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Beneficial effect of wheatgrass juice on some biochemical parameters in Type 2 diabetic subjects with reduced lymphocytes count.

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ABSTRACT

Wheatgrass juice (*Triticumaestivum* Linn) has been known for its high nutritional and healing properties. It is a rich source of essential amino acids, minerals, enzymes, vitamins and chlorophyll. Its chlorophyll content is of about 70% so it is referred as Green blood. The present study aimed to assess the health benefits of Wheatgrass juice (WGJ) on type 2 diabetic subjects with low lymphocytes count. Sixteen males type 2 diabetic subjects were selected in the study. They were asked to consume 150 ml of WGJ daily before breakfast for a period of 21 days. Results showed a significant decrease in the fasting and the postprandial plasma glucose level. Blood hemoglobin level and RBCs count were significantly increased. Total leukocytes count was decreased with significant increase in lymphocytes count. C-reactive protein level was significantly reduced. Despite immunoglobulin M (IgM) was significantly elevated; immunoglobulin G (IgG) was significantly reduced. Intervention with WGJ significantly increased platelets count and reduce prothrombin time. Plasma fibrinogen level showed significant reduction. No significant decrease was observed in plasma level of direct bilirubin and ALT activity; however, AST activity and plasma level of total bilirubin showed significant decrease.WGJ intervention did not affect the concentration of plasma creatinine and uric acid, while plasma level of urea was significantly decreased. In conclusion, our data suggest wheatgrass juice possess beneficial effect on some hematological, biochemical parameters, immune and inflammatory markers of type 2 diabetic subjects with reduced lymphocytes count.

Keywords: Wheatgrass juice, type 2 diabetes, plasma glucose, lymphocytes, anti-inflammatory, immune-stimulant effect.

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INTRODUCTION

Diabetes mellitus (DM) is a leading cause for morbidity and mortality in the world. Oxidative stress in diabetic patients induced the development of macro and micro-vascular complications causing the incidence of atherosclerosis, kidney diseases and neuropathy [1]. Patients with DM are more prone to infections due to lowered immune response [2].

Triticumaestivum Linn. commonly called wheat grass, belongs to the family Poaceae (Gramineae). It is a powerful health food supplement that is a rich source of protein, supplying all the essential amino acids primarily alanine, glutamic acid, serine, arginine and aspartic acid, to the body [3]. It is richin vitamins (A, C, E, K and B complex),minerals (sodium, potassium, calcium, magnesium and iron), active enzymes (protease, amylase, lipase, cytochrome oxidase, transhydrogenase and superoxide dismutase) and chlorophyll[4]. Wheatgrass juice stimulates metabolism, restores alkalinity to the blood as its abundance of alkaline minerals help to reduce over acidity in the blood [5].

Wheatgrass juice is called the green blood. Its most outstanding feature is its high content of chlorophyll at about 70%. Chlorophyll and hemoglobin both are structurally very similar. The pH value of blood and wheat grass juice is about 7; therefore it is quickly absorbed in the blood and replenishes the body with electrolytes that benefit the body. Chlorophyll in wheatgrass is a detoxification agent, builds red blood cells and helps to restore healthy cells growth. It has the ability to neutralize toxins in the body, cleanses the lymphatic system and oxygenates the blood [6].

Wheatgrass has been valued for its high nutritional properties and its abundance in phytochemical constituents including alkaloids, tannins, saponins and phytosterols [7]. It has been also shown that wheatgrass juice had a potent antioxidant activity due to its high content of bioflavonoids and polyphenols [8], which makes it one of the best functional foods that can be used as an excellent adjuvant in therapeutic regimens. It was shown that wheatgrass juice has an anticancer, immunomodulatory, anti-inflammatory and antibacterial activities [9].
The purpose of the present study was to investigate the beneficial effect of wheatgrass juice consumption onsome hematological, biochemical parameters, immune and inflammatory markersof type 2 diabetic patients with poorly controlled blood glucose (HbA_1c level > 8%) with reduced lymphocytes count.

MATERIALS AND METHODS

1. Growing and preparation of wheatgrass juice

The process of wheat grass cultivation consists of two stages: Germination of wheat seeds and cultivation of wheat grass as per the standard procedure described below [10].

A. Germination of wheat seeds

Whole wheat seeds (Triticumaestivum L) used in this study were purchased from local market and washed properly with running tap water to remove dust and dead seeds. Four kilogramsof wheat seeds were soaked overnight for 12 hours to tender the wheat seeds. Water was then strained and the soaked seeds were placed in wet cotton cloth and well covered for a period of 12 hours. Seeds were washed twice a day to prevent mold growth and water was sprinkled over the cotton cloth during the germination period.

B. Cultivation of wheat grass

After 12 hours of germination, the germinated wheat seeds were spread over soil in 12 plastic trays (20×40×10 cm) and further covered with soil and placed in a shady place (indirect sunlight) for growth of grass. Small quantities of water were sprinkled daily over soil to moist the roots. On the fifth day, shoots of wheat seeds started to grow to 30 cm. Around the eighth or ninth day, the wheatgrass reached a length of 17 to 19 cm (plate 1), then harvested right before it splits. At this stage, wheatgrass reach its nutritional peak [11]. Fifty five grams of wheat seeds were required to yield a 100g of wheatgrass. Each tray was cultivated with 330 g seeds and gave a yield of 600 g of grass. Trays were similarly planted at one-day interval to harvest a continuous supply of fresh grass of the same length. On the eighth day, wheatgrass was harvested and cut with scissors ½-1 cm above the root (plate 2).

C. Preparation of wheatgrass juice

Two hundred grams of harvested fresh grass were cut into small pieces, washed and placed in a strainer (plate 3). The juice was then prepared by mincing the chopped grass in the mincer (plate 4A). Other methods could also be used by crushing the grass in an electric juicer or mixer but they yield less amount of juice. The residue was then placed in a stainless steel strainer and squeezed to get the juice out of it (plate 4B).One hundred and fifty ml of fresh wheatgrass juice was obtained and consumed by the subjects without any additives.

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Plate 1: Cultivated wheatgrass in trays

Plate 2: Harvested wheatgrass by cutting above the root by ½ to 1 cm. before mincing

Plate 3: Wheatgrass after washing shoots in a strainer

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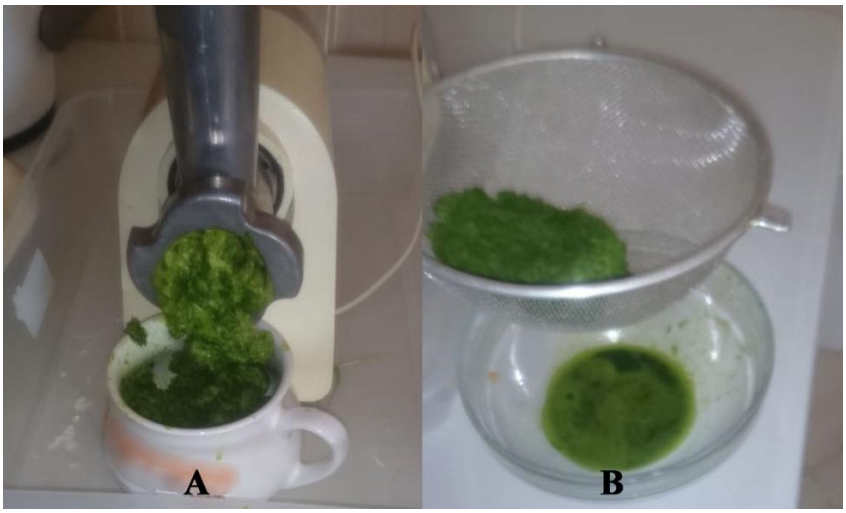

Plate 4: Preparation of wheatgrass juice
A: Mincing; B: Extraction

2. Subjects

Sixteen type 2 diabetic males subjects aged 45 - 55 years anda mean BMI of 35.22 ± 1.21 kg/m² were enrolled in the study for a 21 days trial. Diabetic duration was from 10-15 years. The mean initial baseline fasting and postprandial plasma glucose level of the participants was 184±6.04 mg/dL and 200±6.59 mg/dL. All participants had HbA_1c level > 8% (poorly controlled diabetes), reduced lymphocytes level (mean=12± 0.57) and high C-reactive protein (mean= 11.3± 0.38). The volunteers were enrolled for this study at the National Research Centre (NRC), Cairo, Egypt, after a written informed consent was obtained from each of them. All subjects were asked to consume 150 ml of wheatgrass juice daily before breakfast on an empty stomach in the morning. It is recommended that two glasses of water (600 ml) should be consumed with the juice to reach its maximum nutritional benefits [12]. Subjects were also asked to record their daily diet and physical activity and not to change their drug regimen, daily diet and physical activity during the study period.

A clinical examination was conducted on all subjects at the beginning of the study. All subjects answered a standard questionnaire that gave information about their residential address, age, sex, smoking history, medical history including current drug treatment and how frequent they get viral or bacterial infections. Patients with type 1 diabetes mellitus, severe infectious disease, severe anemia, excessive smoking and kidney disease were excluded. Fasting and postprandial plasma glucose levels were monitored for three days before and after intervention of the wheatgrass juice and the mean values were recorded.

Blood sampling and biochemical measurements

Fasting blood samples were drawn to examine various biochemical measurements at baseline and after 21 days of the intervention period.Part of the blood samples were collected in tubes containing EDTA for quantitative colorimetric determination of glycosylated hemoglobin (HbA1c) according to Niederau and Reinauer [13]. Blood hemoglobin was determined according to Van kampen and Zijlstra [14]. Two ml of venous blood were drawn into EDTA for complete blood count (CBC). Plasma was separated for the determination of fasting and postprandial plasma glucose by using oxidase peroxidase method as described by Trinder [15]. For liver function tests, plasma aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to the method of Reitman and Frankel [16], total and direct bilirubin were determined by the method of Schmidt and Eisenburg[17]. Plasma creatinine [18], uric acid [19] and urea [20] were determined to monitor kidney function. C-reactive protein (a marker of inflammation) was determined by the method of Saxtad*et al.* [21]. Immunoglobulin M (IgM) and immunoglobulin G (IgG) were assessed according to the method of Macini*et al.* [22]. Plasma fibrinogen (a marker of thrombosis and inflammation) was determined by the method of Berne [23]. Prothrombin time was determined according to Yang *et al.* [24].

Statistical analysis

The data are presented as means ± SEM. Statistical Package for the Social Sciences SPSS software for windows (SPSS Inc., Chicago, IL, version 17.0) was used for the statistical analysis. Paired *t*-test was used to compare data from before and after the study intervention. *P*-Value <0.05 indicated a statistically significant difference for all tests.

RESULTS

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Mean fasting and postprandial plasma glucose levels were highly significant reducedafter intervention of WGJ (153±6.82 mg/dL and 169± mg/dL respectively) (P<0.01) (Fig.1).

After WGJ intervention, blood hemoglobin and RBCs significantly increased (P<0.05) in diabetic subjects compared to the same subjects before intervention (Fig.2). There was no significant reduction in total leukocytes count (P>0.05); lymphocytes count was highly significantly elevated (P<0.01); CRP level was highly significant reduced (P<0.01); Despite IgM was significantly elevated, IgG was significantly reduced (P>0.05) (Table 1). Treatment with WGJ produced high significant increase in platelets count (P<0.01); prothrombin time was decreased; fibrinogen level showed a high significant reduction(P<0.01) (Table 2).No significant decrease was observed in plasma level of direct bilirubin, and ALT activity; however, AST activity and plasma level of total bilirubin showed significant decrease (P<0.05) after WGJ intervention (Table 3).WGJ intervention did not affect the concentration of plasma creatinine and uric acid, while it resulted a high significant decrease in plasma level of urea (P<0.01) (Table 4).

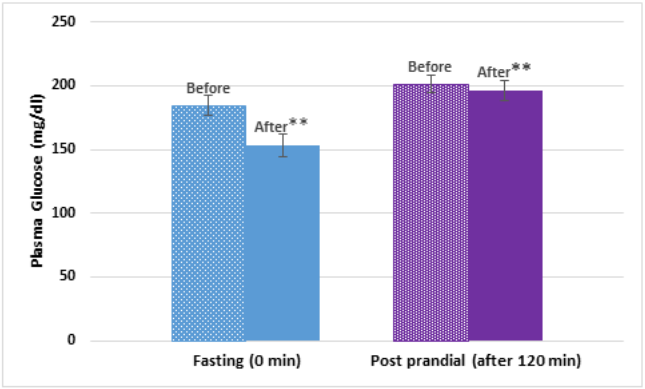

Fig.1. Fasting and postprandial plasma glucose levels of type 2 diabetic subjects before and after intervention of wheatgarass juice. Data are expressed as mean ± SEM, *p<0.05, **p<0.01.

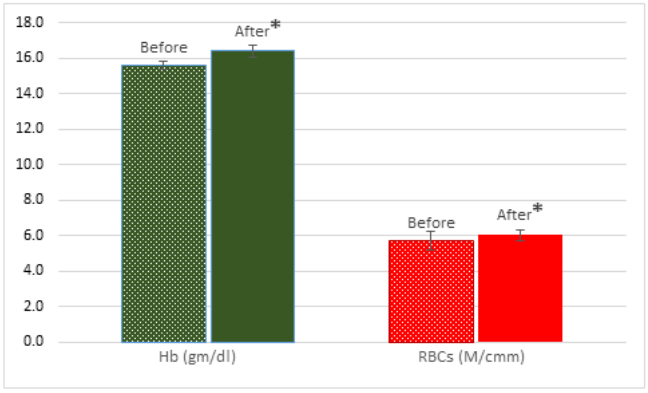

Fig.2. Hb level and RBCs count of type 2 diabetic subjects before and after intervention of wheatgarass juice. Data are expressed as mean ± SEM, *p<0.05.

Table 1: Effect of wheatgrass juice on total leukocytes count, lymphocytes, C-reactive protein, IgM and IgG in type 2 diabetic subjects

Blood and plasma parameters	Before intervention Mean ± SEM	After intervention Mean ± SEM
Leukocytes count (10^3/ µL)	10264 ± 348.51	9660 ± 328.00
Lymphocytes (%)	12 ± 0.57	20 ± 0.92 **
CRP (mg/L)	11.3 ± 0.38	3.9 ± 0.18 **
Ig M (mg/dL)	25 ± 0.85	29 ± 1.33 *
Ig G (mg/dL)	878 ± 29.83	765 ± 23.77 *

Values are expressed as Mean + S.E.M, *- significantly different from group before intervention (p< 0.05),
** - highly significantly different from group before intervention (p< 0.01).

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Table 2: Effect of wheatgrass juice on platelets count, prothrombin time and fibrinogen level in type 2 diabetic subjects

Blood and plasma parameters	Before intervention Mean ± SEM	After intervention Mean ± SEM
Platelets count (10^3/ µL)	187000 ± 596.65	208000 ± 667.00**
Prothrombin time (sec.)	13 ± 0.44	11.9 ± 0.46
Fibrinogen (mg/dL)	483 ± 16.40	395 ± 18.03**

Values are expressed as Mean + S.E.M, *- significantly different from group before intervention (p< 0.05),
** - highly significantly different from group before intervention (p< 0.01).

Table 3: Effect of wheatgrass juice on liver function in type 2 diabetic subjects

Plasma parameters	Before intervention Mean ± SEM	After intervention Mean ± SEM
T. Bilirubin (mg/dL)	0.66 ± 0.02	0.49 ± 0.02*
D. Bilirubin (mg/dL)	0.09 ± 0.01	0.09 ± 0.02
SGOT (AST) (U/L)	19.12 ± 0.65	16.11 ± 0.74*
SGPT (ALT) (U/L)	26.16 ± 0.89	24.17 ± 1.10

Values are expressed as Mean + S.E.M, *- significantly different from group before intervention (p< 0.05).

Table 4: Effect of wheatgrass juice on kidney function in type 2 diabetic subjects

Plasma parameters	Before intervention Mean ± SEM	After intervention Mean ± SEM
Creatinine (mg/dL)	0.81 ± 0.03	0.91 ± 0.04
Uric acid (mg/dL)	4.33 ± 0.15	4.33 ± 0.20
Urea (mg/dL)	34.21 ± 1.16	25.17 ± 1.15*

Values are expressed as Mean + S.E.M, *- significantly different from group before intervention (p< 0.05).

DISCUSSION

Wheatgrass juice has immense medicinal potential and is promoted as a healthy supplement for many years. In our research, beneficial effects of wheatgrass in type 2 diabetic subjects with reduced lymphocytes level have been studied.Many researches showed that lymphocyte levels were reduced due to hyperglycemia. Patients with diabetes mellitus have been suggested to have inadequate proliferationof lymphocytes [25].

In a previous study, the relation between the number of leukocytes and that of lymphocytes was shown to have an important role in determining the integrity of immune function in diabetic patients. The decrease in lymphocytes count might play an important role for the impaired immune function and high incidence of infections in uncontrolled diabetes [26]. The reduced number of lymphocytes in these patients was found to be due to the occurrence of apoptosis[27].

After 3 weeks of interventionwith fresh WGJ (150 ml/day), a significant elevation of hemoglobin, RBCs count, lymphocytes and platelets count was elicited, while there was a reduction in the total leukocytes count compared to the results before the intervention of the juice. These results are in accordance with a study made by Shah *et al.* [28] who found a significant increase in hemoglobin, RBCs count, total and differential WBC's and platelets count in thrombocytopenic rats supplemented with fresh juice of wheatgrass. The beneficial effect of WGJ to increaseRBCs count may be due to its induction of hemopoietic effect [29], as its chlorophyll content resembles the structure of hemoglobin in blood. The only difference between the two is that magnesium is the central metal atom in chlorophyll and it is iron in hemoglobin [30, 31]. It was reported that chlorophyll enhanced the formation of red blood cells and hemoglobin function in anemic animals[32] leading to elevating oxygen supply to all body cells.Furthermore, evidence suggests that natural chlorophyll derivatives are absorbed by human intestine [33].

In a previous study on uncontrolled hyperglycemic patients, levels of IgG and IgM tended to be of low level [34]. In the present study, consumption of WGJ produced a significant increase in lymphocytes count and IgM level in type 2 diabetic subjects. The IgM-type antibodies are predominantly produced by B1 cells (a sub-class of B-cell lymphocytes) that are responsible for the humoral immune response [35]. The ability of WGJ to increase lymphocytes count and IgM level indicates that it has a potent immuno-stimulatory effects which could be attributed to its content of chlorophyll (70%), polysaccharide (WG- PS), maltoheptaose derived from oligosaccharides (WG-PS3) [36], as well as chlorophyll independent properties of WGJ [37]. WGJ being rich in antioxidants is thought to offer a system of protection to the body that can regenerate the diseased cells and maintain the function of the immune system [38, 39].

Type 2 diabetic subjects under investigation were characterized by high levels of C- reactive protein (CRP) (11.3 mg/L) and a high normal value of total leukocytes counts (10,264/ cmm). Xu et al. [40] reported that CRP is a more effective biomarker for the metabolicstate andconditions that cause inflammation than WBCs, as the high CRP level increases the risk of developing heart disease**.** Furthermore, CRP is a sensitive marker of vascular inflammation and an

emerging risk factor for CVD [41]. The present study showed that one advantage of WGJ intake in patients with type 2 diabetes was a significant reduction in plasma levels of the inflammatory marker CRP. Several studies showed that WGJ is rich in natural antioxidants, such as polyphenols and bioflavonoids that have anti-inflammatory effects [42, 43] and have acute beneficial effects on endothelial function [44].This anti-inflammatory effect may provide a possible additional mechanism for the beneficial effect of WGJ in reducing the risk of atherosclerosis [45].

This study indicated that 21 days of WGJ consumption showed a significant reduction in fasting as well as postprandial blood glucose levels of 16.85% and 15.50% respectively in type 2 diabetic subjects. The antidiabetic effect of WGJ is due to its phytochemicals content [46]. It has been suggested that polyphenols, as the main component of WGJ might have hypoglycemic effects that may be related to increased peripheral tissues glucose uptake, reducedgut glucose absorption, increased secretion of insulin from pancreatic β-cells and suppressed glucose release from the liver [47, 48, 49]. Furthermore, the presence of chlorophyll, which is an active component in wheatgrass, acting as an anti-diabetic agent [50].

In the present study, type 2 diabetic subjects had high plasma fibrinogen level which increased their susceptibility to atherosclerosis and its complications [51]. Plasma fibrinogen levels were significantly associated with glycosylated hemoglobin in diabetics. In this study, wheatgrass juice consumption produced a significant reduction in plasma fibrinogen level of type 2 diabetic subjects. This could be explained by the inhibition of fibrinogen function by polyphenols content of WGJ as they bind to fibrinogen; this binding may strongly affects blood coagulation and platelet aggregation [52].

Previous data showed a significant increase in platelets count and a decrease in prothrombin time in rats with thrombocytopenia after treatment with WGJ [28]. In the present study, diabetic patients showed a lower prothrombin time and a higher platelets count after WGJ intervention. These results reveal the beneficial effect of WGJ for thrombocytopenia patients.

Many studies confirmed the significant hepatoprotective action of wheatgrass in CCl4 treated rats. Results showed that supplementation of wheatgrass extract brought down increased activity of ALT, AST, ALP and bilirubin and was shown to be highly effective in controlling hepatotoxicity induced by CCl4 [53, 54]. These results confirmed the improvement of liver function in type 2 diabetic subjects receiving WGJ in the present study. WGJ can therefore be used as a cleansing and detoxificant agent of liver [12]. In the present study, plasma urea was significantly reduced after consumption of WGJ. This result was confirmed by many studies reporting that WGJ possess a beneficial effect on kidney malfunction and inflammation [47].

CONCLUSION

Wheatgrass juice has a beneficial effect in alleviating specific health issues like diabetes and its complications. It reverses hyperglycemia, has an efficacy on hemoglobin level, RBCs and platelets count indicating therapeutic usefulness of wheatgrass in some types of anemia. In addition, it produces an anti-inflammatory and a potent immunomodulatory effects, therefore it could be used as an immune booster in diabetic subjects suffering from low immunity so they can be less susceptible to degenerative and infectious diseases. Therefore, wheatgrass juice should be a part of daily dietary intake in order to explore its maximum benefits.

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