e-ISSN:2322-0139
p-ISSN:2322-0120

RESEARCH AND REVIEWS: JOURNAL OF PHARMACOLOGY AND TOXICOLOGICAL STUDIES

Role of Chromium Histidinate In Glycemic Control, Improving Serum Lipid and Reducing Oxidative Stress in Streptozotocin Induced Type 2 Diabetes Models of Rats.

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Research Article

Received: 02/06/2014
Revised: 20/06/2014
Accepted: 27/06/2014

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Keywords: Chromium Histidinate , Streptozotocin, lipid profile, Malondialdehyde and Type 2 Diabetes Mellitus

ABSTRACT

Hyperglycemia, a hallmark of type 2 Diabetes Mellitus (T2DM), is responsible for hyperlipidemia, oxidative stress and various diabetic related complications. Management aims at minimizing diabetic complications by maintaining near euglycemia. Neonatal Wistar Rats (n = 70, 4-5 days old) were divided into seven groups. All groups except Group I controls were fed a High Fat Diet (HFD)–40% of calories as fat. Group III, IV and V were injected single intra-peritoneal injection of streptozotocin (STZ) (40mg/kg) to induce T2DM. Group IV and VI were supplemented with 4µg/kg of Chromium Histidinate (CrHis) whereas Group V and VII received 8µg/kg of CrHis BW/d for 10 weeks. After 10 weeks the effect of Cr His on glucose, serum lipid and MDA were investigated. Serum glucose showed mild hyperglycemia in Group II and III compared to Group I control rats and also elevation of insulin levels in Group II rats. Group VII rats on administration of 8 µg Cr His, showed significant decrease in serum glucose values as compared to Group VI rats on 4 µg Cr His. Triglyceride (TG) rich lipoproteins were significantly lowered in Group II rats on HFD compared to Group I Controls ($p<0.05$). Group V and VII rats on 8 µg Cr His showed lower levels of TG and Very Low Density Lipoproteins (VLDL) compared to Group IV and VI rats on 4µg Cr His ($p<0.05$). The plasma MDA level was elevated in the stress induced rats in Group II and III but normalized to baseline when Cr His was administered. CrHis controls hyperglycemia, improves lipid profile and reduces oxidative stress in streptozotocin induced type 2 diabetes model in rats.

INTRODUCTION

Chromium (Cr) is a trace metal, and is an essential micronutrient required for optimal insulin activity, normal carbohydrate and lipid metabolism and is known to maintain normal glucose tolerance since decades [1,2]. It exists in various valence state in earth crust, among them trivalent Cr (Cr III) is biologically active. It has been identified as an active component of the Glucose Tolerance Factor (GTF). Cr increases insulin sensitivity, decreases insulin levels and improves glucose disposal in obese with type 2 diabetes mellitus. Chromium Picolinate (CrPic), a combination of the element chromium and picolinic acid (CrPic), is a widely used dietary supplement in the United States. Supplementation of CrPic has been found to have multiple beneficial effects in T2DM, including decrease in body weight gain [3], improving insulin sensitivity and enhancing endothelial function [4] and improving lipid profiles [5, 6].

e-ISSN:2322-0139
p-ISSN:2322-0120

Diabetes mellitus is a heterogeneous metabolic disorder, characterized by chronic hyperglycemia with disturbances of carbohydrate, lipid and protein metabolism. Depending on the etiology of the diabetes mellitus, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. Hyperglycemia, hallmark of type 2 Diabetes Mellitus, is responsible for hyperlipidemia, oxidative stress and various diabetic related complications [7].The metabolic dysregulation associated with diabetes mellitus may cause secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

Chromium Histidinate (CrHis) is another compound that has been seen to positive effects on diabetic rats because of the highest degree of absorption in the GI Tract due to the addition of amino acid histidine. Administration of CrHis significantly decreased lipid peroxidation levels and HSP expression in the kidneys of diabetic rats [8]. Supplementation with CrHis has been found to be protective against obesity in rats fed with high fat diet.

High fat-fed/streptozotocin (STZ)-treated rats is an animal model for T2DM and is considered suitable to test antidiabetic compounds. [9]The present study was undertaken to assess the effect of CrHis supplementation on diabetic profile.

METHODS

Selection of Experimental Animals

Neonatal Wistar Rats (4-5 days old), weighing between 5 and 10 g were produced in the laboratory breeding house of the Department of Pharmacology. The animals were maintained under controlled room temperature (25 ± 2°C) and light and dark (12:12 hr) conditions and were allowed to breast feed. Later at around 6 wks of age, they were given food (chow diet) and water *ad libitum*. The animals of all the groups except Group I controls were given a high fat diet -40% kcals from fat. Group III, VI and V were injected single intra-peritoneal injection of streptozotocin (40mg/kg) to induce Type 2Diabetes Mellitus. Animals of Group IV and VI were supplemented with 4µg/kg where as Group V and VII received 8 µg/kg BW/d of CrHis dissolved in water through mouth by feeding syringe everyday from 6 wks of age till 16 weeks i.e till the time they were sacrificed. (Table I). Before conducting the experiment, ethical clearance was obtained from the local Ethical Committee on Animal Research and ethical guidelines for investigations were followed in accordance with National Science Academy.

Drug and chemicals

The following special drugs were used in these experiments: Streptozotocin (Sigma Chemicals, U.S.A.); Chromium Histidinate (Nutrition 21 U.S.A.); Citric Acid; Sodium Citrate (salt) for the preparation of Citrate buffer at pH 5.5. Streptozotocin was prepared by dissolving in Na-Citrate buffer solution and was administered intraperitoneally (i.p.) in a volume of 40 mg /kg. The standard control was citrate buffer at 5.5 pH.

Experimental design

Groups

Experiment was carried out in Neonatal Wistar rats of age four to five days. Animals were fasted for 4-6 hrs. Type 2 diabetes mellitus was induced by single intra-peritoneal injection of streptozotocin(STZ), 40 mg/kg bw dissolved in a citrate buffer (pH- 5.5). Animals were divided into seven groups with 10 rats in each group.

Table 1: Division of animals into seven groups and treatment given.

Group I: Control rats: i.p. Injection of citrate buffer only (pH-5.5)
Group II: High Fat Diet(HFD) rats (40% KCals from fat)
Group III: High Fat Diet (HFD) rats + STZ (40mg/kg BW i.p.)
Group IV: High Fat Diet(HFD) rats +STZ (40mg/kg BW i.p.) + 4µgCrHis
Group V:High Fat Diet(HFD) rats +STZ (40mg/kg BW i.p.) + 8 µgCrHis
Group VI: High Fat Diet(HFD) rats +4µg CrHis
Group VII: High Fat Diet(HFD) rats +8µg CrHis

e-ISSN:2322-0139
p-ISSN:2322-0120

Analysis of Biochemical parameters

After 10 weeks the animal was euthanized under ether anesthesia and bloodsample collected from draining abdominal aorta. Serum sample was used for estimation of glucose [10] insulin (ELISA)[11] and MDA [12]. Lipid profile like TG, Total Cholesterol, LDL Cholesterol and HDL Cholesterol was estimated by Selectra E chemistry analyzer (Merck)using Kits from Human Diagnostics Germany [13,14].

Statistical analysis

All the data were expressed as Mean ± Standard deviation. Analysis was done using Mann Whitney U test and p <0.05 was considered to be statistically significant.

RESULTS

Table 2: Biochemical Parameters in serum of various groups of experimental rats

Groups	Glucose (mg/dl)	Insulin (µU ml/dl)	T G (mg/dl)	T Ch (mg/dl)	VLDL (mg/dl)	LDL mg/dl)	HDL (mg/dl)
I	306.50 ± 74.93	1.11 ± 0.32	134.50±41.14	79.75 ± 7.77	26.86 ± 8.23	19.76 ± 6.04	33.08 ± 5.01
II	391.25± 35.98*	1.39 ± 0.72*	74.40± 24.86*	65.00±15.94	17.08± 8.94*	20.70± 6.32	29.42±10.30
III	341.75± 88.05*	1.30 ± 0.69	108.33±43.79	79.25±16.50	21.66 ± 8.75	22.83±11.66	34.75± 9.33
IV	324.67 ± 54.75	1.64 ± 0.80 *	122.50±17.20	80.67±14.79	27.20 ± 9.06	23.65±11.87	31.50 ± 6.14
V	230.42 ± 50.64 *	1.34 ± 0.32*	114.83±43.64*	85.42±12.97	22.96 ± 8.72 *	20.87 ± 7.40	33.58 ± 5.94
VI	378.75±78.05*	1.48 ± 0.62*	145.42±41.03	81.75±17.32	29.08±12.00	18.66 ± 6.10	34.00 ± 7.49
VII	196.58±61.16 **	1.84 ± 1.74**	123.58±40.12*	72.17±15.73	21.70±9.44**	20.30±8.22	27.17±7.45

Data are expressed as Mean± SD (n= 10 rats in each group) , also , (*) indicates p < 0.05 & (**) indicates p< 0.001 while comparing among different groups, detail description in the text.

Blood glucose levels of the Group I controls, the Group II rats on a High Fat Diet (HFD) and the Group III rats are shown in Table 2. Serum glucose was 306.50 ± 74.93 mg/dl, 391.25 ± 35.98 /dl and 341.75 ± 88.05 mg/dl in Group I, II and III respectively and shows mild hyperglycemia in Group II and Group III compared to Group I which was statistically significant (p< 0.05).Among them, serum insulin level in these groups was 1.11± 0.32 µU/ml, 1.39 ±0.72 µU/ml and 1.30 ±0.69 µU/ml and are found elevated in Group II and III rats than Group I control. Elevation was statistically significant (p< 0.05) and is due to the fact that high fat diet induces insulin resistance in rodents. Glucose level in Group IV rats on 4 µg CrHis was 324.67 ± 54.75 mg/dl and was similar to Group I controls, how ever serum insulin level found to be elevated than Group I control and was 1.64 ± 0.80µU/ml (p< 0.05). Group V rats on 8 µg CrHis showed lower serum glucose of 230.42 ± 50.64 mg/dl (p<0.05) of glucose with concomitant mild increase in serum insulin of 1.34 ± 0.32 µU/ml (p<0.05) and even Group IV rats who were on 4µg CrHis. (p<0.05) (Table 2)

Group VI rats on HFD and 4 µg CrHis showed serum glucose and insulin level of 378.75 ± 78.05 mg/dl and 1.48 ± 0.62 µU/ml respectively which are higher than Group I control statistically (p<0.05), but similar to Group II and III rats. Group VII rats on HFD and 8 µg CrHis showed serum glucose of 196.58 ± 61.16 mg/dl and insulin of 1.84 ± 1.74 µU/ml significant decrease in serum glucose level compared to all Groups including Group VI rats on 4 µg CrHis (p <0.001) and a significant rise of insulin compared to control (p <0.001) and also among all the groups except Group VI rats.

Insulin level in all the Groups on HFD was significantly higher than the Group I control of 1.11 ± 0.32 µU/ml (p<0.05).

Figure 1. Depicts the level of serum insulin and lipid profile in all groups of rats. TG rich lipoproteins, TG and VLDL level were significantly lowered in Group II rats on HFD compared to Group I Controls and all other Groups of rats too. TG rich values were 74.40 ± 24.86 mg/dl and 17.08 ± 8.94 mg/dl (p<0.05) in the Group II and significantly lower than Control Group. Group V and VII rats on 8 µg CrHis showed 114.83 ± 43.64 mg/dl and 123.58 ± 40.12 mg/dl respectively, a significant lower level of TG and VLDL compared to Group IV and Group VI rats on 4µg CrHis (p<0.05) and no statistical change was observed compared to control.How ever, other lipid parameters showed no significant changes compared to controls and other relevant groups (Table 2).

e-ISSN:2322-0139
p-ISSN:2322-0120

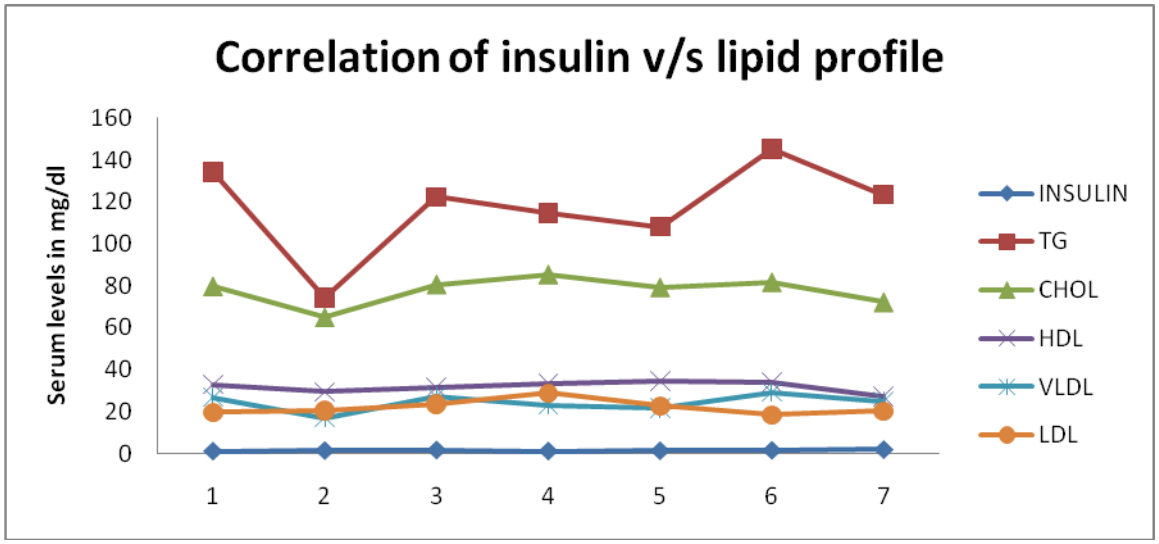

Figure1: Graph showing serum insulin and lipid profile like TG, Cholesterol, VLDL, LDL and HDL in all the seven groups of rats and their correlation.

Figure2. MDA levels in serum in different groups of rats. Serum MDA level was 6.42±3.02 mmol/ml and 7.17 ±3.09 mmol/ml in Group II and III statistically higher than Group I control of 3.92± 3.02 mmol/ml ($p<0.05$). Group V and VII on 8 µg CrHis showed 5.42 ± 2.90 and 3.58 ± 2.70 mmol/ml which at the level of Group I control, indicating no oxidative stress at all in these animals.Group IV and VI on 4 µg CrHis also showed base line level of MDA as Group I which are 4.00 ± 2.86 and 4.12 ± 2.04 mmol/ml respectively. When serum MDA of rats on 4 µg and 8 µg CrHis was compared, no difference was found statistically signifying both the doses are adequate to reduce oxidative stress.

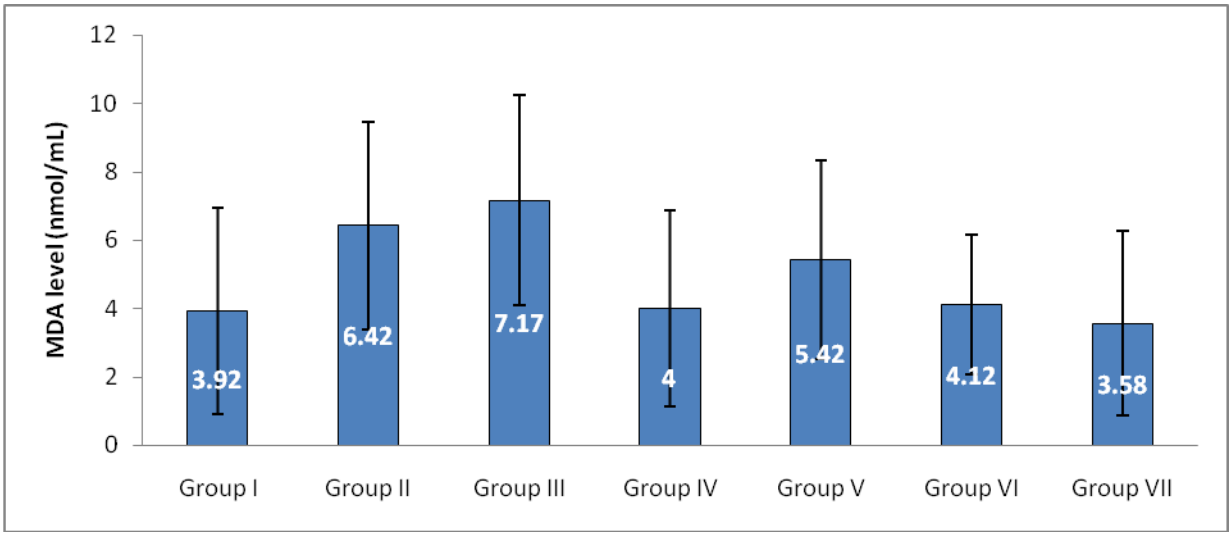

Figure 2: Serum Malondialdehyde levels in mmol/ml in all the seven groups expressed in mean ± SD.

DISCUSSION

Chromium is required for normal metabolism of carbohydrate, protein and lipid through enhanced insulin sensitivity. It reduces plasma cholesterol and triglyceride levels and , inhibits oxidative stress and inflammatory cytokine secretion [15,7].

Chromium is a trace metal, and is an essential micronutrient, used as a medication to treat insulin resistance .When used in appropriate amount, it enhances the glucose tolerance in human beings and is known as glucose tolerance factor for this reason.

In this experiment, mild hyperglycemia and hyper-insulinemia was produced in Group II and Group III rats, mimicking insulin resistance. On treating with 8 µg CrHis to Group VII rats, optimum glucose lowering effect was observed compared to all the groups.

This experiment shows, serum glucose levels in Group II and III rats higher than Group I controls with concomitant increase in serum insulin level. This is because high fat diet induces insulin resistance in rodents [16,17,18].

(Table 2) signifies development of insulin resistance. These statistically significant findings are important to characterize type 2 diabetes mellitus. CrHis in 4µg and 8µg was used in this experiment in both HFD and diabetic rats on HFD. Though both doses of CrHis lowered glucose, from this result it is clear that dose of 8 µg has a beneficial effect over 4 µg and has more glucose lowering activity than 4 µg CrHis(196.58 ± 61.16) P <0.001, (Table 2). This result establishes the glucose regulatory activity of Chromium Picolinate , and is achieved by this new compound too. This glucose lowering role of CrHis is modulated by enhancing the insulin binding to its receptors, thereby increasing tyrosine kinase activity and increasing GLUT-4 translocation to the cell surface.

High-fat diets induce insulin resistance in rodents [16-18] and Insulin resistance and hyperinsulinemia have been shown to predict T2DM. In the current study, group II and III rats had hyperglycemia and high insulin concentrations in serum. Theincreased magnitude was due to greater synthesis and release of insulin from β-cell function to overcome the resistance in the target tissues [19]. (Table 2).

TG levels was 44.68±39.57 %;(p<0.001) reduced in Group II and 19.45±43.22 %(p<0.001) in Group III compared to Group I Controls, also was 8.11±2.47% ;(p<0.05) low in group VII as a part of TG lowering activity of chromium. Even rats on 8 µg CrHis showed lower TG level than rats on 4 µg CrHis and authenticate the fact that CrHis activates 5' Adenosine Monophosphate activated Protein Kinase (AMPK), a major signal that suppresses lipogenesis and diverts the lipid molecule for oxidation [20].

Total cholesterol level was also 9.50±4.66%;(p<0.05) lowered in the in Group VII than Group I Controls and thought to be due to up-regulation of sterol regulatory element-binding protein(SREBP) by chromium.[21] This 8 µg CrHis dose is appropriate to lower the Cholesterol level. However, LDLc and HDLc remain unaffected due to short duration of experiments.

Data from various experiments shows lowering of MDA, a marker of oxidative stress.While examining the MDA level in this experiment, there was significant increased in Group II and III compared to control but was found at the level of control group in rats on 4 and 8 µg CrHis. The MDA level was elevated in plasma in stress- induced rats in Group II and III but normalized to base line control level when CrHis was administered. This experiment proves the reduction on oxidative stress by use of CrHis. It is beyond doubt that Chromium supplementation reduced serum concentration of MDA and has been provenin stressed hens [22,23]. Reports also shows decrease in hepatic thiobarbituric acid–reactive substance formation by supplementation of CrPic and chromium nicotinate in rats [24]. Decrease in MDA levels could be related to inhibition of epinephrine because of the insulinotropic effect of chromium. Changes in biochemical values detected in the present study could be due to the stimulatory effect of CrHis on insulin activity.

Basic nature of histidine facilitates maximum absorption and maintains high chromium level in the tissues. It then promotes insulin facilitatory action which potentiates the signal transduction, enhance tyrosine kinase activity that promote translocation of GLUT-4 to the cell surface, thus facilitating glucose uptake in concerted manner.[25] It also up-regulates insulin receptors as underlying mechanism [26,27].

Among many causes, inadequate dietary intake may play a role in development and progression of Type 2 Diabetes Mellitus as food stuffs are cultivated in Chromium depleted soil and CrHis stands nutritional remedy at this juncture.

ACKNOWLEDGEMENTS

We are grateful to Nutrition21 for providing us with chromium histidinate for the study.

e-ISSN:2322-0139
p-ISSN:2322-0120

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