

PROTECTIVE ROLE OF WHEATGRASS ON OXIDATIVE STRESS IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Diabetes mellitus (DM) is one of the leading causes of death in the world, especially in the developing countries like India. Hyperglycaemia in diabetes generates reactive oxygen species (ROS) and disturbs the endogenous antioxidant defence system that causes oxidative stress. Oxidative stress in turn generates several secondary complications in diabetic patients. Due to this, great interest has been developed towards the treatment of diabetes by controlling the oxidative stress with antioxidants. There are various synthetic antioxidants available in the market but are associated with many side effects. Thus, much of the interest is towards the screening of natural antioxidants from plant 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 the present study is aimed to find the effect of wheatgrass on oxidative stress in diabetes. In our study either blood plasma or haemolysate and tissues (liver, heart, kidney and pancreas) were analysed to evaluate the levels of lipid peroxidative markers thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LH), enzymatic antioxidants; catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and non enzymatic antioxidants; reduced glutathione (GSH) and vitamin C in male albino Wistar rats. The levels of TBARS and LH were significantly increased in diabetic rats, which were found to be reduced on treatment with wheatgrass. The levels of both enzymatic and non enzymatic antioxidants were decreased in diabetic rats, which were found to be reversed on treatment with wheatgrass. From this we conclude that wheatgrass has protective role on oxidative stress in diabetic rats.

Keywords: Type 2 Diabetes mellitus, Wheatgrass, Glucose, Oxidative stress, Antioxidant, Glibenclamide.

INTRODUCTION

Reactive oxygen species (ROS) are recognized as one of the factors responsible for the pathogenesis of various diseases like diabetes mellitus (DM), cancer, infectious disease, atherosclerosis and ageing¹. DM is a chronic metabolic disorder, characterised by hyperglycaemia and hyperlipidaemia, due to insufficient insulin secretion, action or both. It has become a major cause of mortality and morbidity in the world, mainly contributed by developing countries, especially India, which has largest number of diabetic patients higher than China and USA².

Hyperglycaemia, generate ROS and increases the oxidative stress in the body. Oxidative stress plays important role in the development of many secondary complications in diabetes. Various deleterious cellular effects of oxidative stress are DNA oxidation, lipid peroxidation, protein oxidation and abnormal synthesis of nitric oxide³. In normal metabolic conditions ROS are continuously produced within the body. The small concentration of ROS is required for cellular activities and their adverse effects are destroyed by various endogenous defence mechanisms, such as enzymatic and non enzymatic antioxidants. but at higher concentration they are very harmful⁴. In normal physiological conditions the endogenous antioxidant defence mechanism is enough to control the generation of free radicals. In DM, hyperglycaemia and disturbance in the antioxidant defence mechanism increases the production free radicals, causing oxidative stress in the body^{4,5}.

The fact that the free radicals are responsible for the various secondary complications in diabetes, generated the approach of the treatment of diabetes using antioxidants⁶. Antioxidants play an important role in preventing many diseases by inhibiting the oxidation of oxidizable substances in a chain reaction. Thus the use of synthetic antioxidants has increased, but they are toxic and carcinogenic to health. Therefore, it has increased the demand for screening the safer and highly effective medication for diabetes from the natural sources⁷. Plants are the richest source of antioxidants like phenolic compounds, flavonoids, gallotannins and other related polyphenols. Therefore, the search for effective hypoglycaemic agent has been focused on the medicinal plants². In search of such effective and safe medication for the treatment of diabetes we tested the wheatgrass in our study.

Wheatgrass is known as a "health drink" because it contains all the essential nutrients, vitamins, nutrients and antioxidants. Wheatgrass is a rich source of vitamin C and E, β carotene, ferulic acid and vanillic acid⁸. Wheatgrass juice is found to have, healing properties in cancer and degenerative disease, and is known to benefit the blood cell, bone, glands, kidneys and other parts of the body⁹. Wheatgrass extract contains phenolic compounds including flavonoids, which is a very potent antioxidant¹⁰. Wheatgrass has been used for the treatment of diabetes in folk medicine. Till now there is no scientific report on its effect on DM. Hence the present study is aimed to find the effectiveness of wheatgrass in reducing the oxidative stress on streptozotocin induced type 2 diabetic rats. Glibenclamide, a very popular oral hypoglycaemic drug was used to compare the effectiveness of wheatgrass on diabetic rats¹¹.

MATERIALS AND METHODS

Animals

Male albino rats, Wistar strain of body weight 140-160 g 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 fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). It produces a metabolisable energy of 3600KCal.

The animals were housed in plastic cages under controlled condition of 12h light / 12h dark cycles, 50% humidity and at $30 \pm 2^\circ\text{C}$. 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 in Puducherry, India. All chemicals and solvent used were of high purity and analytical grade.

Induction of diabetes

Diabetes was induced in male albino Wistar rats by a single intra peritoneal injection of streptozotocin (40 mg/kg B.Wt, in 0.2 M citrate buffer (pH 4.0)).

Experimental design

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

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

Group 2 Diabetic Mellitus (DM) - Rats were given streptozotocin, intraperitoneally (single dose)

Group 3 Diabetic Mellitus + Wheatgrass (DM+WG) - Rats were given streptozotocin intraperitoneally and 1 ml of 2% wheatgrass (in water) orally.

Group 4 Diabetic Mellitus + Glibenclamide (DM+G) - Rats were given streptozotocin and 1 ml of 6 mg/kg body weight glibenclamide (in water) orally.

At the end of the experimental period (45 days), rats were sacrificed after an overnight fast by cervical dislocation

Preparation of plasma

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

Preparation of haemolysate

After separating the plasma, the packed cells were washed thrice with physiological saline. 0.5 ml of erythrocyte was lysed with hypotonic phosphate buffer, pH7.4. The haemolysate was separated by centrifugation at 2500g for 15 min at 2°C for the estimation of enzymatic antioxidants.

Preparation of tissue homogenate

Tissues (liver, heart, kidney and pancreas) were removed, cleared off blood and immediately transferred to ice cold containers containing 0.9% NaCl solution. A known amount of tissue was weighed and homogenized in appropriate buffer (10%) for the estimation of various biochemical parameters.

Biochemical Estimation

Glycosylated haemoglobin (HbA1C) is estimated by the immunoturbidimetric latex method of Nathan et al.,¹² by using AGAPPE reagent kit. TBARS were estimated by thiobarbituric acid assay method of Niehaus and Samuelsson¹³. The lipid hydroperoxide (LH) was estimated by the method of Jiang et al.,¹⁴. The catalase (CAT) activity was assayed by the method of Sinha¹⁵. Glutathione peroxidase (GPx) was assayed by the method of Rotruck

et al.,¹⁶. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar et al.¹⁷. Vitamin C was estimated by the method of Roe and Kuether¹⁸. Reduced glutathione (GSH) was determined by the method of Ellman¹⁹.

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 $p \leq 0.05$.

RESULTS

Figure 1 shows the elevated levels of HbA1c in diabetic group compared to normal. Administration of wheatgrass and glibenclamide significantly decreased the HbA1c levels in diabetic rats.

Figure 2 and 3 shows the elevated levels of TBARS in the plasma and tissues (liver, heart, kidney and pancreas) of the diabetic rats, respectively. Administration of wheatgrass and glibenclamide showed significant reduction of the TBARS levels in diabetic rats, compared to normal groups.

Figure 4 and 5 represent the levels of LH in the plasma and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. LH was increased significantly in DM group, compared to that of normal group. Administration of wheatgrass and glibenclamide significantly decreased the LH in diabetic rats.

Figure 6 and 7 shows the activity of CAT in the haemolysate and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. CAT activity was found to be decreased in diabetic rats, when compared to normal group. In the wheatgrass treated groups, the activity of CAT increased significantly.

Figure 8 and 9 represents the decreased activity of GPx in the haemolysate and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. Wheatgrass and glibenclamide treatment significantly reversed the effect.

Figure 10 and 11 shows significant reduction in the activity of SOD in haemolysate and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. The SOD activity improved significantly in wheatgrass treated rats.

Figure 12 and 13 shows the decreased level of vitamin C in plasma and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. Treatment with wheatgrass and glibenclamide significantly increased the vitamin C level in diabetic rats.

Figure 14 and 15 shows the significant decrease in the levels of GSH in the plasma and tissues (liver, heart, kidney and pancreas) of diabetic rats, respectively. Wheatgrass and glibenclamide treatment reversed the effect in diabetic rats.

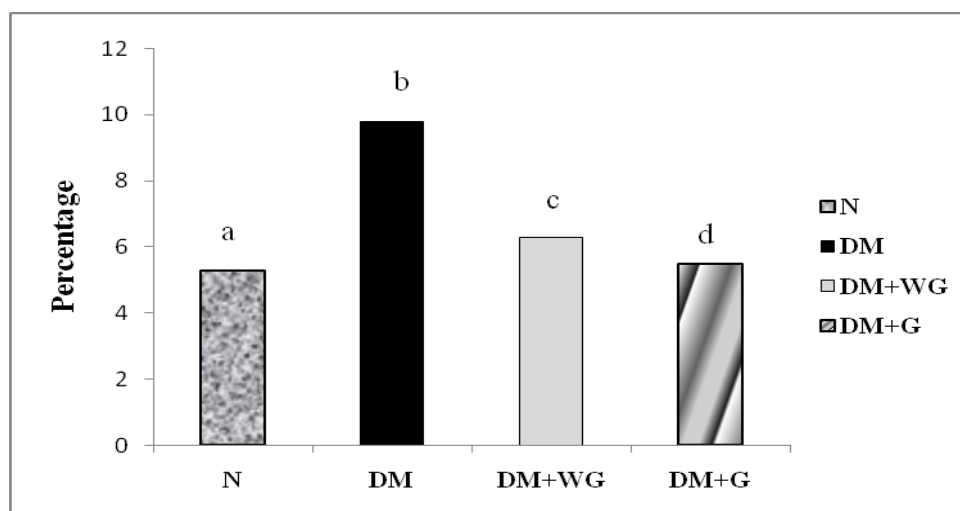


Fig. 1: HbA1c levels in plasma

Values are Mean \pm SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

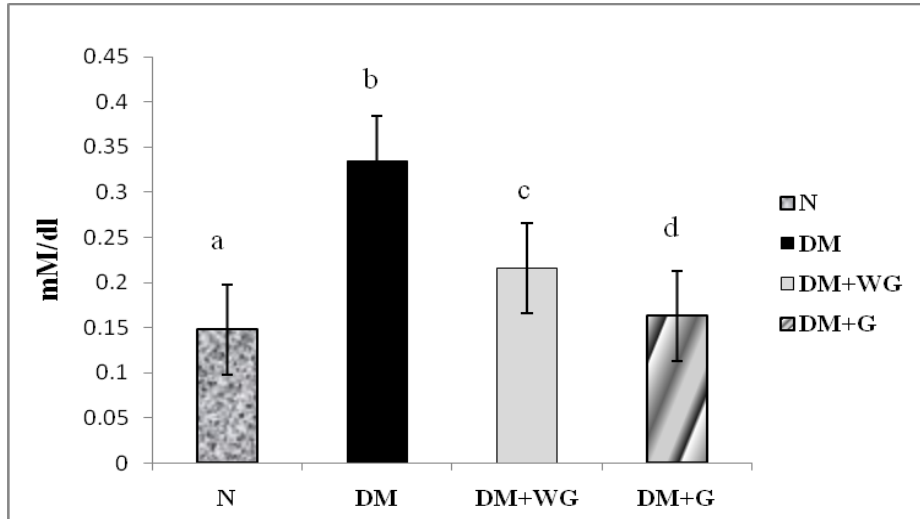


Fig. 2: Levels of thiobarbituric acid reactive substances in plasma

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

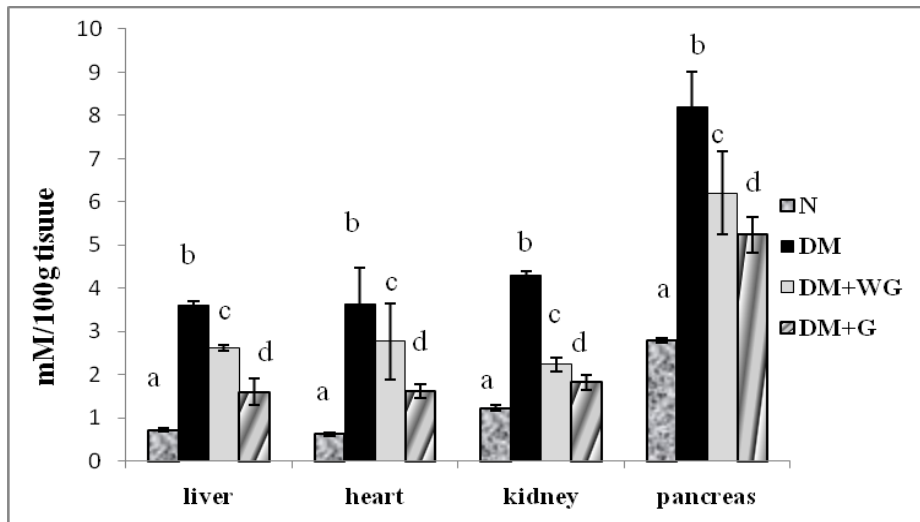


Fig. 3: Levels of Thiobarbituric acid reactive substances in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

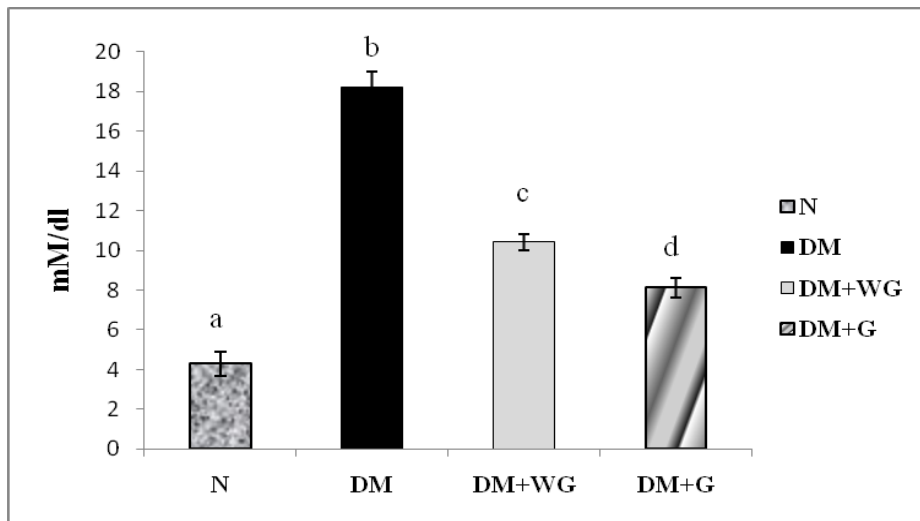


Fig. 4: Levels of lipid hydroperoxides in plasma

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

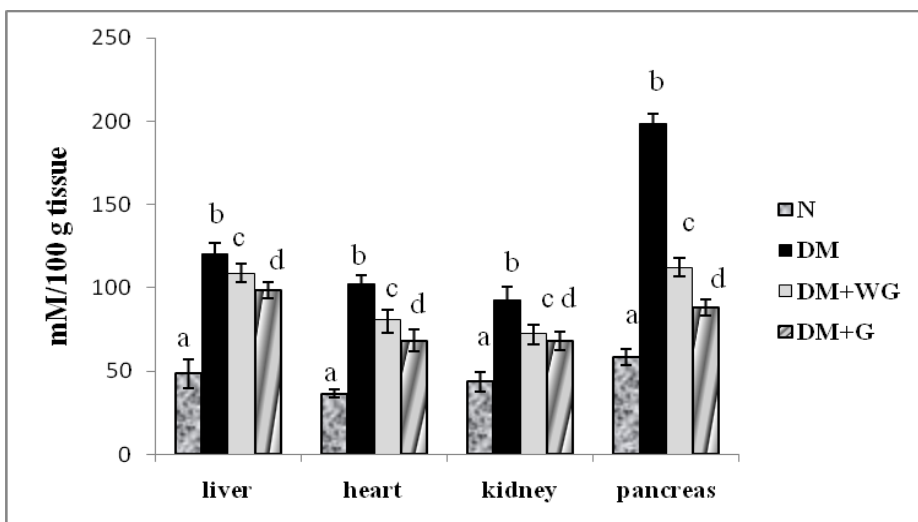


Fig. 5: Levels of lipid hydroperoxides in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05

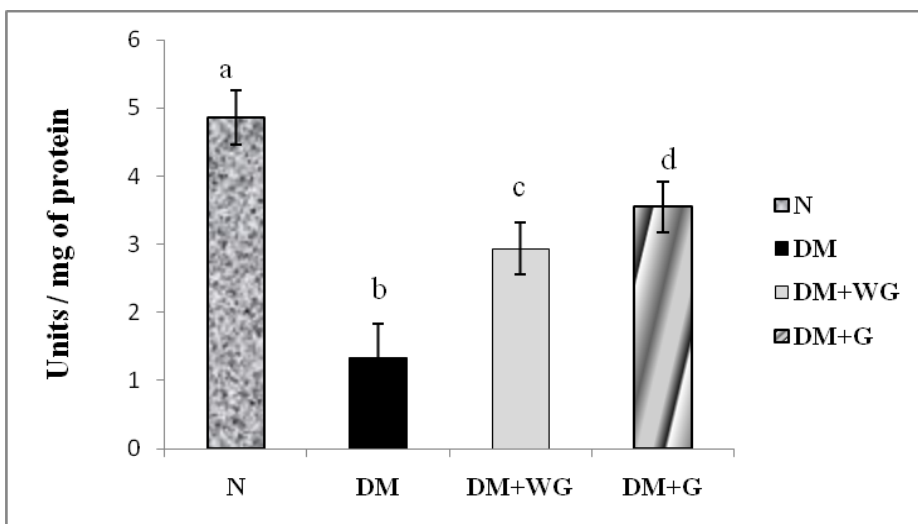


Fig. 6: Catalase activity in haemolysate

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars a common superscript do not differ significantly at P≤0.05
Units - μmoles of H₂O₂ liberated/ minute.

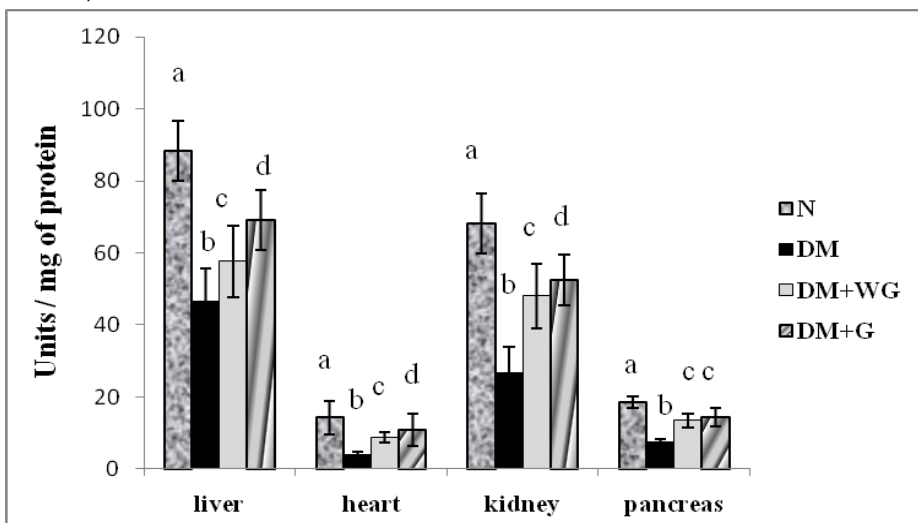


Fig. 7: Catalase activity in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05
Units - μmoles of H₂O₂ liberated/ minute.

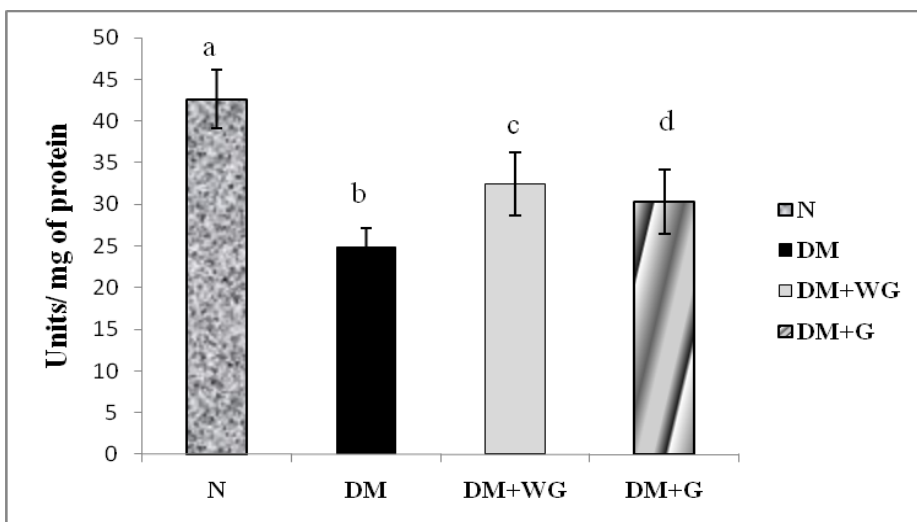


Fig. 8: Glutathione peroxidase activities in haemolysate

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05 Units- mmoles of glutathione liberated / minute.

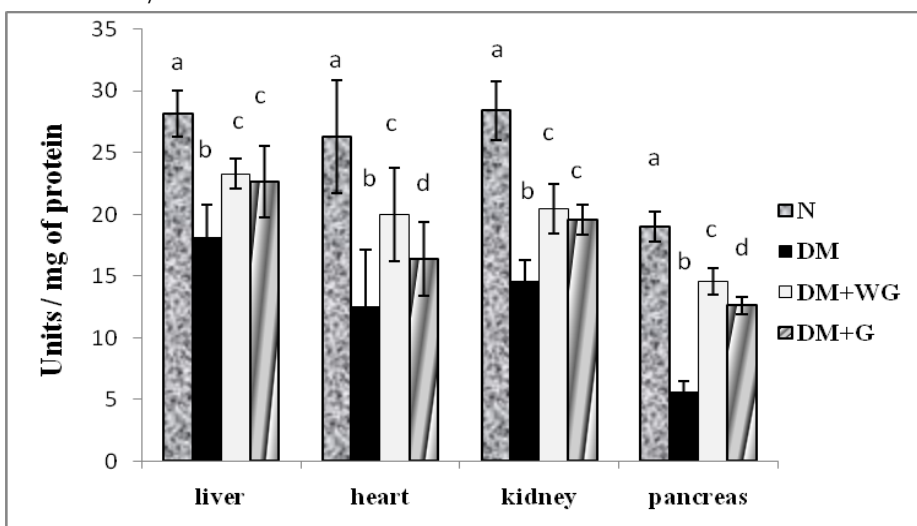


Fig. 9: Glutathione peroxidase activity in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05 Units- mmoles of glutathione liberated / minute

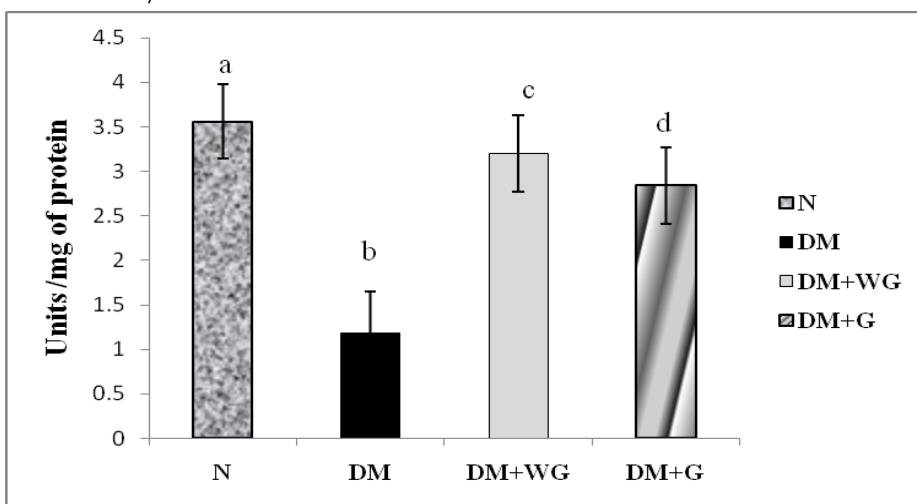


Fig. 10: Superoxide dismutase activity in haemolysate

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05 Units- enzyme reaction which gives 50% inhibition of NBT reduction / minute

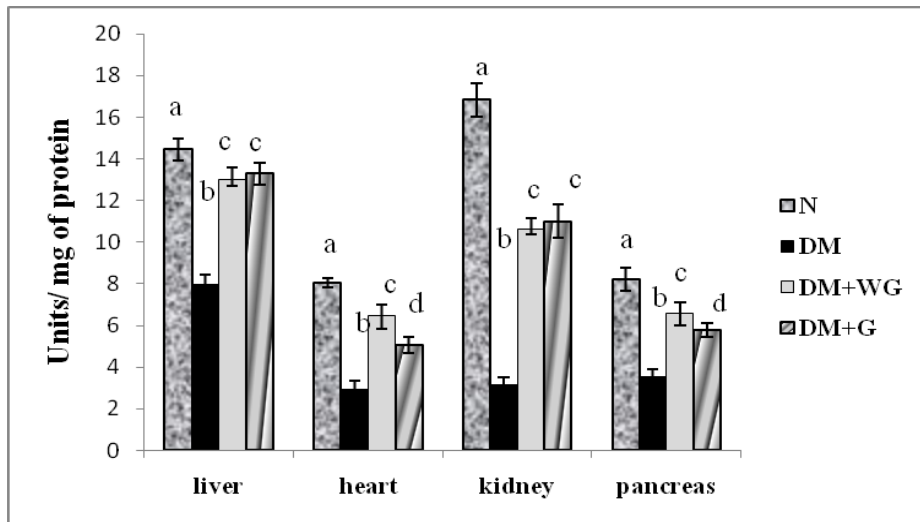


Fig. 11: Superoxide dismutase activity in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05
Units- enzyme reaction which gives 50% inhibition of NBT reduction / minute

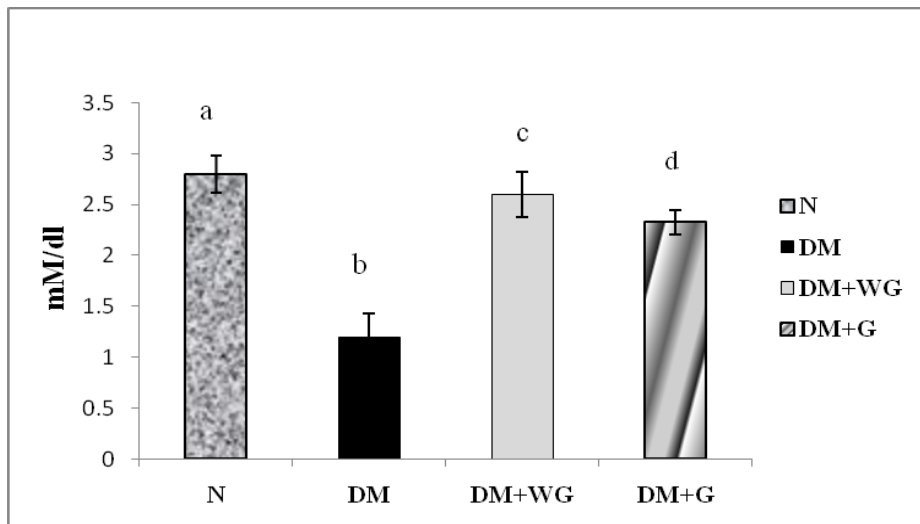


Fig. 12: Levels of vitamin C in plasma

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05

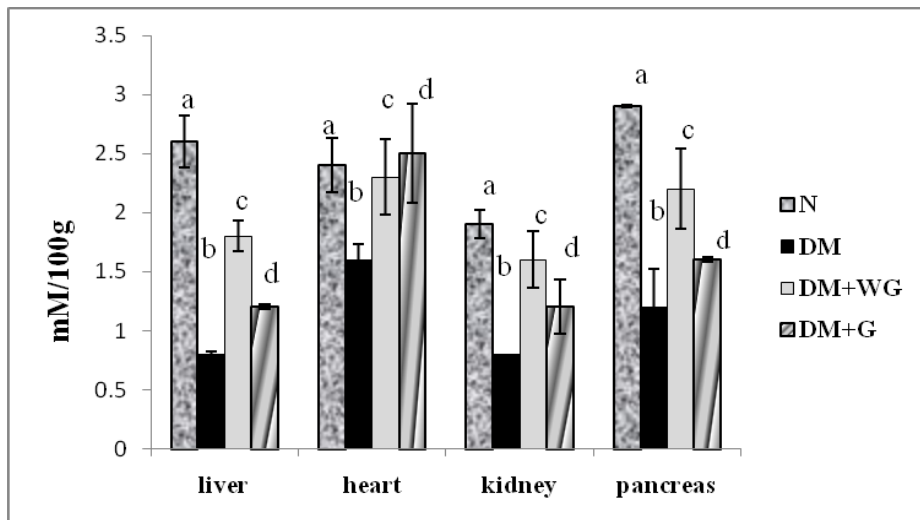


Fig. 13: Levels of vitamin C in tissues

Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at P≤0.05

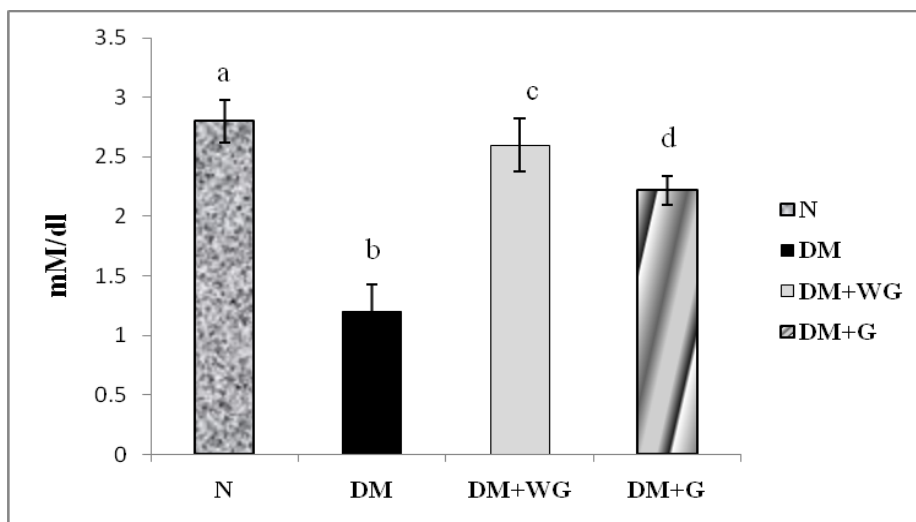


Fig.14: Levels of reduced glutathione in plasma

Values are Mean \pm SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

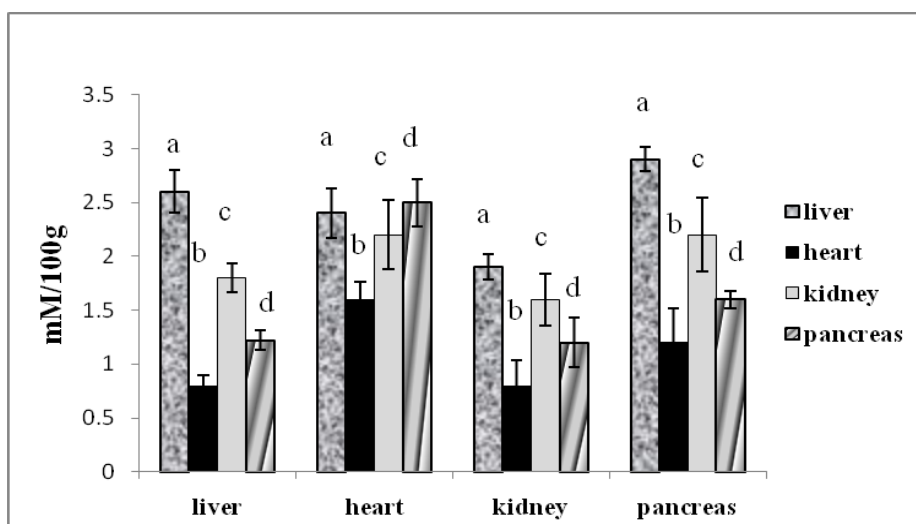


Fig. 15: Levels of reduced glutathione in tissues

Values are Mean \pm SD from 6 rats in each group. ANOVA followed by Tukey's test. Bars sharing a common superscript do not differ significantly at $P \leq 0.05$

DISCUSSION

In diabetes, oxidative stress occur due to increased production of ROS such as superoxide radicals (O_2^-), hydroxide radicals (OH^\cdot), hydrogen peroxide (H_2O_2), which are found to be involved in the destruction of β cell of pancreas, thus decrease the insulin level and increase blood glucose levels^{20,21}. Hyperglycaemia further generates more free radicals by non enzymatic glucose autoxidation and protein glycation⁴.

In our study the blood glucose was determined by estimating the HbA1c, which is directly correlated with blood glucose. HbA1c is produced due to non enzymatic addition of glucose at amino group in haemoglobin. Red blood cell has a life span of 120 days, so HbA1c becomes a good indicator for blood glucose level in preceding 2-3 months²². Wheatgrass treated rats showed significant decrease in plasma HbA1c levels, which means decrease in blood glucose levels.

Decrease in blood glucose levels with wheatgrass treatment, may be due to reduced oxidative stress on β cells of pancreas, which might have increased insulin level and its activity. Increased insulin in turn acts either by increasing glucose regulation or uptake by peripheral tissues². Decrease in glucose also prevents, further generation of free radicals and lipid peroxidation⁴.

Oxidative stress occurs due to imbalance between the production of free radicals and antioxidant defence mechanisms⁴. In our study both the factors, increased production of free radicals and decreased activity of antioxidant defence system, were found to be involved in causing oxidative stress in the diabetic rats. In diabetic conditions, oxidative stress produces many secondary complications and affects various organs⁴. Therefore various organ tissues like liver, heart, kidney and pancreas were also analysed for the oxidative stress status in our study.

High levels of ROS causes lipid peroxidation of cellular structure²³. Variety of long chain polyunsaturated fatty acid interacts with the free radicals and produces LH. This highly reactive and cytotoxic lipid radical, in turn generates more LH, and leads to structural damage²⁴. Malondialdehyde (MDA) is used as a common marker for the estimation of LH²⁵. In our study increased levels of TBARS and LH in diabetic rats were significantly decreased on wheatgrass treatment. This indicates that wheatgrass may act by reducing the generation of ROS, thus decreasing the lipid peroxidation and MDA production.

The biological harmful effects of free radicals are controlled by endogenous antioxidant defence mechanisms, which include enzymatic antioxidants such as SOD, CAT, GPx and non enzymatic

antioxidants such as vitamin C and GSH ²⁶. Persistent hyperglycaemia in DM also disturbs the endogenous antioxidant defence mechanism and prevents the free radical scavenging activity ²⁷. The levels of all these enzymatic and non enzymatic antioxidant were studied to confirm the positive effect of wheatgrass on oxidative status in diabetes.

SOD is one of the important enzymatic antioxidants involved in catalysing superoxide radical (O₂⁻) to hydrogen peroxide (H₂O₂) ²⁸. H₂O₂, a potent free radical is detoxified by another enzymatic antioxidant CAT along with GPx, which convert LH to non toxic alcohols ¹. Wheatgrass treated rats showed increased levels of enzymatic antioxidants, which scavenge the free radicals and prevent lipid peroxidation.

Oxidative stress also affects the levels of GSH, a non enzymatic antioxidant. GSH is involved in scavenging free radicals and plays an important role in maintaining the redox status of the cell. In oxidative stress condition GSH is oxidised to glutathione disulphide (GSSG) by the action of GPx, which is then again reduced to GSH by the enzyme glutathione reductase, with coupling reactions of NADPH to NADP ²⁹.

Vitamin C or ascorbic acid (AsA), another potent non enzymatic antioxidant, also plays an important role in detoxifying free radicals ³⁰. In DM, hyperglycaemia reduces the AsA absorption and oxidative stress, increases the oxidation of AsA to dehydroascorbic acid (DHAA) and decreases the regeneration of ascorbic acid from DHAA ³¹. Due to these effects the level of AsA decreases in DM. Our study showed similar results of decrease in level of AsA in diabetics rats, which reversed after wheatgrass treatment. Wheatgrass may act by preventing oxidation of AsA and by increasing insulin level and activity, which in turn increase absorption of AsA along with glucose ³², thus increase in AsA levels.

On the whole wheatgrass treatment showed an increase in antioxidant defence mechanism by increasing enzymatic and non enzymatic antioxidants. This could be due to its rich source of vitamin C and E, β carotene, ferulic acid, vanillic acid, and phenolic compounds including flavonoids, which are very potent antioxidants. Due to the presence of such potent antioxidants in wheatgrass, it might have contributed to the free radicals scavenging activity and hence improved the enzymatic and non enzymatic antioxidants level.

The present study showed the positive effect of wheatgrass on antioxidant status and glucose tolerance. Thus wheatgrass is found to be beneficial in controlling the oxidative stress in diabetes either by preventing ROS generation or by activating endogenous antioxidant defence mechanisms or both. Hence we conclude that, being a natural product, wheatgrass could be a very effective cure for diabetes.

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