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ASCORBIC ACID AND CHROMIUM PICOLINATE COMBINATION POTENTIATES ANTIDIABETIC ACTIVITY IN RATS

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ABSTRACT

Diabetes mellitus is a major cause of morbidity and mortality in worldwide, with an increasing prevalence. Now days there are various approaches for the treatment if diabetes like Non-Pharmacological approaches such as diet, caloric content, Macronutrients, dietary fibres and physical activity and Pharmaccological approaches like insulin treatment and antidiabetic drugs. Still there is search for new chemical entities. Literature survey reveals that (Cr (III)) is required in trace amounts for sugar and lipid metabolism. Increase oxidative stress and decrease antioxidant levels are the leading cause of diabetes and diabetic complications. Vitamin C plays a role in many oxidative and metabolic reactions. So a suitable plan has been designed for the evaluation of anti-diabetic activity of chromium picolinate and Ascorbic acid mixture with the objective of Supplementation of anti-oxidant may be useful in controlling the glucose level and postpone the occurrence the diabetic complication. Oral glucose tolerance test followed by Plasma Glucose, Triglycerides and Total Cholesterol were evaluated for the establishment of antidiabetic activity. The study indicates that additive action of nutrient L-Ascorbic acid and Chromium picolinate combination as well as influence of L-Ascorbic acid and Chromium picolinate on hypoglycaemic activity of Metformin. These nutrients alone as well as combination with Metformin were reduced total cholesterol and triglyceride, more significantly as compared to alone Metformin. Thus, it may be helpful in hyperlipedaemia associated with hyperglycaemia. Apart from that further work on human patients is required to confirm the safety and efficacy in diabetic condition and usefulness of these supplement for improvement of blood glucose as well as blood lipid and lipoprotein profile including free cholesterol, triglyceride, HDL-c, LDL-c and VLDL-c when administrated along with Metformin.

Key words: *Ascorbic acid, Chromium picolinate, Total Cholesterol, Triglyceride, Diabetis*

INTRODUCTION

Diabetes mellitus is a major cause of morbidity and mortality in worldwide, with an increasing prevalence. In 2009 there were around 228,000 people registered as having diabetes in Scotland, an increase of 3.6% from the preceding year[1]. Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. Diabetes mellitus (Greek: diabetes- a siphon or running through; mellitus- sweet). The rare disease diabetes insipidus has similar symptoms as diabetes mellitus, but without disturbances in the sugar metabolism (*insipidus* means "without taste" in Latin) [2]. Diabetes results in abnormal levels of glucose in the bloodstream. This can cause severe short-term and long term consequences ranging from brain damage to amputations and heart disease [3]. Another major micro vascular complications i.e. (retinopathy, nephropathy and neuropathy) of is also well-established [4]. Now days there are various approaches for the treatment if diabetes like Non-Pharmacological approaches such as diet, caloric content, Macronutrients, dietary fibres and physical activity and Pharmaccological approaches like insulin treatment and antidiabetic drugs. Still there is search for new chemical entities. Literature survey reveals that (Cr (III)) is required in trace amounts for sugar and lipid metabolism. Further

chromium supplementation, in the form of chromium picolinate, (1,000 µg per day) decreased symptoms in type II diabetes patients. This beneficial effect of chromium picolinate administration has been attributed to increased insulin action rather than to increased secretion[5]. Increase oxidative stress and decrease antioxidant levels are the leading cause of diabetes and diabetic complications. Vitamin C plays a role in many oxidative and metabolic reactions[6]. So a suitable plan has been designed for the evaluation of anti-diabetic activity of chromium picolinate and Ascorbic acid mixture with the objective of Supplementation of anti-oxidant may be useful in controlling the glucose level and postpone the occurrence the diabetic complication.

MATERIALS AND METHODS:

Drugs, chemicals and Diagnostic Kits

Drugs like Metformin obtained from Aarti drugs private Limited Mumbai as Active pharmaceutical ingredients (API). Chromium picolinate (Bal pharma *CP-200* marketed) was used while L-ascorbic acid was obtained from Central Drug Store of S.L.T. Institute of Pharmaceutical Sciences, G.G.V, Bilaspur (Chhattisgarh). Chemicals like Diethyl ether, Xylene and alloxan monohydrate was also obtained from department laboratory and plasma TC, TG, and glucose was determined by using Span Diagnostics Ltd had purchased from local dealers.

Antidiabetic Activity

Animals

Wistar albino rats (180-200g) purchased from Kolkata was maintained in the Department animal house for experimental purpose. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 25 ± 1^0 C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet (Pranav Agro Industries Ltd, Vadodara, India), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of SLT Institute of Pharmaceutical Sciences, Bilaspur (Chhattisgarh) was taken prior to the experiments. The protocol of the experiment was approved by the Institutional Animal Ethical Committee **(Reference No: IAEC/Pharmacy/2012/55)** as per the guidance of the Committee for the Purpose of Control and Supervision for experiments on Animals **(994/a/GO/06/CPCSEA)**. The approval was taken prior to the experiments[7].

Treatment Protocol

Fourty two adult wistar rats of either sex weighing between 180-200g were randomly divided into seven groups with six rats in each group. Group I received distilled water as non-diabetic control (2ml/kg body weight p.o.), Group II received alloxan as diabetic control (120mg/kg body weight i.p.), Group III received L-Ascorbic acid (60mg/kg body weight p.o.), Group IV received Chromium picolinate (80µg/kg body weight p.o.), Group V received L-Ascorbic acid + Chromium picolinate (40mg/kg + 60µg/kg body weight p.o. respectively), Group VI received L-Ascorbic acid + Chromium picolinate + Metformin (30mg/kg + 50µg/kg + 80mg/kg body weight p.o. respectively) and Group VII received Metformin only (120mg/g body weight p.o.). The treatment of animal began on the 3rd day after alloxan injection and this was considered as 1st day of treatment. Treatment was done for 3 weeks and Blood samples were collected from the tail vein of each rat at 1 (0, 1.5, 3.0, 4.5, 6 and 24 hour), 7, 14 and 21 days after drug administration. Blood glucose levels were determined by using portable glucometer (Johnson and Johnson one touch Glucometer). Finally at 21st day's blood sample were collected from cardiac puncture of each rat which was earlier

anaesthetized by diethyl ether and measured blood glucose levels through GOD/POD method by using UV spectrophotometer. These blood samples were also taken for blood cholesterol and triglyceride measurement. Body weights of the animals were also measured twice a week up to three weeks [8].

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was performed in rats after induction of diabetes. Each group of rats was subjected to oral administration of glucose at a dose of 2g/kg body weight of rat, 16^{th} days after induction of diabetes. Animals were kept on 12 hour fasting before administration of glucose. Finally blood sample of each rat were collected from tip of tail before 1 hour and after 0.5, 1.0 and 2.0 hour after glucose administration and subjected to blood glucose level measurement. Test and standard compounds were administrated before administration of glucose [9].

Preparation of Plasma sample

Blood sample of each animal poured into previously cleaned centrifuge tube. Before this EDTA (ethylenediaminetetraacetic acid) was added at a concentration of 1.5-2.0mg/ml of blood, it inhibits the clotting process by removing calcium from the blood [10, 11] (Excessive amounts of EDTA could not to be added because it produces morphological changes in blood cell). All tubes were inverted several times (8-10) to ensure thorough mixing and, therefore, proper anticoagulation [12]. Each centrifuge tube was placed into centrifuge at 4000/rpm for 10 minute. Centrifugation was carried out to settle down blood cells and thus, supernatant liquid was plasma. After 10 minute each centrifuge tube carefully removed from centrifugation so as to prevent mixing of blood cells with plasma.

Estimation of Plasma Glucose, Triglycerides and Total Cholesterol:

Blood glucose was estimated by the method described Barham and Trinder, 1972. The red colour so developed was measured spectrophotometrically at 505 nm. The intensity of red colour is proportional to the concentration of the glucose present in the specimen. The concentration of Glucose was calculated using the following formula: Glucose concentration (mg/dl) = (Absorbance of sample/Absorbance of standard) × 100. The concentration of Triglycerides was determined by the method of Balch and Phyllis A. 2006. The content was calculated using the following formula: Triglycerides concentration (mg/dl) = (Absorbance of sample/Absorbance of standard) × 200. Total cholesterol (TC) was estimated by the enzymatic method as described by Allain et al. (1974).The concentration of total Cholesterol was calculated using the following formula: Cholesterol concentration (mg/dl) = (Absorbance of sample/Absorbance of standard) × 200.

RESULT

Blood Glucose level in diabetic rats (Acute treatment)

All treated group was compared with diabetic control. Presence of L-Ascorbic acid at a dose 60mg/kg body weight orally administrated produced 27.2% blood glucose reduction at 1.5 hour as peak effects. Chromium picolinate at a dose of 80µg/kg body weight orally administrated produced 31.6% blood glucose reduction at 3 hour as peak effects. Thus, Chromium picolinate is more effective and longer acting as compared to L-Ascorbic acid. Combination of L-Ascorbic acid + Chromium picolinate/ L-Ascorbic acid + Chromium picolinate + Metformin produced 35.8% and 45.9% blood glucose reduction, respectively at 4.5 hour as peak effects. Metformin at a dose of 120mg/kg body weight alone produced 36.4% blood glucose reduction at 3.0 hour as peak effects. Thus, combination of L-Ascorbic acid and Chromium picolinate was longer acting as compared to standard Metformin and when it was combined with Metformin, duration as well as activity of Metformin increased.

Oral Glucose Tolerance Test (OGTT) in Normal and Diabetic rats

Oral Glucose Tolerance test used to determine the peripheral insulin resistance in normal and diabetic rats. Table 1 shows that; in diabetic rats significantly increase blood glucose level after 2g/kg p.o. glucose administration as compared to normal control (P<0.001) and reached maximum level at 60 minute. Diabetic rats treated with L-Ascorbic acid /Chromium picolinate/ L-Ascorbic acid + Chromium picolinate/ L-Ascorbic acid + chromium picolinate + Metformin/ Metformin significantly decrease blood glucose level as compared to diabetic control. Combination of L-Ascorbic acid + Chromium picolinate + Metformin was found to be maximum blood glucose reduction as compared to all treated group including standard Metformin. These indicate that this combination having maximum glucose utilisation capacity. However normal rats were utilised maximum amount of glucose and reached to approximately normal level in 120 minute.

Serum Cholesterol and Triglyceride level in Diabetic rats

Diabetic rats were increased significant amount of plasma cholesterol and triglyceride level (P<0.001) as compared to normal control. Diabetic control was found to be 27.8% increase cholesterol level and 50.9% triglyceride level as compared to normal control. Diabetic rats treated with L-Ascorbic acid + Chromium picolinate/ L-Ascorbic acid + Chromium picolinate + Metformin/ Metformin significantly decrease blood cholesterol and triglyceride level as compared to diabetic control. However, individually L-Ascorbic acid and Chromium picolinate were not significantly reduced blood triglyceride level. Combination of L-Ascorbic acid + Chromium picolinate + Metformin was found to be 25.7% cholesterol and 34.6% triglyceride reduction as compared to diabetic control and it was maximum cholesterol and triglyceride reduction as compared to all treated group. L-Ascorbic acid + Chromium picolinate combination and Metformin alone were approximately equally effective for cholesterol and triglyceride reduction as shown in table-2.

Table: 1 Oral Glucose Tolerance Test (OGTT) in diabetic and normal rats after 16th days of treatment.

Plasma Blood Glucose level expressed in mg/dl

Experimental groups	Zero time	30 min	60 min	120 min
Normal control	82.4±1.99	147.3±65.6	115.7±33.9	99±16.5
Diabetic control	389.8±7.5^{a}	488.3±6.8^{a}	505±20^{a}	480±8.8^{a}
Diabetic + Aa	226.5±7.6$^{a, d}$	293.1±47.3^{f}	259.8±19$^{b, d}$	243±39.7$^{b, d}$
Diabetic+ Cp	223.8±6.6$^{a, d}$	307.2±49^{f}	257.2±15.8$^{b, d}$	240.5±14.7$^{b, d}$
Diabetic + Aa + Cp	206±3.0$^{a, d}$	272.7±33.8^{e}	239.3±20.8$^{c, d}$	222.7±31.2^{c}, ^{d}
Diabetic + Aa + Cp +Metformin	191.2±16.6$^{a, d}$	251.1±19^{e}	217.8±26.3^{d}	192.8±27.9^{d}
Diabetic + Metformin	198.3±10.8$^{a, d}$	276.7±21.8^{e}	243.3±24.7$^{b, d}$	210±31.09^{d}

N=6 animals in each group, Values are expressed as mean ± SEM. aP< 0.001; bP<0.01; cP < 0.05 Vs normal control. dP< 0.001; eP< 0.01; fP < 0.05 Vs Diabetic control. Data analyzed by one-way ANOVA followed by Tukey multiple test.

Table: 2 Effect of Aa/ Cp/ Aa + Cp/ Aa + Cp + Metformin combination on Cholesterol and Triglyceride level in diabetic albino rats.

Plasma Cholesterol and Triglyceride level expressed in mg/dl

Experimental groups	Cholesterol	% Change	Triglyceride	%Change
Normal control	110.0±2.9	--------	70.0±8.0	--------
Diabetic control	152.5±4.4[a]	27.8↑	137.5±13.9 [a]	50.9↑
Diabetic + Aa	135.0±3.4[a, f]	11.5↓	116.7±5.6 [b]	15.2↓
Diabetic+ Cp	131.7±4.8[b, e]	13.6↓	110.0±4.5 [c]	20.0↓
Diabetic + Aa + Cp	126.7±3.3[c, d]	16.9↓	100.8±5.2[f]	26.7↓
Diabetic + Aa + Cp +Metformin	113.3±2.1[d]	25.7↓	90.0±6.3[e]	34.6↓
Diabetic + Metformin	120.0±3.6[d]	21.3↓	96.7±4.2[e]	29.7↓

N=6 animals in each group. Values are expressed as mean ± SEM. [a]P< 0.001; [b]P<0.01; [c]P < 0.05 Vs normal control. [d]P< 0.001; [e]P< 0.01; [f]P < 0.05 Vs Diabetic control. Data analyzed by one-way ANOVA followed by Tukey multiple test.

DISCUSSION

Diabetes poses a major health problem globally and is one of the top five leading causes of death in most developed countries. Indeed, by the year 2025, three-quarters of the world's 300 million adults with diabetes will be in developing countries and almost a third in India and China alone[13,14]. The prevalence of diabetes is rising all over the world due to population growth, aging, urbanisation and an increase of obesity and physical inactivity. The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030. According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. The primary goal in the management of diabetes mellitus is the attainment of near-normal glycaemia. In India, more than half of patients have poor glycaemic control and have vascular complications. Therefore, there is an urgent need to develop novel therapeutic agents of diabetes without the development and progression of complications or compromising on safety. Glucagon-like peptide-1 (GLP-1) analogues and dipeptidyl peptidase-4 (DPP-4) are novel agents that show promising results [76]. There are various research going on herbal preparations and nutrient supplement, both are effective medication in diabetic patients without any complication but no one surely control diabetes up to the satisfactory level. There are several synthetic oral hypoglycaemic agents having more glycaemic control than herbal and nutrient supplement but they have somewhat side effects. Therefore in present investigation we decided to take combination of nutrient supplement and Metformin as oral hypoglycaemic agents so that increase the effectiveness of Metformin as well as reduction of side effect. Side effect may be reduced due to decrease the dose of Metformin when combined with nutrient supplement. We have taken combination of L-Ascorbic acid and Chromium picolinate and their effect with Metformin. However, in future other oral hypoglycaemic agents may also taken in study but drug interaction studies should be conducted in animal models before applied in humans. Reactive oxygen species (ROS) are thought to be implicated in the pathogenesis of diabetes as well as other diseases [15]. Reactive oxygen species usually comprise radicals that have the ability to oxidize and damage DNA, proteins and carbohydrates. Hyperglycaemia appears to induce oxidative stress on cells and this can cause an increase in the production of free radicals [78]. Human antioxidant enzymes are mobilized during hyperglycaemia, but they cannot meet the continued demand due to increased oxidative stress. This problem is either due to decreased intake of needed precursors or an inability to synthesise the antioxidant enzymes

[16] Antioxidant supplementation may provide the only means to reverse this process. Use of typical antioxidants alone or in combination may retard or even prevent the normal progression of diabetic complications. It was reported that L-Ascorbic acid levels were decreased in diabetic patients and rats. So it is felt that L-Ascorbic acid supplementation may help in the treatment of diabetes mellitus. The cellular uptake of vitamin C (L-Ascorbic acid, ASC) is promoted by insulin and inhibited by hyperglycemia. If a rise in plasma ASC is uncoupled from insulin replacement in insulin-dependent diabetes mellitus (IDDM) then the degree of hyperglycemia could account for "tissue scurvy" in IDDM. Leukocyte ASC is lower in IDDMs compared with nondiabetics when vitamin C consumption is adequate. The cellular uptake of vitamin C is regulated by both glucose and insulin and the renal reabsorption of ASC is impaired by hyperglycemia [17]. Evidence also suggests that vitamin C supplementation may be beneficial in countering the pathophysiologies resulting from the chronic hyperglycemia of insulin-dependent diabetes mellitus (IDDM). Cromium is reported to be an essential element required for normal carbohydrate and lipid metabolism [18]. Lack of dietary chromium has been reported to lead to the development of abnormal glucose tolerance in rats. Moreover, it is reported that the impairment of glucose tolerance was the earliest recognised symptom of a low chromium state in animals. It has been reported that chromium deficiency is difficult to produce in animals since it requires strict control of dietary (from food and water) as well as environmental sources of chromium (like steel cages and lids, nozzles of drinking water bottles, pipes supplying drinking water, etc.). Furthermore, it develops slowly over a period of several months[19]. In the present investigation, the intake of chromium by the experimental animals from these sources was not controlled. Although earlier studies have reported that individuals with diabetes mellitus were found to have lower chromium levels in serum, hair and tissues [20] as compared to non-diabetic subjects. In the present investigation alloxan induced diabetic albino rats treated with chromium picolinate improve glycaemic control as well as cholesterol and triglyceride. Chromium picolinate were also tested for OGTT and found to be insulin like action.

Chromium deficiency in diabetic patients is a debatable problem. The prevailing opinion suggests the presence of low serum concentrations in such patients and therefore an early, long-term addition of chromium to the standard therapy is recommended [21] Chromium supplements are widely used as an alternative remedy for type 2 diabetes mellitus (T2DM). In vitro study findings show that chromium picolinate (CrPic) may improve insulin sensitivity by enhancing intracellular insulin receptor.

Within the last 5 years chromium (Cr) has been shown to play a role in glucose intolerance, Type 2 diabetes mellitus (Type 2 DM), and gestational diabetes. In addition, diabetes and the neuropathy of a patient on home parenteral nutrition were alleviated when supplemental Cr was added to total parenteral nutrition (TPN) solutions. In a study conducted in China that has been supported by studies in the United States, supplemental Cr as Cr picolinate improved the blood glucose, insulin, cholesterol, and haemoglobin[22]. In the present investigation both L-Ascorbic acid and Chromium picolinate when combined with Metformin, additive effect was found. These combinations increase the effectiveness, duration of action and improve insulin resistance of Metformin. Thus, this combination may postpone the occurrence of diabetic complications. These nutrients alone as well as combination with Metformin were reduced total cholesterol and triglyceride, more significantly as compared to alone Metformin. Thus, it may be helpful in hyperlipedaemia associated with hyperglycaemia. However several other parameters associated with diabetes like glycated haemoglobin, HDL-c, LDL-c and VLDL-c, insulin level, glucose transport in muscle and adipocytes etc. need to be evaluated.

CONCLUSION

The study indicates that additive action of nutrient L-Ascorbic acid and Chromium picolinate combination as well as influence of L-Ascorbic acid and Chromium picolinate on hypoglycaemic activity of Metformin. These nutrients alone as well as combination with Metformin were reduced total cholesterol and triglyceride, more significantly as compared to alone Metformin. Thus, it may be helpful in hyperlipedaemia associated with hyperglycaemia. Apart from that further work on human patients is required to confirm the safety and efficacy in diabetic condition and usefulness of these supplement for improvement of blood glucose as well as blood lipid and lipoprotein profile including free cholesterol, triglyceride, HDL-c, LDL-c and VLDL-c when administrated along with Metformin.

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