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ANTIDIABETIC, HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF *MOMORDICA CHARANTIA* ON TYPE-II DIABETIC PATIENT IN ALLAHABAD INDIA.

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ABSTRACT

The present study was undertaken to investigate the antidiabetic, hypolipidemic and antioxidant effects of *Momordica charantia extract on diabetic humen patients.* The blood glucose level was significantly ($p< 0.05$) decreased in diabetic human patients after 4 and 8 weeks of treatment ascompared to diabetic control. .The administration of *Momordica charantia* extract on diabetic human patients for 4- 8 weeks, the total cholesterol and triglycerides levels were significantly decreased where as HDL level was increased as compared to non treated patients.. **.** The enzymatic antioxidants levels were decreased in diabetic control as compared to normal control. Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of superoxide dismutase (8.95±0.34, 13.43±0.33 unit/mg protien) and catalase (2.28±0.14, 3.56±0.45 unit/mg protien) were increased significantly ($p<0.05$) as compared to diabetic control.. The non-enzymatic antioxidants such as ascorbic acid and reduced glutathione level also decrease ($p<0.05$) in diabetic test groups when compared to the corresponding normal control groups. The oxidative stress marker melanodialdehyde (MDA) was significant increase ($p<0.05$) in diabetic group as compare to the normal control group. Compared to the diabetic control there was a significant deccrease in melanodialdehyde of diabetic human patients administered fruit juice of *Momordica charantia* for 4week and 8 weeks. Thus, this study indicates that extract of *Momordica charantia* have favorable effect in hypolipidemic and hepatoprotective activities.

KEYWORDS: Diabetes, Lipid profile, *Momordica charantia*, Oxidative Stress, Antioxidant

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INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterized by elevated blood glucose due to absolute or relative deficiency of insulin secretion from pancreatic cells[1,2]. Noninsulin dependent diabetes mellitus (NIDDM) or type II diabetes has been increasing alarmingly worldwide. The worldwide prevalence of diabetes mellitus is expected to increase by 42% from 51 to 72 million in the developed countries and by 170% from 84 to 228 million in the developing countries by the year 2025.[3]. Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental factors and chemicals. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications in human beings[4]. In diabetes mellitus, chronic hyperglycemia produces multiple biochemical squeal, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease[5].Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defense potential[6]. Elevated generation of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation[7]. Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production[8]. The antioxidant defense system represents a complex network with interactions, synergy and specific tasks for a given antioxidant[9]. Many of the complications of diabetes mellitus, atherosclerotic vascular disease, the leading cause of mortality in diabetes mellitus, have been linked to oxidative stress, and antioxidants have been considered as treatments[10]. Plants often contain substantial amounts of antioxidants, flavonoids and tannins and the present study suggests that antioxidant action may be an important property of plant medicines associated with the hypoglycaemic effect on diabetes mellitus[11]. The Bitter melon (*Momordica charantia*) or Bittergourd fruit (BF), commonly known as karalla (L), family: Cucurbitaceae), is grown in tropical countries in South Asia, South America and Africa. The juice of bittergourd fruit has been proved for hypoglycaemic effects in experimental type 1 diabetes and in type 2 human diabetes[12,13]. The juice of bittergourd can increase glucose uptake by tissues *in vitro*[14]. Many species of the genus in the Cucurbitaceace family, like Momordica Charantia[15,16,17] and Momordica Charantia[18] have been reported for significant antidiabetic effects.

MATERIALS AND METHODS

Preparation of Momordica Charantia fruit juice

Momordica Charantia fruits were obtained from the local market, washed thoroughly, and fresh juice was prepared on a juicer eliminating seeds. It was centrifuged at 1000 rpm on a tabletop refrigerated centrifuge for 10 min at 4°C. The clear supernatant was considered as 100% *Momordica Charantia* fruit juice (BFJ) which was diluted with autoclaved distilled water to make 25 and 50% juice or any other desired concentrations. The 100% juice was stored at 4°C[19].

Selection of patients and dose

The present study will be carried out by collection of random blood samples of 100 subjects. Total subjects were divided into two groups. Group-I was 50 known type-II diabetic patients and group-II 50 normal healthy individual without no any metabolic disorder. In group I (50) person will take 100 ml fruit juice of *Momordica charantia* (karela) in once a day during fasting condition. During the research work no any antihyperglycemic, Antihyperlipidemic medicine taken by the group I diabetic patient. The biochemical parameters should be analyzed before the supplementation

4 weeks then after the supplementation of 8 weeks.

Collection of blood for biochemical assays

For determination of biochemical parameter fasting blood samples were collected from the anticubital vein of diabetic patients and blood was collected in the sterile glass test tubes. The blood containing tubes were placed in a slanting position at room temperature for 4 hours. The tubes were then incubated overnight in the refrigerator (4°C). The serum samples were separated and centrifuged to get rid of unwanted blood cells.

Estimation of Blood Glucose level

For acute study, blood samples were collected by anticubital vein of diabetic human patient and the blood glucose determinations were carried out by using a single touch glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase method.

Estimation of Oxidative stress marker (MDA)

Melanodialdehyde (MDA) was determined by colorimetric method[20]. Briefly, to 0.5 ml plasma, 2.5ml of 20% Tricholoro Acetic Acid (TCA) in 2M sodium sulfate is added. After precipiting the protein with TCA and washing with 0.05 sulfuric acid. It is incubated in a boiling water bath for 30 min. After cooling, the samples are extracted with n-butanol and centrifuged at 3500 rpm.The absorbance of samples is determined at 530 nm.

Estimation of Enzymatic antioxidant level

Superoxide dismutase (SOD)

The superoxide dismutase (SOD) was determined by colorimetric method[21]**.** The reaction mixture composed of 1.0 ml carbonate buffer (0.2 M, PH 10.2), 0.8 ml KCl (0.015 M),0.1 ml of diluted blood and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025 M). Change in absorbance was recorded at 480 nm at 15 sec interval for 1 min at 25^{o}C (UV- 1800 SHIMADZU) Suitable control lacking enzyme preparation was run simultaneously.

Catalase (CAT)

The catalase activity was determined by colorimetric method[22]. The assay is based on the disappearance of H_2O_2 in the presence of the enzyme source at 26^{o}C. In brief the hemolysate is prepared from lysed RBC suspension,further dilute by phosphate buffer (PH-7.0). Here the Reaction mixture containing 0.05M phosphate buffer (PH-7.0), 1.2mM H_2O_2 and 0.2ml of diluted hemolysate is allowed to stand for 25 minuts. At the end of which reaction is stopped by the addition of 2.5ml peroxidase reagent containing peroxidase and the red coloured compound chromogen system. peroxidase reduced the H_2O_2 to give a compound and an absorbance measure at 505 nm.

Estimation of Non Enzymatic antioxidant level

Ascorbic acid

The ascorbic acid was determined by the colorimetric method[23]. Take the 0.5 ml blood or granulation tissue in test tube and added 1.5 ml of 4% TCA solution and mixed properly and left for 5 minutes and centrifuge at 2500 rpm for minutes. After centrifugation the supernatant to be discarded in other clean tube and filtered the solution by Whatman filter paper No 42. Take the above filtered solution 0.5 ml in test tube and added 1.0 ml of 2% 2-4 dinitrophenyl hydrazine in 9N sulphuric acid to be added. The sample is incubated at 37^{o}C for 3 hours and after 3 hr, the sample is to be cooled in ice bath. Added the 5 ml of 85 % sulphuric acid in each tubes a mixed properly. The sample kept at room temperature for 30 min and absorbance to be measured at 540 nm.

Reduced Glutathione (GSH)

The reduced glutathione was estimated by colorimetric method[24]. Preparation of Erythrocytes. The 2.0ml of prepared hemolysate mix with , 0.8ml of Na_2HPO_4 reagent (0.3 mol /l) and 1.0ml of 5 5' Dithiobis-2 nitrobenzoic acid (DTNB) reagent in cuvette mark as a test. In Blank cuvette 1.2ml of precipitating solution, 0.8ml of distilled water was taken. Then added 8.0ml of Na_2HPO_4, & 1.0ml of DTNB reagent in both the cuvettes and mix well.

Read absorbance at 412nm within 4mins. Reduced glutathione (GSH) calibrators curve was plotted by assaying different GSH calibrators (50, 10, 5, 4, 2 mg/dl) to determine GSH concentration of blood sample from the calibrator curve.

Estimation of Lipid Profile

Total cholesterol

Cholesterol was estimated (CHOD-PAP method) by commercially available kit (ERBA diagnostic Mannheim, Transasia Bio-Medicals Limited, Solan, India)[25]. The reaction mixture contained 1000 µl Cholesterol reagent and 20 µl serum, mix well and incubate at 37°C for 10 minutes. Blank and standard contained 20 µl water and standard Cholesterol in place of sample respectively. Blank the spectrophotometer with reagent blank, read the absorbance of standard and each sample at 505 nm. Calculate the Cholesterol concentration in mg/dl.

Triglycerides (TG)

Triglycerides (TG) were estimated by commercially available kit (ERBA diagnostic Mannheim, Transasia Bio-Medicals Limited, Solan, India)[26]. The reaction mixture contained 2000 µl glucose reagent and 20 µl serum, mix well and incubate at 37°C for 10 minutes. Blank and standard contained 20 µl water and standard Triglycerides in place of sample respectively. Blank the spectrophotometer with reagent blank, read the absorbance of standard and each sample at 546 nm. Calculate the Triglycerides concentration in mg/dl. The absorbance of dye is directly proportional to TG concentration in the sample.

High density lipoprotein (HDL)

HDL Cholesterol was estimated by commercially available kit (ERBA diagnostic Mannheim, Transasia Bio-Medicals Limited, Solan, India)[27]. Chylomicrons, LDL, VLDL were precipited from serum by phosphotungustate in the presence of divalent cations such as Mg^{2+}. The reaction mixture contained 1000 µl Precipitating reagent and 50 µl serum, mix well and incubate at 37°C for 10 minutes. Blank and standard contained 50 µl water and standard HDL Cholesterol in place of sample respectively. Blank the spectrophotometer with reagent blank, read the absorbance of standard and each sample at 505 nm. Calculate the HDL Cholesterol concentration in mg/dl.

RESULTS AND DISCUSSION

Blood glucose level

The blood glucose levels of both normal and diabetic pateints before and after 4 & 8 weeks of treatment are shown in Table 1 The blood glucose level was increased in diabetic control (230.04±42.31 mg/dl) patient as compare to normal control (95.52±7.96 mg/dl). Following oral administration of fruit extract of *Momordica charantia* after 4 weeks (165.90±28.31 mg/dl) the blood glucose level was significantly reduced ($P<0.05$). Meanwhile, treated patients after 8 weeks (142.54±26.75 mg/dl) also had significant decreased on the blood glucose level as compared to diabetic untreated patients. In the present study, the oral treatment of *Momordica charantia* fruits extract decreased the blood glucose levels in diabetic human patients. It has been reported that using medicinal plant extract to treat diabetic patients results in activation of *ß*-cells and insulinogenic effects[28].

Table 1

Supplementation effect of Momordica Charantia on routine investigations of Type-II diabetes mellitus and non diabetic patients.

S.NO	PARAMETERS	Group-I	Group-II	Group-V	Group-VI	P.VALUE
		N.C.	D.C.	S-4 Week	S-8 Week	
1.	Glucose (mg/dl)	95.52±7.96	95.52±7.96	95.52±7.96	142.54±26.75	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*
2.	Protien (gm/dl)	7.08±0.54	5.35±0.46	5.92±0.38	7.20±0.40 I-II*	I-V*,I-VI**,II-V*,II-VI*,V-VI*

**significant, ** Non significant*

Lipid Profile

The lipid profile total cholesterol, HDL, and triglycerides levels normal control and diabetic patients before and after four & eight weeks treatment were represented in Table 2**.** The Total cholesterol and Ttriglycerides levels were increased in diabetic control (302.48±35.13 and 345.65±41.88 mg/dl) as compared to normal control (191.10±28.87 and 175.10±41.07 mg/dl) respectively.While the HDL level is decreased in diabetic control (28.05±20.89mg/dl) as compared to normal control (38.765±5.89). Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of total cholesterol (261.14±20.07, 228.54±14.86 mg/dl) and Triglycerides (276.05±26.77, 195.10±24.88 mg/dl) were decreased significantly ($p<0.05$) as compared to diabetic control respectively**.** Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of HDL (36.92±13.04, 41.56±11.29 mg/dl) increased as compared to diabetic control**.** Abnormalities in lipoproteins are very common in both NIDDM and IDDM. Diabetes leads to alterations in the plasma lipid and lipoprotein profile and increases risk of coronary heart disease. In patients with type 2 diabetes hyper triglyceridemia and low HDL-cholesterol levels are common[29]

Table 2
Supplementation effect of Momordica Charantia on lipid Profiles of Type-II diabetes mellitus and non diabetic patients.

S.NO.	PARAMETERS	Group-I	Group-II	Group-V	Group-VI	P.VALUE
		N.C.	D.C.	S-4 Week	S-8 Week	
1.	Total Cholesterol (mg/dl)	191.0±28.87	302.48±35.13	261.14±20.07	228.54±14.86	I-II*,I-V*.I-VI*, II-V*,II-VI*, V-VI*
2.	HDL (mg/dl)	38.76±5.89	28.05±20.89	36.92±13.04	41.56±11.29	I-II*,I-V**.I-VI**,II-V*,II-VI*, V-VI**
3.	Triglycerides(mg/dl)	175.10±41.07	345.65±41.88	276.05±26.77	195.10±24.88	I-II*,I-V*.I-VI*,II-V*,II-VI*,V-VI*

significant, ** Non significant

Melanodialdehyde (MDA)

Melanodialdehyde (MDA) level of normal control and diabetic patients before and after four & eight weeks treatment were represented in Table 3. The melanodialdehyde (MDA) level is increased in diabetic control (6.12±0.46nmol/ml) as compared to normal control (1.32±0.56nmol/ml). Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of MDA level is decreased (3.86±0.24, 2.10±0.23nmol/ml) as compared to diabetic control**.** MDA is a lipid peroxidation product which is formed during oxidative process of PUFA by reactive oxygen species. All of the major classes of biomolecules may be attacked by free radicals but lipids are the most susceptible. Cell membranes are rich sources of polyunsaturated fatty acids which are readily attacked by oxidizing radicals. The oxidative destruction of PUFA by deleterious free radical reactions is known as lipid peroxidation. Lipid peroxidation has been implicated in a wide range of cell and tissue damages, diseases, biological variables and life habits[30].

Table 3
Supplementation effect of Momordica Charantia on oxidative marker (MDA level) of Type-II diabetes mellitus and non diabetic patients.

S.NO.	PARAMETERS	Group-I	Group-II	Group-V	Group-VI	P.VALUE
		N.C.	D.C.	S-4 Week	S-8 Week	
1.	MDA (nmol/ml)	1.32±0.56	6.12±0.46	3.86±0.24	2.10±0.23	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*

significant, ** Non significant

Non Enzymatic Antioxidant

The non enzymatic antioxidant ascorbic acid and reduced glutathione level of normal control and diabetic patients before and after four & eight weeks treatment were represented in Table 4**.** The ascorbic acid and reduced glutathione levels were decreased in diabetic control (1.15±0.262 mg/dl) (33.14±4.08µmol/mg protien) as compared to normal control (3.09±0.54 mg/dl) and (54.88±1.77 µmol/mg protien) respectively. Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of ascorbic acid (1.86±0.14, 2.73±0.15 mg/dl) and reduced glutathione (42.91±1.97, 49.20±1.94 µmol/mg protien) were increased significantly ($p<0.05$) as compared to diabetic control respectively**.** Vitamin C, a potent water soluble non-enzymic antioxidant effectively intercept oxidants in the aqueous phase before they attack and cause detectable oxidative damage[31]**.** Vitamin C acts as a potent antioxidant by scavenging physiologically relevant reactive oxygen, chlorine, and nitrogen species[32,33]. Vitamin C can also be a prooxidant in vitro under certain circumstances[34,35] and can glycate protein [36]. Plasma vitamin C is often subnormal in diabetic persons[37,38]. Glutathione is one of the most abundant tripeptide, nonenzymatic antioxidant present in liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Moreover, it is substrate for glutathione peroxidase [39]**.** A major function of GSH in the erythrocyte is to reductively eliminate H_2O_2 and organic peroxides, which are reactive oxygen metabolites that can irreversibly damage hemoglobin and cleave the C-C bonds in the phospholipids tails of cell membranes [40].

Table 4

Supplementation effect of Momordica Charantia on non enzymatic antioxidants oxidative marker of Type-II diabetes mellitus and non diabetic patients.

S.NO.	PARAMETERS	Group-I	Group-II	Group-V	Group-VI	P.VALUE
		N.C.	D.C.	S-4 Week	S-8 Week	
1.	Ascorbic Acid (mg/dl)	3.09±0.54	1.15±0.262	1.86±0.14	2.73±0.15	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*
2.	Reduced Glutathione (µmol/mg protein)	54.88±1.77	33.14±4.08	42.91±1.97	49.20±1.94	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*

*significant, ** Non significant

Enzymatic Antioxidants

The enzymatic antioxidant superoxide dismutase (SOD) and catalase (CAT) level of normal control and diabetic patients before and after four & eight weeks treatment were represented in Table 5. The superoxide dismutase (SOD) and catalase level were decreased in diabetic control (5.89±0.30, 1.31±0.14 unit/mg protien) as compared to normal control (14.35±0.32 , 4.08±0.32 unit/mg protien) respectively. Diabetic patients treated with fruit extract of *Momordica charantia* for four and eight weeks the level of superoxide dismutase (8.95±0.34, 13.43±0.33 unit/mg protien) and catalase (2.28±0.14, 3.56±0.45 unit/mg protien) were increased significantly ($p<0.05$) as compared to diabetic control respectively**.** Free radical scavenging enzymes such as superoxide dismutase (SOD) protect the biological systems from oxidative stress. SOD and CAT provide the first defense against oxygen toxicity by catalyzing the dismutation of superoxide anion to hydrogen peroxide and decomposition of hydrogen peroxide to water and molecular oxygen[41]. Catalase is a heame protein, which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. This decrease in catalase activity could result from inactivation by glycation of the enzyme[42]

Table 5
Supplementation effect of Momordica Charantia enzymatic antioxidants oxidative marker of Type-II diabetes mellitus and non diabetic patients.

S.NO.	PARAMETERS	Group-I	Group-II	Group-V	Group-VI	P.VALUE
		N.C.	D.C.	S-4 Week	S-8 Week	
1.	Superoxide dismutase (Unit/mg protein)	14.35±0.32	5.89±0.30	8.95±0.34	13.43±033	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*
2.	Catalase (Unit/mg protein)	4.08±0.32	1.31±0.14	2.28±0.14	3.56±0.45	I-II*,I-V*,I-VI*,II-V*,II-VI*,V-VI*

significant, ** Non significant

CONCLUSION

Our present investigation shows that the fruit extract of *Momordica charantia* extract possesses antidiabetic, antioxidant and antihyperlipidaemic effects in type-II diabetic patient. Therefore, *Momordica charantia* fruits show therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in type-II diabetic patient it's worth emphasing that *Momordica charantia* has considerable potential for improving public health if used on a regular basis. Further pharmacological and biochemical investigations are underway to find out the active constituent responsible for the antidiabetic activity and to elucidate its mechanism of action.

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