Antihyperglycaemic and antihyperlipidaemic effect of wheatgrass on streptozotocin induced type 2 diabetic rats

Garima Shakya, Madhu Sudhana Rao K, Sankar P, Rukkumani R*

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India.

Received on: 12-02-2012; Revised on: 14-03-2012; Accepted on: 22-04-2012

ABSTRACT

Diabetes mellitus (DM), especially type 2 DM, is a major cause of morbidity and mortality in the world. Cardiovascular disease is one of the serious complications in DM due to dyslipidaemia. Management of dyslipidaemia in DM is one of the major issues. High risk and low efficacy of oral hypoglycaemic drugs diverted much of interest towards the natural products. Several researches are going on to find the effective treatment of DM from the natural sources. Wheatgrass has a potent antioxidant efficacy and has been used as a health drink in everyday’s life and is used to cure DM in folk medicine. Hence we planned to test the effect of wheatgrass on DM. Present study is aimed to find the effect of wheatgrass on lipid profiles in diabetes induced rats. Either blood plasma or organ tissues (liver and heart) or both were used to study the levels of free fatty acids, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, and phospholipids in diabetic rats. Wheatgrass treatment showed decrease in the levels of free fatty acids, triglyceride, total cholesterol, LDL cholesterol and phospholipids and increase HDL cholesterol, in diabetic rats. Wheatgrass increases the HDL-c secretion, which reduce bad cholesterol and thus prevents cardiovascular disease. It also increases insulin activity and sensitivity in peripheral tissue, which reduce the blood glucose levels. Wheatgrass is found to be very effective in lowering the lipid levels in diabetic rats.

Key words: Type 2 diabetes mellitus; Wheatgrass; Glucose; Lipids; Glibenclamide

INTRODUCTION

Diabetes mellitus (DM) is recognized as a leading cause of death in the world [1]. It is a chronic disorder of glucose metabolism, which occurs due to defect in insulin secretion, action, or both. It results in impaired utilization of glucose for energy purposes and elevated level of the blood glucose [2]. This in turn affects the lipid metabolism and leads to hyperlipidaemia [3]. DM is characterised by hyperglycaemia and hyperlipidaemia [4]. Hyperlipidaemia in DM leads to cardiovascular diseases, which is 3-5 times more prevalent in type 2 diabetes. Type 2 diabetes is one of the major causes of morbidity and mortality in the world, already affecting 5% population worldwide [5]. Patients with DM are found to have abnormal lipid profile such as decreased levels of high density lipoprotein-cholesterol (HDL-c) and increased levels of plasma triglycerides (TG) [6], low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein (VLDL). Dyslipidaemia has been identified among the several other factors that contribute for increased risk for cardiovascular diseases [6]. Management of hyperglycaemia and dyslipidaemia is an important criteria in DM.

Low therapeutic efficacy and safety of various available oral hypoglycaemic drugs increased the demand for screening the safer and highly effective medication for diabetes management[7]. Plants are found to be highly effective in reducing hyperglycaemia and hyperlipidaemia and are rich sources of antioxidants [11]. Various herbs are widely used in traditional medicine for the treatment of DM. Therefore, numerous studies have been done to evaluate natural product from the medicinal plants for the remedy of diabetes[8]. In search of such effective and safe medication for the treatment of diabetes we tested the effect of wheatgrass in our study.

Wheatgrass is known as a 'complete food' because it contains all the essential nutrients and minerals. Wheatgrass is the rich source of vitamin C and E, β carotene, ferulic acid, vanillic acid and phenolic compounds [9]. Wheatgrass juice has been found to have healing properties in cancer, degenerative diseases and benefit the blood cell, bone, glands, kidney and other parts of the body [10]. Various studies have shown that wheatgrass extract contains phenolic compounds especially flavonoids [10]. Wheatgrass has been used for the treatment of DM in folk medicine. Till now there is no scientific report on its effect on DM. Hence the present study is aimed to evaluate the effect of wheatgrass on lipid profiles in type 2 DM.

Glibenclamide is a very popular and widely used oral hypoglycaemic drug [10]. It is potent, long acting, sulfonylurea drugs, which act by stimulating insulin secretion [11]. Glibenclamide treated rats were used to compare of effectiveness of wheatgrass in diabetic rats.

MATERIALS AND METHODS

Animals

Male albino rats, Wistar strain of body weight 140-160 gm bred in Central animal House, Pondicherry University were used in this study. The animals were fed on the standard pellet diet (Hindustan Lever Limited, Mumbai, India). Water was given ad libitum. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). It produces a metabolisable energy of 3600 K Cal.

The animals were housed in plastic cages under controlled condition of 12h light / 12h dark cycles, 50% humidity and at 30 ± 2°C. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Institutional Animal Ethical Committee, Pondicherry University.

Chemicals

Streptozotocin and glibenclamide were purchased from Sigma–Aldrich, Bangalore, India. Wheatgrass powder was purchased from local market Puducherry,

*Corresponding author.
Dr. R. Rukkumani,
Assistant Professor,
Department of Biochemistry and Molecular Biology,
School of Life Sciences,
Pondicherry University,
Puducherry- 605014. India.
India. All chemicals and solvents used were of high purity and analytical grade.

**Induction of diabetes**

Diabetes was induced by a single intraperitoneal injection of streptozotocin (40 mg/kg B. Wt, in 0.2 M citrate buffer, pH 4.0).

**Experimental design**

The animals were randomized into five groups of six rats each.

Group 1 Normal (N) - Rats were given normal pellet diet.

Group 2 Diabetes Mellitus (DM) - Rats were given single dose of streptozotocin (i.p).

Group 3 Diabetes Mellitus + Wheatgrass (DM+WG) - Rats were given single dose of streptozotocin (i.p) and 1 ml of 2% wheatgrass in water orally every day.

Group 4 Wheatgrass (WG) - Rats were given 1ml of 2% wheatgrass in water orally every day.

Group 5 Diabetes Mellitus + Glibenclamide (DM+G) - Rats were given single dose of streptozotocin and 1 ml of 6 mg/kg body weight glibenclamide in water orally every day.

At the end of the experimental period (45 days), rats were sacrificed after an overnight fast by cervical dislocation. The blood was collected and the organs liver and heart, were removed, cleared off blood and collected in ice-cold containers containing 0.9% NaCl for various estimations.

**Preparation of plasma**

Blood was collected in heparinised tubes and plasma was separated by centrifugation at 1000g for 15 minutes for various biochemical estimations.

**Extraction of lipids**

Tissue lipids were extracted according to the method of Folch et al.,[12]. The tissue lipids were rinsed in cold physiological saline thoroughly and dried by pressing between the folds of filter paper. They were then weighed and homogenized in cold chloroform – methanol mixture (2:1 V/V) and contents were extracted after 24 hours. The extraction was repeated four times. The combined filtrate was washed with 0.7% potassium chloride and the aqueous layer discarded. The organic layer was made up to a known volume with chloroform and used for various estimations.

**Biochemical Assays**

Blood glucose was estimated by ortho-toluidine method of Dubowski[11]. Plasma and tissues cholesterol were estimated by the CHOD-PAP method of Allain et al.,[14] by using AGAPPE reagent kit. LDL-c was estimated by the enzyme selective protection method of Gordon et al.,[15] by using AGAPPE reagent kit. HDL-c was estimated by the immune inhibition method of Gordon et al.,[15] by using AGAPPE reagent kit. Plasma TG was estimated by the GPO-PAP methodology of Buccolo et al.,[16] by using AGAPPE kit. TG in tissues was estimated by the method of Foster & Dumm[17]. Free fatty acid (FFA) in plasma and tissue were estimated by the method of Falholt et al.,[18]. Plasma and and tissue phospholipids (PL) were estimated by the method of Zilversmit & Davis[19].

**Statistical analysis**

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Tukey’s test. The level of statistical significance was set at \( p < 0.05 \).

**RESULTS**

Figure 1 shows the effect of wheatgrass on blood glucose levels. The blood glucose levels in diabetic group increased significantly compared to normal. Treatment with wheatgrass and glibenclamide significantly reduced the blood glucose levels compared to normal.

Figure 2 shows the plasma levels of cholesterol, HDL-c, LDL-c, and figure 3 shows the levels of plasma TG, PL and FFA in normal and experimental rats. The levels of cholesterol, LDL-c, TG FFA and PL were significantly increased in the diabetic rats compared with the normal rats. Treatment with the wheatgrass and glibenclamide reversed all these effects. The plasma levels of HDL-c showed a significant decrease in diabetic rats and treatment...
with wheatgrass and glibenclamide significantly increased the plasma levels of HDL-c.

Figure 4 and 5 represent the levels of cholesterol, TG, FFA and PL in liver and heart respectively. Liver and heart tissues of diabetic rats showed a significant elevation in the levels of cholesterol, TG, FFA and PL when compared to normal rats, which were significantly decreased on treatment with wheatgrass and glibenclamide.

**DISCUSSION**

Diabetes mellitus is a complex mixture of various syndromes like hyperglycaemia, dyslipidaemia which alters carbohydrate, protein and lipid metabolism and also causes various secondary complications[28]. The present study was focused to analyze the effects of wheatgrass on lipid profile in DM.

In our results there was decrease in blood glucose level in diabetic rats with wheatgrass treatment. This may be either due to increase in insulin activity, which act by inhibiting the gluconeogenesis in hepatic cell or by improving its sensitivity to peripheral tissue which act by increasing the glucose uptake of HDL-c.

Increased activity of hormone sensitive lipases in diabetic patients causes high lipolysis of peripheral fats, which leads to elevated levels of FFA in circulation[2]. Increased FFA in the blood causes insulin resistance, defective insulin signal pathway and ß cell destruction. Plasma FFA further increases with the increase in blood glucose and hepatic glucose production in DM[21]. In our study diabetic rats showed the similar effect of elevated plasma FFA level, which decreased with wheatgrass treatment.

FFA is directly taken to liver through portal circulation, which is an important organ in regulating the lipid metabolism. In diabetes condition there is excess influx of FFA in liver, which produces high levels of TG leads to excess deposition of fat in liver. In our study, the increase in hepatic fats (cholesterol, TG, FFA and PL) of diabetic rats was reversed with the wheatgrass treatment. This effect of wheatgrass treatment may be due to increase in glucose regulation and enhance lipid clearance by increase in HDL-c level in diabetic rats[12, 31].

High level of FFA in liver produces high levels of VLDL, which in turn increases the serum TG level, and responsible for hypertriglyceridaemia[22]. The decrease in the level of TG in wheatgrass treated diabetic rats suggests that wheatgrass treatment reduced the FFA influx in liver and hence decreased the VLDL synthesis and reduced TG production. This indicates that wheatgrass act by increasing insulin sensitivity in peripheral tissue, which may be mediated by increasing FFA uptake and utilization[23].

In DM the high level of TG in circulation, primarily (TG rich) VLDL, is involved in the exchange of neutral lipids with LDL via cholesterol ester transfer protein. The TG rich LDL is then metabolised to small dense LDL-c by the action of hepatic lipase[22]. Oxidation of these highly susceptible small dense LDL during oxidative stress plays significant role in the pathogenesis of atherosclerosis[24, 25]. Wheatgrass has potent antioxidant property and is a rich source of vitamin C and E, β carotene, ferric acid, vanillic acid and phenolic compounds[26]. Antioxidant effect of wheatgrass may be one of the key factors in preventing the oxidation of LDL-c and thus could be helpful in preventing dyslipidaemia in DM.

HDL-c is an important lipoprotein in the body, and has negative correlation with the coronary heart disease[26]. They are involved in the removal of excess amount of cholesterol from the peripheral cell to the liver or to other tissues, which require cholesterol, by reverse cholesterol transport. Due to association of antioxidant enzyme, HDL-c has antioxidant properties and hence prevents LDL-c oxidation[27]. HDL-c is also involved in reduction of monocyte adhesion and chemotaxis and inhibition of macrophages production[28]. Due to all these properties HDL-c acts as an anti-atherogenic agent. Our result was very well correlated with this fact, as the level of plasma HDL-c increased, the heart lipids (cholesterol, TG, FFA and PL) decreased simultaneously with wheatgrass treatment. Thus wheatgrass could mediate hypcholesterolemic and thus cardioprotective effect in DM, by its own antioxidant potency and by increasing HDL-c level in treated rats.

The present study showed improvement of glycemic control and decrease in total cholesterol, LDL-c, TG, FFA and PL with wheatgrass treatment. These results suggest that some diabetes induced changes in insulin resistance and insulin secretion could be reversed by treatment with wheatgrass. Thus the conclusion from our study is that, treatment with the wheatgrass is found to be as effective as glibenclamide in reducing the blood glucose and lipid levels in diabetic rats. Being a natural product, it could be a good source for the treatment of DM.

**REFERENCES**

2. Saravanan G and Ponmurugan P. Ameliorative potential of S-


Source of support: Nil, Conflict of interest: None Declared

Journal of Pharmacy Research Vol.5 Issue 5 May 2012

2580-2583