 and respectively. But Chromium picolinate (0.2mg/kg) and selenium (18µg/kg) after repeated
218 administration for 28 days does not showed the reduction in blood glucose levels. Statistical
219 significant difference ($p < 0.05$) was found at 7, 14, 21 and 28th day while compared with
220 corresponding BG values of vehicle control diabetic group. (Table No 2).

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TABLE NO-2

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

237 **streptozotocin induced diabetic rats**

Days	Nondiabetic Control	Nondiabetic Test V+S	Nondiabetic Test M+S	Nondiabetic Test C+S	Diabetic Control	Diabetic Test V+S	Diabetic Test M+S	Diabetic Test C+S	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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239 Values are expressed as mean ± S.E.M; n=6.

240 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.

Table-3

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

Parameter	Nondiabetic Control	Diabetic Control	Diabetic Standard 10mg/kg	Diabetic Test V+S	Diabetic Test S M+	Diabetic Test Cr + S
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)

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 V+S	Diabetic Test M+S	Diabetic Test Cr + S
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)

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306 **5: Effect of Combined trace elements on Glycogen of diabetic and nondiabetic rats.**

307 Combined effect of Vanadium (100mg/kg) and selenium (18µg/kg), Magnesium (0.46gm/kg)

308 and selenium (18µg/kg), Chromium picolinate (0.2mg/kg) and selenium (18µg/kg) showed

309 increased in glycogen by 19.6%, 24.14% and 3.50% respectively. Treatment of Glibenclamide

310 10mg/kg showed 46.86% increased in glycogen.

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313 **Table No-5**

314 **Effect of Combined trace elements on Glycogen of diabetic and nondiabetic rats.**

Parameter	Nondiabetic Control	Diabetic Control	Diabetic Standard 10mg/kg	Diabetic Test V+S	Diabetic Test M+S	Diabetic Test Cr + S
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*

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

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

318 * Represents statistical significance Vs control (P<0.05)

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325 **6: Effect of Combined trace elements on Glutathione of diabetic and nondiabetic rats.**

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

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331 **Table No-6 Effect of Combined trace elements on Glutathione of diabetic and nondiabetic**
 332 **rats.**

Parameter	Nondiabetic Control	Diabetic Control	Diabetic Standard 10mg/kg	Diabetic Test V+S	Diabetic Test M+S	Diabetic Test Cr + S
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

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

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

336 * Represents statistical significance Vs control (P<0.05)

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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 M+S	Diabetic Test Cr + S
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)

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368 **8: Effect of Combined trace elements on Catalase of diabetic and nondiabetic rats.**

369 Combined effect of Vanadium (100mg/kg) and selenium (18µg/kg), Magnesium (0.46gm/kg)
 370 and selenium (18µg/kg), Chromium picolinate (0.2mg/kg) and selenium (18µg/kg) showed
 371 increased in catalase by 5.66%, 8.48% and 2.01% respectively. Treatment of glibenclamide
 372 10mg/kg showed 15.39% increased in catalase.

373 **Table No-8**

374 **Effect of Combined trace elements on Catalase of diabetic and nondiabetic rats.**

Parameter	Nondiabetic Control	Diabetic Control	Diabetic Standard 10mg/kg	Diabetic Test V+S	Diabetic Test M+S	Diabetic Test Cr + S
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

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

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

378 * Represents statistical significance vs control (P<0.05)

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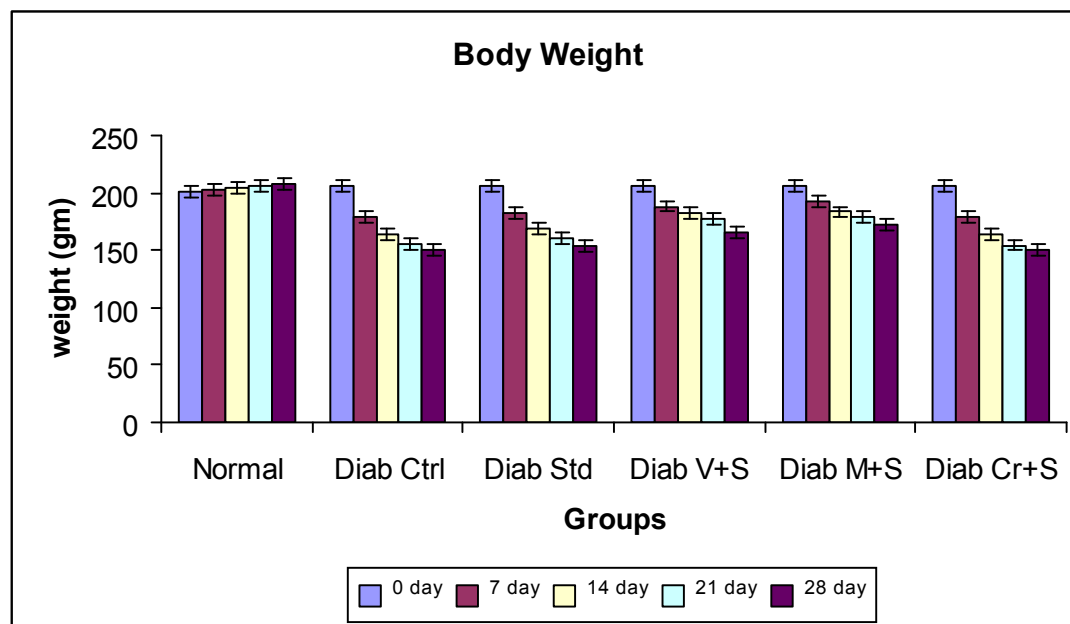
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9. Effect of Combined trace elements on body weight of streptozotocin induced diabetic rats.

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 -1

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



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402 HISTOPATHOLOGICAL STUDY

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

409 **Figure No-02 Pancreas tissue of Normal Vehicle Control rat. (H & E 10 X)**

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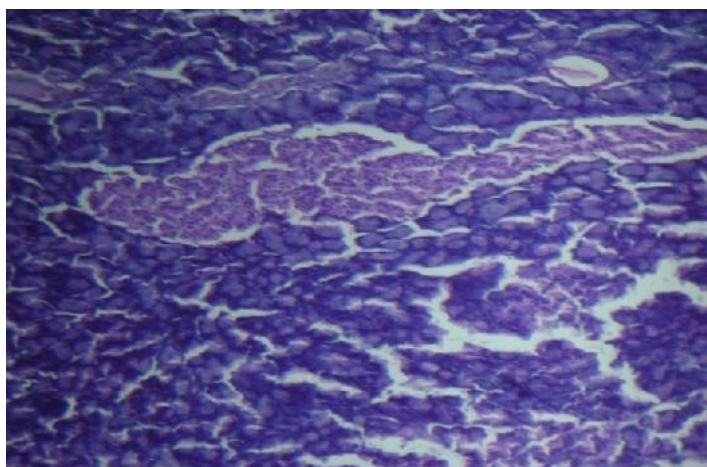
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418 **Figure No-03 Pancreas tissue of Diabetic Vehicle Control rat. (H & E 10 X)**

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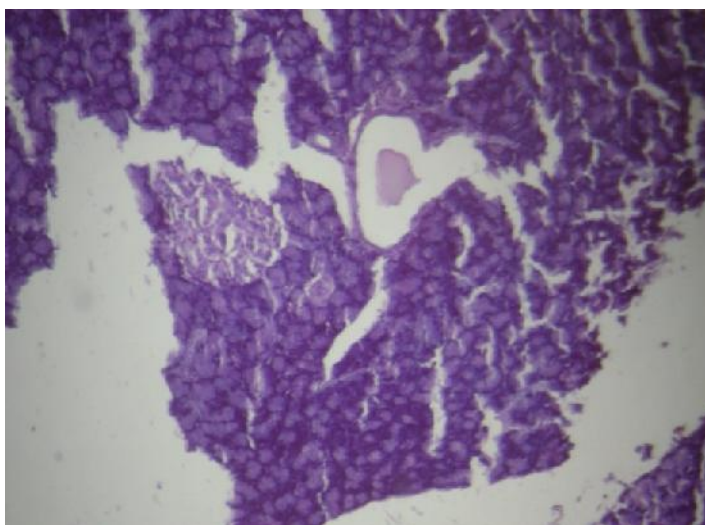


Figure No-04 Pancreas tissue of Combined Vanadium + Selenium Treated Diabetic rat. (H & E 10 X)

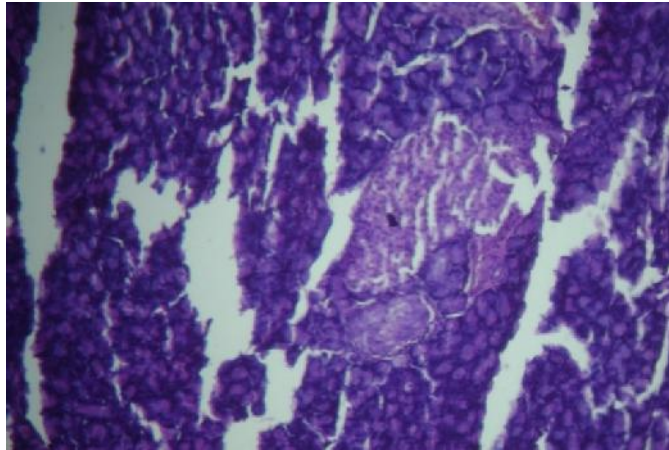


Figure No-05 Pancreas tissue of Combined Magnesium + Selenium Treated Diabetic rat. (H & E 10 X)

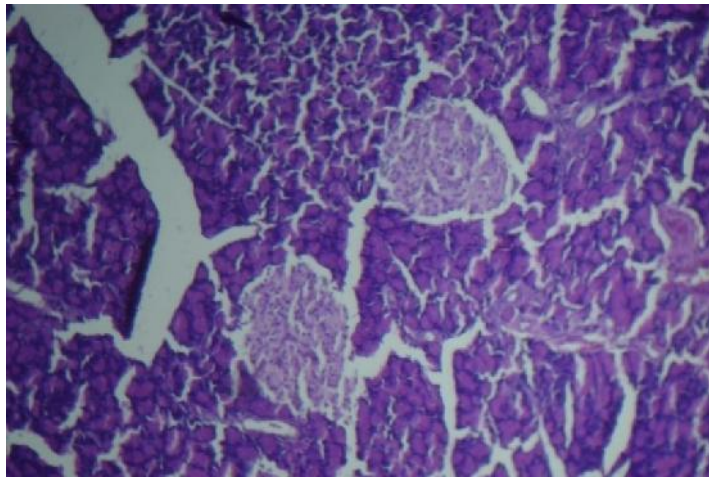


Figure No-06 Pancreas tissue of Combined Chromium + Selenium Treated Diabetic rat. (H & E 10 X)

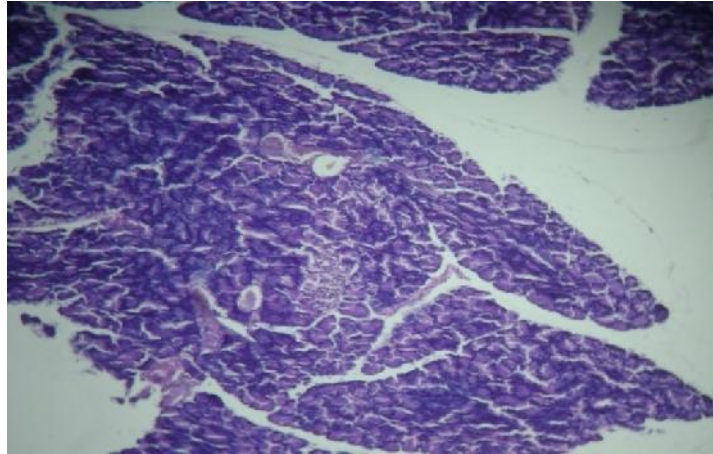
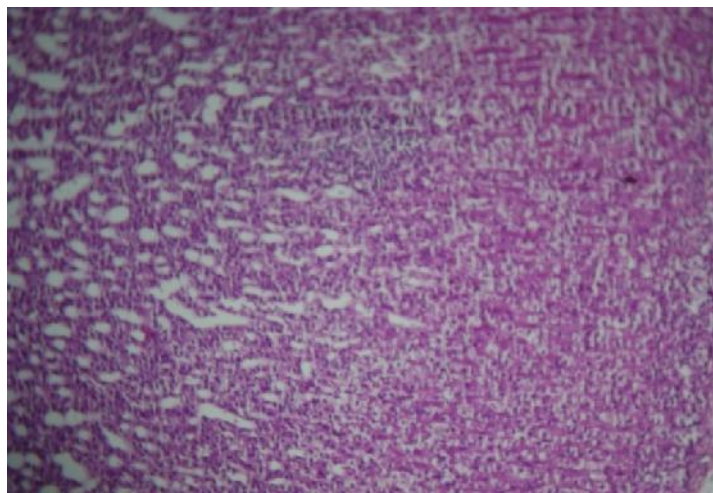


Figure No-07 Pancreas tissue of Glibenclamide (10 mg/kg) Treated Diabetic rat. (H & E 10 X)



468 DISCUSSION

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

486 The abnormally high concentration of lipids in diabetes mellitus is mainly due to an increase in
 487 the mobilization of free fatty acids from the peripheral fat depots, Excess of fatty acids in plasma
 488 produced by streptozotocin promotes the liver conversion of some fatty acids to phospholipids
 489 and cholesterol.²⁷ These two substances, along with excess of TG formed in the liver, may be
 490 discharged into lipoproteins in the blood²⁸. As a result serum phospholipid is elevated.
 491 Administration of Vanadium + selenium and magnesium + selenium to diabetic rats reversed all
 492 the above mentioned changes it not only lowered TC, TG and LDL but also enhanced the cardio
 493 protective lipid HDL (table no - 3). In Diabetes increased oxidative stress and elevated oxidative
 494 stress plays an important role in pathogenesis of diabetic complication. Increased oxidative stress
 495 in diabetes is postulated to promote the development of neuropathy, nephropathy, myocardial
 496 injury and retinopathy²⁹. The possible source of oxidative stress in diabetes might include auto

497 oxidation of glucose, shift in redox balance. Oxidative stress in diabetes may partially be reduced
498 by antioxidants have been prescribed to reduce the long term complication of diabetes.

499 Liver is the main organ responsible for the maintenance of blood glucose homeostasis where
500 glucose-6-phosphatase plays an important role²⁰. To focus the underlying biochemical
501 mechanism of the action of antidiabetogenic activities of these combined trace elements, we
502 measured the enzyme activities in liver in different groups. After the supplementation of these
503 three combined trace elements, out of this two combination such as vanadium + selenium and
504 magnesium + selenium showed a significant recovery of the enzyme activity that may be another
505 possible way for antidiabetogenic potency of these trace elements (table no - 6, 7, 8).

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

510 Diabetes is also associated with lipid peroxidation as insulin secretion is closely associated with
511 lipooxygenase derived peroxides²⁴. Elevation in lipid peroxidation in diabetic state has been
512 supported here from the elevation in TBARS, important indicator of oxidative stress condition in
513 liver²⁷. The elevation in lipid peroxidation in above tissue in diabetic state has been supported
514 here by the diminution of Catalase and glutathione, important antioxidant enzymes in liver.
515 Supplementation of combined vanadium and selenium and magnesium and selenium results
516 significant elevation or restoration of the antioxidant enzymes followed by diminution of
517 TBARS levels to the control which proposed the another mechanism of antidiabetic effect of this
518 trace elements.

519 The combination of chromium and selenium does not show any significant recovery in above
520 mention activities. Thus the present study shows that administration of combination of trace
521 elements such as vanadium and selenium and magnesium and selenium is more effective than
522 combination of chromium and selenium to challenge the diabetic state. This combination of trace
523 elements may recover the disorders in carbohydrate metabolism noted in diabetic state by
524 protecting the oxidative stress induced damage for such disorder and or by stimulating those
525 carbohydrate regulatory enzyme activities in target organ or by stimulating and regenerating β

526 cells in pancreas. The actual mechanism is not clear and further biochemical and
527 pharmacological investigations are needed.

528 It is widely accepted that the most challenging goal in the management of diabetes is to achieve
529 blood glucose levels as close to normal as possible. α -Amylases, endoglucanases that the
530 catalyze hydrolysis of the internal α -1, 4- glucosidic linkage in starch and other related
531 polysaccharides, have also been targets for suppression of postprandial hyperglycemia²⁹. So in
532 this study, the combination of trace elements is used to determine the inhibitory effects on α -
533 amylase. The Significant difference was notice in relation to inhibition of α - amylase of
534 vanadium and selenium, that shows 92.2% inhibition at 12.5 μ g/ml.

535 The number of functionally intact β -cells in the islet organ is of decisive importance in the
536 development course and outcome of DM. The renewal of β -cells in diabetes has been studied in
537 many animal models³⁰. Selenium as an antioxidant and a free radical scavenger prevent auto play
538 (ADP-ribosyl)-ation of PARP, thereby stabilizing Rege gene transcriptional complex and
539 resulting in the regeneration of β -cells and protection of pancreatic islets against STZ³¹. After
540 treatment schedule histopathology study (fig no – 2to7) of pancreas were done to determine
541 potential of Vanadium + selenium and magnesium + selenium to recover from necrosis which
542 was produced by streptozotocin. The result of the present study revealed that there is a partial
543 restoration of β -cells normal after oral administration of Vanadium + selenium and magnesium +
544 selenium for 28 days.

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