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EVALUATION OF ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY OF CHITOOLIGOSACCHARIDES IN ALLOXAN-INDUCED DIABETES MELLITUS IN MICE

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ABSTRACT

This study aims to investigate the therapeutic effects of chitooligosaccharides (COS) on alloxan-induced type II insulin dependent, diabetes mellitus in mice. The effect of aqueous solution of chitooligosaccharides at the dose of 10 mg/kg body weight was found to be a potent antidiabetic agent that reduces blood glucose significantly in diabetic mice. Serum lipids (triglycerides, total cholesterol, Low density lipoprptein and Very Low density lipoprptein cholesterol), urea, creatinine and various enzymes of glucose metabolism (Serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase and alkaline phosphatases) showed significant decrease in the diabetic group treated with aqueous solution of COS when compared with the diabetic group. The study reveals that COS has significant antidiabetic activity and hypolipidemic activity in alloxan induced and normal mice. The COS solution seems promising for the development of a medicine for diabetes mellitus.

KEYWORDS

alloxan; diabetic hypolipidemic; Chitooligosaccharides

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease with complex underlying etiologies. The incidence of diabetes mellitus is on the rise world wise. Based on the World Health Organization (WHO) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030[1]. As a devastating disease, diabetes is affecting approximately 3% of the population worldwide[2]. Increasing evidences from both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of DM. Free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins[3]. Abnormally high level of free radicals and the simultaneous decline in antioxidant defense mechanisms may lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance[4]. Current drugs used for the treatment of diabetes are associated with several side effects and hence there is need for effective, safe and better oral hypoglycemic agents[5]. In the indigenous, system of medicine many compounds isolated from these plants is used in combinational therapy for diabetes [6] but only a few have been scientifically evaluated and their active principles have been isolated [7].

Chitosan is a polycationic copolymer consisting of β-1, 4-linked 2-acetamido- D-glucose and β-1, 4-linked 2-amino-D-glucose units. Which is prepared from one of the most widespread natural biopolymers, chitin, is increasingly widely used in many fields owing to its valuable properties. Crab and shrimp shell wastes are currently utilized as the major industrial source of biomass for large-scale production of chitosan. Chitosan which is biodegradable, non-toxic and biocompatible has been shown to be particularly useful in many fields[8], including food, cosmetics, biomedicine, agriculture and environmental protection. Furthermore, it can be used as a bioactive material due to its biodegradable, non-toxic and non-allergenic natures. However, chitosan shows its biological activity only in acidic medium because of its poor solubility at pH above 6.5 and low absorbability of non-digestible and high molecular polysaccharides. Therefore, recent studies on chitosan have attracted interest in converting it to chitooligosaccharides (COS), because COS not only are water-soluble but also possess versatile functional properties such as antitumor enhancing properties, immunostimulating effects[9], antimicrobial activity[10], free radical scavenging activity [11,12], arthritis controlling activity [13]. However, little attention has been paid to its activity in diabetes mellitus and related mode of action. In the present study, soluble chitooligosaccharides with low molecular weight were used to see the effect on diabetic mice.

The purpose of this study was to examine the effect of chitooligosaccharides on hyperglycemia and hyperlipidemia.

MATERIALS AND METHODS

i) Drugs and reagents

Alloxan was purchased SD Fine chemicals (Mumbai, India). Glucose Analyzer and strips were purchased from Roche Diagnostics GmbH, Germany. All other chemicals used for this study were of analytical grade and

obtained from HIMEDIA (India), SRL (India), CDH (India) and Qualigens (India/ Germany). Kits for the estimation of total cholesterol, triglyceride and HDL-cholesterol were purchased from by Span Diagnostic Ltd.; Surat; India

ii) COS preparation

Chitooligosaccharides was used in this study obtained from the chitosan isolated from microalgae Diatoms. The 1% chitosan solution was prepared in acetate buffer. In the present study, water soluble chitooligosaccharides with low molecular weight were prepared by enzymatic hydrolysis of chitosan with immobilsed in chitin papain as previously described [14].

(iii)Animal care and monitoring:

Healthy male Swiss albino mice (*Mus musculus*) (4-5 months old, weighing 20-30 g) were procured from Banaras Hindu University, Varanasi. They were housed in polypropylene cages, under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). The animals were provided standard mice pellet feed and tap water *ad libitum.* Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

(iv) Induction of diabetes mellitus in mice

The mice were made diabetic by a single intra-peritoneal injection of alloxan monohydrate, 150 mg/kg body wt, freshly dissolved in cold sterile normal saline [15]. The mice were then kept for the next 24 hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia. After a fortnight, mice with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 250 – 300 mg/dl were used for the experiment. Blood was collected from the tail vein puncture for the estimation of the blood glucose.

(v) Experimental design and treatment

In the experiment a total number of 36 mice (18 diabetic induced mice and 18 normal mice) were used. Diabetes was induced in mice two weeks before starting the treatment. The mice were divided into six groups as follows after the induction of alloxan diabetes and each group comprised of six mice.

Group1: Normal control mice received only distilled water during the experimental period.

Group 2: Diabetic control- freshly prepared alloxan in normal saline was administered in a single dose of 150 mg/kg bw through intra-peritoneal to overnight fasted mice and the animals were allowed to develop diabetes for two weeks.

Group 3: Diabetic mice were daily administered with COS (LD) at a dose of 5 mg/kg body weight (125µg/25 µl) inta-peritonially for 21st days

Group 4: Diabetic mice were daily administered with COS (HD) at a dose of 10 mg/kg body weight (250µg/25 µl) intaperitonially for 21st days

Group 5: Normal mice were daily administered with COS (LD) at a dose of 5 mg/kg body weight intaperitonially for 21st days

Group 6: Normal mice were daily administered with COS (HD) at a dose of 10 mg/kg body weight (250µg/25 µl) intaperitonially for 21st days

(vi)Fasting Blood Glucose Level and Weight of Animals

The fasting glucose level was monitored periodically during the treatment with the tail vein prick method using Accucheck Active Glucometer (Roche Diagnostics GmbH, Germany) and compatible blood glucose strips. The blood glucose level was measured in milligram per deciliter. The weights of the animals were checked before and after treatment.

(vii) Quantification of glycogen

Hepatic glycogen content was measured according to the anthrone-H_2SO_4 method, with glucose as standard [16].

(viii) Estimation of serum triglyceride (TG): Serum level of triglyceride was measured by standard protocol of cholesterol oxidase / peroxidase (CHOD/POD)-phosphotungstate method (Kit supplied by Span Diagnostic Ltd.; Surat; India). The absorbance was noted at 520 nm and the TG level in serum was expressed in mg/dl [17].

(ix) Estimation of serum total cholesterol (TC): Serum TC was quantified by spectrophotometer method [18] by the addition of enzyme present in the reagent kit. The absorbance of red quinoneimine complex was determined at 505 nm. The value of TC present in serum was expressed in mg/dl.

(x) Estimation of serum lipoprotein cholesterol:

Serum low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were measured according to a standard protocol [19].VLDL-cholesterol = Serum triglyceride /5; High density lipoprotein cholesterol (HDLc) was measured biochemically [20]. LDL-cholesterol = Serum total-cholesterol –VLDL-cholesterol – HDL-cholesterol. Results were expressed in mg/dl.

(xi) Biochemical assay of GOT, GPT activities from serum:

Specific kits were used (Span Diagnostic Ltd.; Surat; India) for this purpose. The activities of these enzymes were expressed as relative units [21].

(xii) Statistical Analysis

All the grouped data were statistically evaluated with SPSS/13.00 software. Hypothesis testing methods included one-way Analysis of Variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered to indicate statistical significance. All the results were expressed as mean ± SE for six mice in each group.

RESULTS

Effect of COS on body weight

The alloxan-induced diabetic mice exhibited loss of body weight. Before embarking on the experiment, all the groups had no significant difference in body weight ($P > 0.05$). A significant ($P < 0.05$) decrease in body weight was detected in the Diabetic, D+COS (LD), D+COS (HD) groups as compared to the normal control group from 7 days after alloxan injection. However, the body weights in the D+COS (HD) and D+COS (LD) groups were significantly ($P < 0.05$) and dose-dependently increased as compared to those of the diabetic control from 21 days after administration, which is comparable to that of the diabetic group. The results were shown in Table1.

Table 1

Changes in Body Weight of Alloxan induced diabetic and COS treated mice

Groups	Treatments	Body weight (gm)		
		Initial	7 days after Alloxan administration	21 days After COS administration
I	Control	24.3 ± .88	29.1 ± .40	29.6 ± .21
II	Diabetic	27.0 ± 1.63 [a]	22.1 ± .94 [a*]	21.1 ± .90 [a*]
III	D + COS(LD)	25.8 ± .83 [b]	23.0 ± .57 [b]	25.8 ± 1.22 [b*]
IV	D + COS(HD)	27.0 ± .88 [b]	20.8 ± .94 [b]	24.8 ± .30 [b*]
V	COS(LD)	25.6 ± .83 [c]	27.6 ± .91 [c]	29.5 ± .56 [c]
VI	COS(HD)	26.1 ± .41 [c]	28.5 ± .50 [c]	29.1 ± .60 [c]

*Alloxan (150 mg/kg b.w.) was injected to the group II, III and IV for induction of diabetes. COS 5 mg/kg b.w.(LD) and 10 mg/kg b.w.(HD) were administered to the diabetic and normal mice (Group III to VI). Data are expressed as mean ± S.E of 6 mice in each group; [a] alloxan induced diabetic group vs control; [b]D+COS (LD) and D+COS (HD) group vs alloxan induced diabetic group; [c]COS (LD) and COS (HD) vs control group. In column figures bearing * is significantly different at p<0.05 between the groups.*

Effect of COS on Serum glucose and Glycogen levels

Glucose levels on day zero showed no significant intra-group variation. Seventy-two hours after administration of alloxan, they increased approximately fourfold ($p<0.05$) while remaining unchanged in control non-diabetic (Table2). The extract administration decreased plasma glucose levels by 54.1% on day 21st of the experiment ($p<0.05$). Hepatic glycogen content decreased significantly by 80.0 % in diabetic controls as compared to non-diabetic animals.COS (HD) significantly increased glycogen content by 78.3 % as compared to diabetic group (Table 2). But this was not restored up to normal level

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Table 2

Effect of COS on blood glucose level in diabetic and normal mice

Groups	Treatments	Liver Glycogen mg/g tissue weight	Blood Glucose in (mg/dl)		
			Initial	7 days after Alloxan administration	21 days After COS administration
I	Control	50.0 ± .73	105.6± 3.72	97.5 ± 3.29	100.8 ± 2.67
II	Diabetic	9.8 ± .69 [a*]	95.3 ± 6.87 [a]	282.3 ± 6.05 [a*]	311.6 ± 8.15 [a*]
III	D + COS(LD)	37.1 ± 1.85 [b*]	95.6 ± 4.18 [b]	282.6 ± 6.20 [b*]	168 ± 10.7 [b*]
IV	D + COS(HD)	44.9 ± .58 [b*]	106 ± 5.23 [b]	275.6 ± 6.56 [b*]	126.5 ± 3.81 [b*]
V	COS(LD)	51.3 ± 1.57 [c]	102.3 ± 2.97 [c]	102.3 ± 3.12 [c]	102.6 ± 3.07 [c]
VI	COS(HD)	43.9 ± .81 [c]	97.3 ± 5.12	98.8 ± 3.68 [c]	93.3 ± 4.27 [c]

Alloxan (150 mg/kg b.w.) was injected to the group II, III and IV for induction of diabetes. COS 5 mg/kg b.w.(LD) and 10 mg/kg b.w.(HD) were administered to the diabetic and normal mice (Group III to VI). Data are expressed as mean ± S.E of 6 mice in each group; [a] alloxan induced diabetic group vs control; [b]D+COS (LD) and D+COS (HD) group vs alloxan induced diabetic group; [c]COS (LD) and COS (HD) vs control group. In column figures bearing * is significantly different at $p<0.05$ between the groups.

Activity of Serum GOT and GPT

Serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity were increased significantly in alloxan-induced diabetic mice in respect to control group. These two parameters in serum were come towards control level after treatment this solution in separate manner. COS (HD) treatment resulted, an in significant variation in the level of these parameters when compared to control (Table 3).

Effect of COS on Serum lipids levels

Diabetes mellitus is usually complicated with hyperlipoproteinemia. The present results showed that the TC and TG levels were significantly elevated in the diabetic control group as compared to the normal control group ($P< 0.05$). After administration with COS, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels and same time increase in HDL-c in diabetic mice. The response was better in D+ COS (HD) group compared to the others group. The results were shown in Table 4.

Table 3

Effect of COS on serum transaminases, urea and creatinine on diabetic and normal mice

Treatments (COS mg/kg body weight)	SGOT (IU/L)	SGPT (IU/ L)	ALP (IU/ L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	56.0 ± 1.54	38.4 ± .78	89.7 ± 2.85	19.4250 ± .53	0.88 ± 0.03
Diabetic	143.8 ± 5.65 a*	96.4 ± 4.73 a*	200.8 ± 2.98 a*	44.2000 ± 1.35 a*	2.6 ± 0.21 a*
D + COS(LD)	108.4 ± 3.48 b*	66.2 ± 1.70 b*	134.5 ± 2.34 b*	24.3617 ± 0.65 b*	1.1 ± 0.10 b*
D + COS(HD)	83.5 ± 2.76 b*	53.7 ± 2.17 b*	114.5 ± 1.94 b*	20.8667 ± 0.40 b*	0.84 ±0.03 b*
COS(LD)	60.5 ± 2.92 c	39.9 ± 2.75 c	93.8 ± 1.57 c	27.3667 ± 0.61 c	0.79 ± 0.03 c
COS(HD)	59.2 ± 3.80 c	44.2 ± 3.20 c	99.0 ± 1.59 c	28.8833 ± 0.34 c	0.73 ± 0.05 c

Alloxan (150 mg/kg b.w.) was injected to the group II, III and IV for induction of diabetes. COS 5 mg/kg b.w.(LD) and 10 mg/kg b.w.(HD) were administered to the diabetic and normal mice (Group III to VI). Data are expressed as mean ± S.E of 6 mice in each group; [a] alloxan induced diabetic group vs control; [b]D+COS (LD) and D+COS (HD) group vs alloxan induced diabetic group; [c]COS (LD) and COS (HD) vs control group. In column figures bearing * is significantly different at $p<0.05$ between the groups.

Table 4

Effect of COS on lipid profile (mg/dl) on diabetic and normal mice

Groups	Treatments (mg/Kg body weight)	Total Cholesterol	Triglyceride	LDL	VLDL	HDL
I	Control	144.5 ± 4.64	120.5 ± 4.46	76.4 ± 3.11	24.1 ± .89	43.9 ± 2.55
II	Diabetic	375.8 ± 13.31 a*	239.8 + 3.31 a*	308 ± 11.29 a*	47.9 ± .66 a*	19.7 ± 2.44 a*
III	D + COS(LD)	182.3 ± 4.30 b*	173.1 ± 4.00 b*	117.7 ± 3.67 b*	34.6 ± .80 b*	30.0 ± 2.85 b*
IV	D + COS(HD)	153.6 ± 3.26 b*	168.5 ±10.55 b*	87 ± 2.35 b*	33.7 ± 2.11 b*	32.8 ± 2.69 b*
V	COS(LD)	142.3 ± 4.02 c	133 ± 5.91 c	75 ± 5.52 c	26.7 ± 2.89 c	40.5 ± 2.18 c
VI	COS(HD)	148.3 ± 3.21 c	129.3 ± 6.00 c	87.1 ± 5.20 c	25.8 ± 1.20 c	35.3 ± 2.38 c

Alloxan (150 mg/kg b.w.) was injected to the group II, III and IV for induction of diabetes. COS 5 mg/kg b.w.(LD) and 10 mg/kg b.w.(HD) were administered to the diabetic and normal mice (Group III to VI). Data are expressed as mean ± S.E of 6 mice in each group; [a] alloxan induced diabetic group vs control; [b]D+COS (LD) and D+COS (HD) group vs alloxan induced diabetic group; [c]COS (LD) and COS (HD) vs control group. In column figures bearing * is significantly different at $p<0.05$ between the groups.

DISCUSSION

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs [22]. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc.These complications have long been assumed to be related to chronically elevated glucose level in blood [23]. Alloxan has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycemic[24]. The increased blood glucose in diabetic mice (group II) as compare to control (group I) might be use to glycogenolysis and / or glyconeogenesis. Body weight, which is another important parameter in diabetes, also increased with the treatment of COS. There was a gradual increase in the body weight of controls and treated mice while the diabetic mice continued to lose weight.

Diabetes is also associated with hyperlipidemia [25].The levels of TC, TG LDL and VLDL have been decreased and HDL increased significantly in diabetic mice after the COS treatments.

In the present study the activities of SGOT, SGPT and ALP in serum were altered in DM. In diabetic animals, the changes in the levels of SGOT, SGPT and ALP are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis. The restoration of SGOT and ALT to their respective normal level was observed in the COS treated groups. This is consistent with our previous report of the extracts of *Chinese juniper* berries [26]. SGOT and SGPT levels also act as an indicator of liver function hence restoration of normal level of these enzymes indicates that the normal functioning of liver. Increased activities of serum ALP have been observed in alloxan diabetic mice. Alloxan treated diabetes caused lipid peroxide mediated tissue damage in the pancreas, liver, kidney, and heart [27]. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and then migrating into the blood stream [28] and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic mice. In COS treated groups, the cell damage might be reverted and which may leads to the decreased activities of ALP. Therefore, the present study clearly indicates that COS possess hypoglycemic and hypolipidaemic activities in alloxan induced DM mice.

CONCLUSION

In conclusion, COS possess various biological activities and can be used in the treatment of diabetes mellitus. COS can increase insulin secretion of pancreatic cells and improve the overgrowth of β cells and therefore have beneficial effects in diabetes mellitus that holds the hope of new generation of antidiabetic drugs.

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