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 reversed 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.
**Induction of diabetes**

Diabetes was induced in male albino Wistar rats 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 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, pH 7.4. 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. by using AGAPPE reagent kit. TBARS were estimated by thiobarbituric acid assay method of Niehaus and Samuelsson. The lipid hydroperoxide (LH) was estimated by the method of Jiang et al. The catalyse (CAT) activity was assayed by the method of Sinha. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar et al. Vitamin C was estimated by the method of Roe and Kuether. Reduced glutathione (GSH) was determined by the method of Ellman.

**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 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 ± 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. 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 $P \leq 0.05$.

Fig. 6: Catalase activity in haemolysate
Values are Mean ± SD from 6 rats in each group. ANOVA followed by Tukey’s test. Bars with a common superscript do not differ significantly at $P \leq 0.05$.
Units - μmoles of H$_2$O$_2$ 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 $P \leq 0.05$.
Units - μmoles of H$_2$O$_2$ 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
DISCUSSION

In diabetes, oxidative stress occurs due to increased production of ROS such as superoxide radicals (O2•−), hydroxide radicals (OH•), hydrogen peroxide (H2O2), which are found to be involved in the destruction of β-cell of pancreas, thus decrease the insulin level and increase blood glucose levels. Hyperglycaemia further generates more free radicals by non-enzymatic glucose autoxidation and protein glycation. 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 months. Wheatgrass treated rats showed significant decrease in plasma HbA1c levels, which means decrease in blood glucose levels.

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 tissues. Decrease in glucose also prevents, further generation of free radicals and lipid peroxidation by various organ tissues like liver, heart, kidney and pancreas were also analysed for the oxidative stress status in our study.

Oxidative stress occurs due to imbalance between the production of free radicals and antioxidant defense mechanisms. In our study both the factors, increased production of free radicals and decreased activity of antioxidant defense 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 organs. 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 structure. 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 damage. Malondialdehyde (MDA) is used as a common marker for the estimation of LH. 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 defense mechanisms, which include enzymatic antioxidants such as SOD, CAT, GPx and non-enzymatic
antioxidants such as vitamin C and GSH. Persistent hyperglycaemia in DM also disturbs the endogenous antioxidant defence mechanism and prevents the free radical scavenging activity. The levels of all these enzymatic and non enzymatic antioxidants 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 (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ is a potent free radical is detoxified by another enzymatic antioxidant CAT along with GPx, which convert LH to non toxic alcohols. 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.

Vitamin C or ascorbic acid (AsA), another potent non enzymatic antioxidant, also plays an important role in detoxifying free radicals. 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. 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, thus increase in AsA levels.

On the whole wheatgrass treatment showed an increase in antioxidative 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 antioxidative 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 antioxidative defence mechanisms or both. Hence we conclude that wheatgrass could be a very effective cure for diabetes.

REFERENCES


