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Effect of Wheatgrass on DNA Damage, Oxidative Stress Index and Histological Findings in Diabetic Rats

Efecto del Pasto de Trigo sobre el Daño del ADN, el Índice de Estrés Oxidativo y los Hallazgos Histológicos en Ratas Diabéticas

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SUMMARY: This study was aimed to search the effect of wheatgrass on the Total Antioxidan (TAS)-Oxidan Status (TOS) and DNA damage in rat with diabetes. The rats used in the study were randomly divided into 4 groups that each of has 10 rats: Control group; 1 ml single dose phosphate-citrate buffer injected i.p (pH: 4.5), Diabetes group; 45 mg/kg single dose streptozotocin injected i.p., Wheatgrass group; was given oral wheatgrass (10 ml/kg/day) for 6 weeks, Diabetes +Wheatgrass group; 45 mg/kg single dose streptozotocin injected i.p. and wheatgrass (10 ml/kg/day) was given by oral during 6 weeks. After the process of experiment during 6 weeks, blood sample and pancreas tissue were taken. The analysis were done of blood glucose levels, TAS, TOS levels by colorimetric kits; DNA damage by ELISA kits in serum. The pancreas tissues were examined histopathologically. In the group of Diabetes+Wheatgrass was determined that the levels of glucose levels ($p<0.001$),TOS ($p<0.05$) and OSI ($p<0.01$) statistically decreased and heal histopatolojical compared to diabetes group. In the group of Wheatgrass was determined that the levels of TAS $p<0.05$ statistically increased from other groups. The statistical significance were not found in the level of serum 8OHdG differences between the groups. The beta cells were seen to increase in the group receiving wheatgrass for therapeutic purposes.As a conclusion, it was determined that wheatgrass strengthened the anti-oxidant defense system and reduced the glucose level in diabetic rats.

KEY WORDS: Wheat Grass; Diabetes; DNA damage; TAS; Beta cell.

INDRODUCTION

Wheatgrass has important effects on human health as it contains most of the required nutrients for humans. It has become one of the important supplemental nutrients for a healthy life (Ashish *et al*., 2012). Wheatgrass contains high amounts of chlorophyll, vitamins, minerals, amino-acids and enzymes (Rana *et al*., 2011).

Diabetes mellitus (DM) is a chronic disease, which leads to carbohydrate, fat and protein metabolism disorders. Micro-vascular, macro-vascular and neuropathic complications may develop in the course of the disease (Akcay & Akarsu, 2000).

Many researchers have emphasized that this condition leads to macro- and micro-vascular complications of diabetes. Diabetes is an increased oxidative stress status beside metabolic disorders. Increased free radicals may lead to loss of membrane integrity and genetic mutations. The organism possesses anti-oxidant defense systems. The effects of free radicals may be overcome with administration of exogenous anti-oxidants in diabetes (Vincent *et al*., 2004).

Preservation of DNA integrity and avoidance of developing injury are of great importance for survival. DNA damage may develop in various diseases. 8-OhdG is an indicator of DNA oxidation (Piconi *et al*., 2003). Streptozotocin (STZ) and alloxan are widely used to induce experimental DM in test animals. STZ is preferred more in laboratory studies as it has more specific beta-cell cytotoxicity (Gorogawa *et al*., 2002).

The present study was planned to investigate the effect that wheatgrass supplementation has on DNA damage, TAS, TOS and pancreatic tissue in rats with experimentally induced diabetes.

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MATERIAL AND METHOD

In this study were used total of 40 Wistar albino rats weighing between 200 and 250 g in a climate-controlled animal care facility, with a 12 h light/dark cycle. They were divided into 4 groups each containing 10 rats. It was injected ip 0,1 ml a single dose citrate buffer to Control (A) group. Diabetes (C) group and diabetes +Wheatgrass (D)groups a single dose of a freshly prepared solution (45 mg/kg of body weight to 0.1 M cold citrate buffer, pH 4.5) of STZ were injected i.p. in the rats. After 48 h blood glucose levels were determined. The animals were considered diabetic if blood glucose values were > 200 mg/dl the animals were used for diabetic groups. Wheat Grass (B) and Wheatgrass + Diabetes (D) groups were given Wheatgrass extract (10 ml/kg/day,) with orally for 6 weeks.

At the end of six weeks, blood and pancreas samples were collected.Blood samples were taken from hearts with sterile injector and placed into tubes with coagulated. Blood glucose levels were determined (eB sensor).The oxidative DNA damage 8- hydroxy-2'-deoxyguanosine (8OHdG) levels were analysis by ELISA kit (Enzo life sciences, USA); TAS, TOS values using a novel automated measurement by colorimetric kits (Rel Assay, Türkiye) in serum. The oxidative stress index (OSI) was calculated with the ratio of TOS to TAS.

Pancreas tissues obtained from all animals in all groups were fixed in 10 % neutral buffered formalin for 24-48 h. They were blocked in paraffin after routine histological tissue follow-up. Tissue sections of 5 μm thickness were placed on polysin-coated slides. Immune-histochemical staining was performed in accordance with the ABC method (Kanter *et al*., 2006). The sections were stored in 3 % H_2O_2 for 30 min in order to inhibit peroxidase activity following de-paraffinization and washed with distilled water. Protein block was made in normal goat serum prepared with PBS in 1⁄4 dilution in order to prevent non-specific binding. The sections were stored in monoclonal insulin protein (18-0066,Zymed Sanancisco,CA) in 1/40 concentration for one night (4 ºC). The sections were washed with PBS for 30 min. Biotin anti-mouse IgG was kept for 30x2 min and then washed with PBSD. AEC (Aminoethylcarbazole Substrate Kit, Zymed Labaratouries) was kept for 10 min and washed with tap water. Contrasting staining was carried out with hematoxylin-eosin and sealed with water-based adhesive. Required photos were obtained with Leica ICC 50.

Randomly selected 20 pancreatic islands from each group were examined and beta cells were scored semi-quantitatively according to anti-insulin staining, staining severity and density as (+) weak, (++) moderate, (+++) strong, and (++++) very strong (Table II).

The Kruskall-Wallis test was used for comparison of the groups with regard to characteristics. A p level of 5 % was accepted as statistically significant and calculations were made using SPSS.

RESULTS

The serum TAS, TOS, OSI, 80HdG and glucose values of all groups have been displayed in Table I.

While the serum TOS values ($p<0.05$) and the OSI values ($p<0.001$) of the rats in diabetes group were found to be statistically significantly higher than those of the other groups, these values were found to be close to the control group in the rats in the Diabetes + wheatgrass group. However, the TAS values were determined to be higher in the wheatgrass group ($p<0.05$). The serum 80HdG level was higher in Diabetes group; however, the difference was statistically insignificant. While the serum glucose value was very high in the Diabetes group, it was seen to decrease in the wheatgrass + Diabetes group (Table I).

Taking the control group into consideration, the beta cells in the endocrine islands were examined immune-histochemically (Fig. 1A). The beta cells in the wheatgrass group were found to have a distribution close to that of the control group (Fig. 1B). The beta cells were seen to decrease in the diabetic group according to blood glucose levels (Fig. 1C). The beta cells were seen to increase in the group receiving wheatgrass for therapeutic purposes (Fig. 1D).

Table I. The values of serum glucose TAS-TOS-OSI 8OHdG in all groups.

Parameters	Control	Wheatgrass	Diabetes	Wheatgrass+Diabetes	P value
8OHdG	12.47±5.45	15.22±3.51	16.45±3.65	14.29±4.23	≥0.5
TAS	0.55±0.26^{b}	0.98±0.35^{a}	0.47±0.43^{b}	0.68±0.19^{a}	≤0.5
TOS	7.25±1.46^{b}	5.41±1.24^{b}	9.11±1.82^{a}	6.15±1.35^{b}	≤0.5
OS_	1.31±0.55^{b}	0.55±0.36^{c}	1.93±0.41^{a}	1.04±0.70^{b}	≤0.01
Glucose	81.15±4.26^{c}	87.34±9.46^{c}	475.20±72.36^{a}	250.00±48.25^{b}	≤0.001

a,b,c: in the same line values with different letters show statistically significant differences.

Table II. Semi-quantitative assessment of staining grades according to the groups.

Konrol group	Wheatgrass group	Diabetes group	Diabetes+Wheatgrass Group
(++++)	(++)/(+++)	(-)/(+)	(+)/(++)

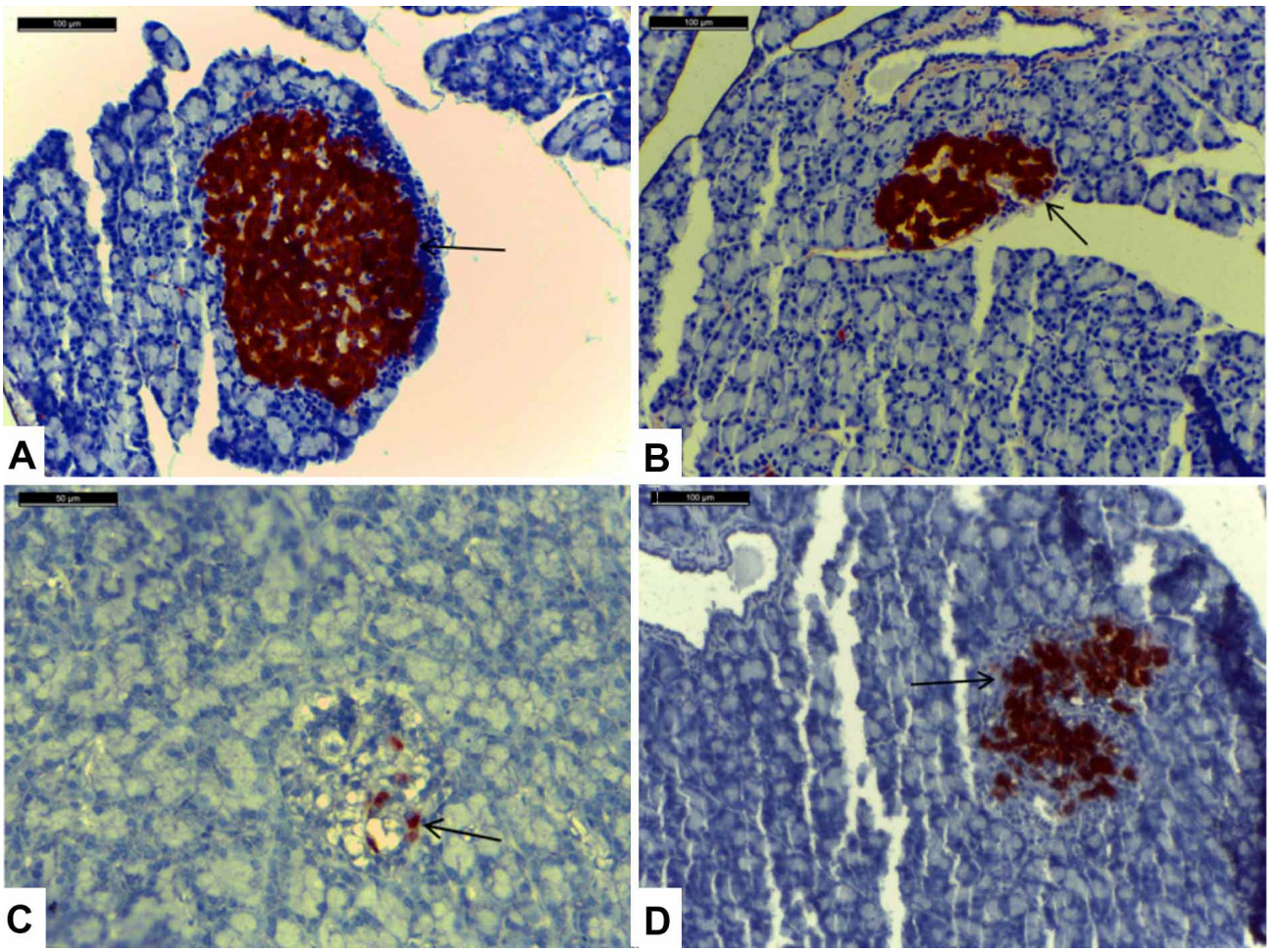

Fig.1. (A) Control, (B) Wheatgras, (C) Diabetes, (D) Diabetes+Wheatgrass (A, B, D Bar 100μm, C, Bar 50 μm).

DISCUSSION

Wheatgrass is recommended as supportive therapy in various diseases such as digestive problems, asthma, hypertension, thalassemia, diabetes, Parkinson's, ulcer, bronchitis, eczema and cancer (Singh *et al*., 2012; Ashish *et al*.; Durairaj *et al*., 2014). It is believed to strengthen the immune system and prolong the lifespan of cancer patients by suppressing the invasion of cancer cells (Durairaj *et al*.). Durairaj *et al*. found that wheatgrass extract was rich in alkaloids, mainly gamma sitosterol, squalene, cariofilene and amyrin, flavonoids, saponine, tannins, coumarin, phenols, alkaloids and terpenoids. Chlorophyll, which is one of the active components of wheatgrass extract, inhibits the metabolic activation of carcinogens (Kulkarni *et al*., 2006).

Researches have revealed that anti-oxidants prevent cell damage by neutralizing free radicals. Free radicals are suggested to be the main cause of atherosclerosis and cardiac diseases. Free radicals also affect the nucleic acids of free radicals. Excessive cell death leads to early aging and form cell sequences that lead to cancer and other diseases (Floyd, 1990) Free radical formation, which initiates some life-threatening chronic diseases as a result of oxidation, has increased the importance of anti-oxidant

compounds that work against them. Cytotoxic aldehydes formed as a result of lipid peroxidation lead to injury through binding to proteins and DNA (Simic, 1994).

Previous studies have reported decreased glutathione reductase, serum glutathione peroxidase and catalase activity in DM (Komosinska-Vassev *et al*., 2005).

Kutlu *et al*. (2005) reported that vitamin E and C supplementation reduced lipid peroxidation in diabetic rats. Another study reported that 800 IU/day vitamin E supplementation given for one month reduced all lipids and lipid fractions, the fasting plasma glucose and the fructosamine levels, TBARS levels, and increased insulin and C peptide levels, glutathione peroxidase and SOD activities; vitamin E is particularly beneficial in the prevention and treatment of type 2 Diabetes (Gökkusu *et al*., 2001).

Many methods and medications are currently used for management of Diabetes. They act through different mechanisms by increasing the insulin production or reducing the glucose production, minimizing the insulin resistance in related cell receptors and thereby aim at balancing the glucose homeostasis (Baxter, 2008).

Use of anti-oxidant substances or anti-diabetics with anti-oxidant properties is recommended to overcome oxidative stress (Memisogullari, 2005).

In their study investigating the anti-proliferative, apoptotic ad anti-oxidant activities of wheatgrass extract in CML (K562) cell series, Aydos *et al*. (2011) determined that the most apoptotic and anti-proliferative effect was seen in cell sequences treated with the water extract of wheatgrass at the 48th hour and that the MDA level, CAT and SOD activities increased ($p<0.001$). Wheatgrass extract has anti-oxidant activity and has been reported to inhibit the proliferation of leukemia cells and induce apoptosis.

Karadag *et al*. (2007) investigated the effects of wheatgrass on CML cells. They stated that apoptosis decreased due to the decrease in ROS in the cells treated with wheatgrass. Wheatgrass was determined to increase anti-oxidant enzymes, SOD and CAT.

Similar to with many previous studies, we determined higher serum TOS and OSI levels in rats in the Diabetes group. These values were found to be close to those of the control group in rats in the Diabetes + wheatgrass group. However, the TAS values in the wheatgrass group were found to be higher than those in the other groups.

Many researchers have shown that oxidative stress leads to DNA damage as a result of experimental diabetes and in vitro studies. As an indicator of oxidative damage, 8OHdG increases in tissues and body fluids in diabetic subjects (Park *et al*., 2001; Andican & Burçak, 2005). Another study has revealed that lycopene, which has anti-oxidant effects, shows a DNA protective effect through inhibiting comet formation and reducing the 8OHdG level (Huang & Hu, 2011). On the other hand, wheatgrass is also effective in suppression of superoxide radicals, which cause many diseases beside the oxidative DNA-damage-protective effects (Falcioni *et al*., 2002).

In the present study, the serum 8OHdG level was higher in the Diabetes group compared to the other groups; however, the difference was not statistically significant.

Drinking wheatgrass water was reported to reduce the need for blood-producing drugs and to help them reach healthy blood values (Bar-Sela *et al*., 2007). Another study reported that the use of wheatgrass water significantly reduced the general disease activity and rectal hemorrhage severity in the treatment of active distal ulcerative colitis (Ben-Arye *et al*., 2002).

The fresh juice of triticum aestivum leaves administered via the per-oral route showed a significant hypolipidemic effect in normal mice (Kothari *et al*., 2008). Mohan *et al*. (2013) reported that wheatgrass extract demonstrated significant anti-hyperglycemic, hypo-lipidemic and anti-oxidant activity in diabetic rats induced with streptozotocin. Rana *et al*. reported that wheatgrass was effective in removing toxins, balancing plasma glucose, preventing tooth decay, maintaining healthy hair and reducing high blood pressure. In the present study, while the serum glucose level was very high in the Diabetes group, it decreased in the wheatgrass + Diabetes group, consistent with these studies.

STZ-induced degeneration in pancreatic beta-cells was determined immune-histochemically. It was concluded that wheatgrass increased the free radical amount and could protect the beta cells against beta cell damage in the wheatgrass + Diabetes group. This result is similar to the study of Karaca *et al*. (2010) investigating the protective effect of ginseng, green tea and green tea + ginseng in STZ-induced diabetic rats.

CONCLUSION

Diabetes is a metabolic disease, which has a gradually increasing prevalence. Many mechanisms have been suggested in the pathogenesis of diabetes and its

complications. Among these, the most accepted mechanism is regarding the free radicals. Diabetes-induced oxidative stress may be overcome by increasing the anti-oxidant capacity. The number of studies on anti-oxidants and the effects on diabetes is increasing day by day. There are studies on the protective and therapeutic effect of wheatgrass in cancer; however, there is a limited number of studies regarding the effects of wheatgrass on diabetes. In this study, we determined that wheatgrass strengthened the anti-oxidant defense system and reduced the glucose level in diabetic rats. Wheatgrass may contribute to diabetes management through reduction in oxidative stress and prevention of diabetic complications. Therefore, we recommend wheatgrass to overcome oxidative stress in diabetes. We suggest that wheatgrass would also be beneficial due to its anti-hyperglycemic effect.

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RESUMEN: El objetivo de este estudio fue buscar el efecto del pasto de trigo sobre el estado total de antioxidantes (TAS) -Oxidan Status (TOS) y el daño del ADN en ratas con diabetes. Las ratas analizadas en el estudio se dividieron aleatoriamente en 4 grupos de 10 ejemplares cada uno: grupo control; 1 ml de tampón fosfato-citrato de dosis única inyectado i.p. (pH: 4,5)., Grupo diabetes; 45 mg / kg de estreptozotocina en dosis única inyectada i.p., grupo pasto de trigo; se administró pasto de trigo oral (10 ml / kg / día) durante 6 semanas, grupo diabetes + pasto de trigo; 45 mg / kg de estreptozotocina en dosis única inyectada i.p. y pasto de trigo (10 ml / kg / día) por vía oral durante 6 semanas. Después del proceso experimental durante 6 semanas, se tomaron muestras de sangre y tejido de páncreas. Se midieron los niveles de glucosa en sangre, TAS, y TOS mediante kits colorimétricos; El daño al ADN fue realizado por kits de ELISA en suero. Los tejidos del páncreas se examinaron histopatológicamente. En el grupo de diabetes + pasto de trigo se determinó que los niveles de glucosa ($p < 0,001$), TOS ($p < 0,05$) y OSI ($p < 0,01$) disminuyeron estadísticamente y curaron histopatológicamente en comparación con el grupo de diabetes. En el grupo de pasto de trigo se determinó que los niveles de TAS $p < 0,05$ se incrementaron estadísticamente con respecto a otros grupos. No fue estadísticamente significativo el nivel de las diferencias séricas de 8OHdG entre los grupos. Se observó que las células beta aumentaron en el grupo que recibió pasto de trigo con fines terapéuticos. Como conclusión, se determinó que el pasto de trigo fortaleció el sistema de defensa antioxidante y redujo el nivel de glucosa en las ratas diabéticas.

PALABRAS CLAVE: Pasto de trigo; Diabetes; Daño en el ADN; TAS; Célula beta.

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