Original Research

Barley microgreen incorporation in diet-controlled diabetes and counteracted aflatoxicosis in rats

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Impact statement

Chronic diseases like diabetes mellitus type 2 affect a wide range of populations worldwide. The causes behind the increased incidence of these diseases are still being investigated. Although it is still under study, exposure to environmental contaminants like aflatoxins in food was linked to an increased incidence of diabetes mellitus. Understanding the mechanistic effect of aflatoxicosis on the initiation and deterioration of diabetes would help the discovery of novel interventions. A healthy natural diet containing functional dietary ingredients is gaining wide popularity in the management of diabetes and lowering the use of synthetic medicine. Several plants at different stages of their growth are studied for possible incorporation in the diet of patients suffering from diabetes mellitus. Our study shows that barley microgreen alleviated the hyperglycemia and reduced oxidative stress in treated rats. Therefore, barley microgreen is a promising candidate in the management of diabetes with or without aflatoxicosis.

Abstract

Increased environmental pollution and unhealthy lifestyle are blamed for escalated chronic diseases. Exposure to aflatoxins was recently suggested to have a role in the increased incidence of type 2 diabetes mellitus. Diet modification and consumption of different functional food are now gaining attention, especially in diabetes management. This study investigates the effect of a diet containing barley microgreen against diabetes induced by streptozotocin with or without aflatoxin administration in rats. Barley microgreen was rich in 3′-Benzyloxy-5,6,7,4′-tetramethoxyflavone (48.8% of total) followed by 5β,7βH,10α-Eudesm-11-en-1α-ol (18.46%). Streptozotocin injection and/or aflatoxin administration significantly elevated glucose level, decreased insulin level, decreased β-cell function, deteriorated liver and kidney function parameters, and induced oxidative stress in the liver. Histopathology revealed irregular small-sized islets and decreased area % of insulin-positive beta cells in the pancreas, hepatic degeneration, nephropathy, and neuropathy in diabetic and/or aflatoxin administered rats compared to control. Barley microgreen diet fed to diabetic rats with or without aflatoxin alleviated all evaluated parameters. Barley microgreen diet also ameliorated the toxic effect of aflatoxin. In conclusion, exposure to aflatoxin aggravated diabetes and its complication. The incorporation of barley microgreen in the diet was able to control type 2 diabetes mellitus and the improved outcomes observed with barley microgreen treatments involved or occurred in conjunction with improved biomarkers of oxidative stress.

Keywords: Diabetes, aflatoxin, barley, histopathology, insulin, oxidative stress

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Introduction

Type 2 diabetes mellitus (DM) remains one of the most prevalent diseases worldwide. It is a chronic metabolic disorder in which there are insulin resistance, hyperglycemia, and relative insulin deficiency.[1,2] Insulin sensitivity of target tissues, like the liver, muscle, and adipose tissue is impaired in type 2 DM resulting in an inability of the cells to utilize glucose and a compensatory increase in beta-cell insulin production.[3,4] Subsequently, metabolic disorders like dyslipidemia, visceral adiposity, endothelial dysfunction, hypertension, hyperuricemia, and elevated inflammatory markers ensue.

Genetics and lifestyle factors such as lack of exercise, obesity, cigarette smoking, and drinking alcohol are

found to be crucial in the development of the disease.[5] Recently, environmental toxins were also blamed for the increased incidence of type 2 DM.[1] Aflatoxin (AF) is one of the major mycotoxins that contaminate food during pre or post-harvest.[6] Aflatoxin B1, the most toxic and prevalent AF, generates free radicals and induces lipid peroxidation resulting in oxidative stress and cellular damage in animals or humans.[7,8] AFB1 is therefore highly toxic and carcinogenic due to its bio-activation by microsomal cytochrome P450 into 8,9-epoxide.[9] Moreover, AFB1 exposure in type 1 diabetic mice disrupted lipid, and oxidative phosphorylation, gluconeogenesis, and reduced major urinary protein 1 (a major insulin sensitivity indicator), with subsequent elevation of blood glucose level.[10] Therefore, the discovery of a new natural intervention becomes crucial.[11]

A diet that contains high dietary fiber (DF) can improve the control of DM.[12] Various functional foods are investigated for their ability to partially compensate for the use of medicine.[12,13] Barley (*Hordeum vulgare* L.), one of the cereals with a high DF 29.5% such as *β*-glucan, was able to reduce blood glucose levels.[13,14] Barley grass is also rich in many functional ingredients such as vitamins (A and C), minerals (Ca, S, Cr, Fe, Mg, and K), chlorophyll, SOD, catalase (CAT), lutonarin, saponarin, gamma-aminobutyric acid, and tryptophan which makes it a potential candidate for prevention and therapy of chronic diseases.[15] Barley extracts also have high total phenolic contents and total flavonoid contents relative to their anti-oxidant potential.[16]

This study aims to evaluate the protective efficiency of barley microgreen (BM) against AF intoxication and diabetes.

Materials and methods

Microgreens of barley

Barley (*H. vulgare*) is a member of the grass family. Its microgreens (BM) were grown in an open field and harvested at the fully expended green cotyledons stage which was 14 days from seed soaking, washing, and hulling.[17] Harvested BM was air-dried for three days according to a previous study[18] and ground into powder. The phytochemical compounds present in BM powder were determined according to a previous method using gas chromatography-mass spectrometry GC/MS/MS technique.[19] The analysis was carried out using a GC (Agilent Technology 7890A) coupled with a mass selective detector (MSD, Agilent 7000 Triple Quad) equipped with Agilent HP-5ms capillary column. The identification of components was based on a comparison of their mass spectra with the authentic compounds and by computer matching with the NIST library as well as by comparison of the fragmentation pattern of the mass spectral data with those registered in the literature.

AF preparation

The strain of *Aspergillus flavus* (NRRL 3357) was kindly provided by the laboratory of Mycotoxin, National Research Center (Dokki, Cairo). The media prepared for fungal growth were Czapek's agar medium and liquid yeast medium.[20] The lyophilized strain of *A. flavus* was grown on slants of Czapek's agar media, using a platinum loop under a complete aseptic condition, and incubated at 25–29°C for nine days. Spores of nine days old of *A. flavus* culture were transferred by adding 2 mL sterile distilled water to each *A. flavus* slant and then added to a cooled sterilized flask containing prepared liquid yeast medium and incubated for nine days at 25–29°C. The filtrate was stored in tightly wrapped bottles in aluminum foil at 4°C. Total AF concentration was determined using a slightly modified immunoaffinity method based on the Association of Official Analytic Chemists method.[21] The concentration was measured in a precalibrated VICAMSeries-4 fluorometer set at 360 nm excitation and 450 nm emission. AF was identified by a modification of the HPLC – AFLATEST procedure Agillent 1200 series USA (HPLC equipment with two pumps, column C18, Lichrospher 100 RP-18).

Animals

The animals obtained from the National Research Center (El Dokki, El Giza, Egypt) were housed in plastic cages (three rats per cage) and acclimated for two weeks. The temperature and relative humidity were adjusted at 25 ± 2°C, 50–60%, respectively. Animals were fed a pelleted diet and had free access to water. This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University (Vet CU01102020224) and was carried out in accordance with the guidelines of Care and Use of Laboratory Animals stated by the National Institutes of Health, USPHS.

Induction of type 2 DM

Type 2 diabetes was induced by a high-fat diet (HFD) and low-dose of streptozotocin (STZ) treatment.[22] Briefly, the rats were fed with HFD *ad libitum* for two weeks and then injected with a low dose of STZ (single dose of 30 mg kg^{-1}, i.p.). Seven days after STZ injection, the fasting blood glucose levels of all the rats were estimated; those having blood glucose levels $\geq$200 mg dL^{-1} were considered diabetic and were selected for further experimentation. These rats were continued on HFD until the end of the experimental period.

Diets and their preparation

Four different diets were formulated: a control diet according to the AIN-76, a BM diet with 10% BM powder replacing corn starch, an HFD with 20% palm oil, an HF and BM diet with 20% palm oil and 10% BM (Table 1). Diets were manufactured into pellets.

Experimental design

Forty-eight male albino rats were randomly assigned to eight groups (six rats each). G1 is a normal control. G2 rats were fed a BM diet. G3 rats were administered AF (30 μg/kg) three days/week orally. G4 rats were administered AF and fed BM. G5 are diabetic rats and fed a HFD. G6 are diabetic rats fed HFD with BM. G7 are diabetic rats

Table 1. Composition of diets.

Ingredients[b]	Control diet	BM diet	High-fat diet	High-fat and BM diet
Casein	20.0	20.0	20.0	20.0
Corn starch	65.0	55.0	50.0	40.0
Mineral mix[a]	3.5	3.5	3.5	3.5
Vitamin mix[a]	1.0	1.0	1.0	1.0
DL-Methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Cellulose powder	5.0	5.0	5.0	5.0
palm oil	5.0	5.0	20.0	20.0
Barley microgreen	–	10.0	–	10.0

BM: barley microgreen.
[a]Based on AIN-76.
[b]Weight percentage.

fed HFD and administered AF. G8 are diabetic rats fed HFD with BM and administered AF orally. All rats were weighed at the beginning and end of the experiment to record the initial weight and final weight, respectively. The percentage of body weight gain/loss in each group was calculated by the following equation: Bodyweight gain/loss % = final weight – initial weight/initial weight × 100. The animals were euthanized after six weeks and serum, blood, and tissue samples were collected.

Determination of glucose level in plasma

Plasma glucose level was determined enzymatically and calorimetrically according to a previous method,[23] in which glucose oxidase catalyzed the oxidation of glucose to gluconic acid.

Determination of serum insulin hormone

The serum insulin in rats was measured by a rat insulin ELISA which is a solid phase two-site enzyme immunoassay (Thermo Fisher Scientific, Waltham, MA). The procedure was performed according to manufacturer protocol.[24] The plate was read at 450 nm using a plate reader. The absorbance values obtained for the standards were plotted against the insulin concentration on a log–log paper for constructing a standard curve. The insulin concentration of the samples was calculated using the standard curve.

$$\text{HOMA-IR} = [\text{fasting insulin (mU/l)} \times \text{fasting glucose (mg/dl)} \times 0.0555]/22.5$$

$$\text{HOMA-\%B} = [20 \times \text{fasting insulin (mU/l)}]/[(\text{fasting glucose (mg/dl)} \times 0.055) - 3.5].$$

$$\text{HOMA -\%S} = [1/\text{HOMA -IR}] \times 100$$

$$\text{Disposition index 1} = (\text{HOMA-\%S}/100) \times (\text{HOMA-\%B}/100).$$

Determination of liver function

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activity were measured in serum.[25,26] Total bilirubin and total protein were determined in serum according to previous methods.[27,28]

Determination of kidney function parameters and lactate dehydrogenase activity

Lactate dehydrogenase activity (LDH) was estimated in serum.[29] Enzymatic determination of serum urea and creatinine was carried out according to previous methods.[30,31]

Histopathology

Tissue specimens from the pancreas, liver, kidneys, and brain of rats in all groups were fixed in 10% neutral buffered formalin. Specimens were then dehydrated, cleared, embedded in paraffin, sectioned, and stained by hematoxylin and eosin stain. Tissue slides were examined by light microscopy and photographed using a digital camera.

Immunohistochemistry

Insulin was immunohistochemically stained in paraffin-embedded tissue sections of the pancreas using anti-insulin antibodies (Invitrogen, Thermo-Fisher Scientific, USA) and the avidin–biotin–peroxidase complex according to kit manufacturer protocol (Dako, North America, Inc., MI, USA). 3,3′-Diaminobenzidine was used to develop the color. The area % of positive insulin in beta cells of the pancreatic islets was measured in three photos/rats in each group at a 400× magnification power using Image J.

Determination of oxidative stress in liver

Reduced glutathione (GSH) was calorimetrically determined according to a method described previously.[32] Superoxide dismutase (SOD) activity was determined according to a previous method.[33] Malondialdehyde (MDA) was estimated according to Ohkawa *et al*.[34] and CAT activity according to the method of Aebi.[35]

Statistical analysis

Statistical analysis was performed using the statistical package for the social science program version.[36] Standard deviation "SD" and standard error "SE" were calculated. Least significant difference (LSD) test was used to compare the significant difference between means of treatment.[37] The area % of insulin-positive cells was tested for homogeneity and analyzed by ANOVA test to detect significance followed by Tamahne's test and Duncan test to detect significance between groups.

Results

Phytochemical content of BM

Egyptian barley microgreen aged 14 days showed 3′-Benzyloxy-5,6,7,4′-tetramethoxyflavone as the major natural phytochemicals (48.8% of total) followed by 5β,7βH,10α-Eudesm-11-en-1α-ol (18.46%), 4′,6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside (7.33%), citronellyl tiglate (4.48%), 3,4-dihydrocoumarin (1.36%) and phytanic acid (1.29%) (Suppl. Table).

Bodyweight gain/loss

The body weight gain (BWG) of rats was significantly reduced in the STZ group and STZ-AF group with the highest decrease in the STZ-AF group compared to the control. The BWG in the STZ-AF-BM group was higher than the STZ-AF group. The BWG recorded no significant difference in the STZ-BM group compared to the STZ group but recorded a significant increase in the STZ-AF-BM group compared to the STZ-AF group. The highest BWG was recorded in the BM group (Table 2).

Blood glucose and insulin level

The glucose level of rats was increased significantly in the AF group, STZ group, and STZ-AF group compared to control and BM groups. Feeding BM was able to significantly decrease the level of glucose in the AF-BM group, STZ-BM group, and AF-STZ-BM group compared to groups not fed BM (Table 3). The insulin level was significantly lower in the STZ group compared to the control, whereas it was slightly decreased without significant difference in the AF group. The lowest significant decrease of serum insulin was recorded in the STZ-AF group. The insulin level was not significantly different in BM-treated groups compared to untreated groups.

Table 2. Body weight gain of rats at the end of six-week experimental period.

Treatment	Initial weight g	Final weight g	BWG/L % of control (G1)
G1 control	118.8 ± 8.91^{b}	150.57 ± 10.94^{d}	100
G2 barley	128.38 ± 2.97^{ab}	220.56 ± 17.95^{b}	290
G3 aflatoxin	124.40 ± 14.09^{ab}	168.78 ± 6.35^{c}	140
G4 aflatoxin BM	128.70 ± 2.35^{ab}	200.45 ± 9.76^{a}	226
G5 STZ	124.80 ± 14.39^{ab}	132.27 ± 2.77^{e}	24
G6 STZ-BM	137.80 ± 0.88^{a}	132.48 ± 6.35^{e}	–17
G7 STZ-aflatoxin	135.73 ± 18.69^{a}	93.33 ± 17.68^{f}	–133
G8 STZ-aflatoxin-BM	134.96 ± 5.68^{a}	128.24 ± 2.78^{e}	–21

Note: All values are represented as mean ± S.D.[not in alphabetical order.] Means with different letters superscript are significantly different ($p < 0.05$). BM: barley microgreen; BWG/L: body weight gain/loss.

Table 3. Blood glucose in different experimental animal groups at the end of the experimental period.

Groups	Treatment	Blood glucose	
		mg/dL	m mol/dL
G1	Control	100 ± 8.1^{e}	5.56 ± 0.45^{e}
G2	BM	99 ± 8.9^{e}	5.50 ± 0.49^{e}
G3	Aflatoxin	131 ± 11.1^{c}	7.28 ± 0.62^{c}
G4	aflatoxin BM	112 ± 9.9^{d}	6.22 ± 0.55^{d}
G5	STZ	150 ± 8.2^{b}	8.33 ± 0.46^{b}
G6	STZ BM	124 ± 7.7^{cd}	$6.89 \pm 0.42c^{d}$
G7	STZ aflatoxin	176 ± 8.3^{a}	9.78 ± 0.46^{a}
G8	STZ, aflatoxin, BM	150 ± 11.1^{b}	8.33 ± 0.62^{b}

Note: All values are represented as mean ± S.D. Means with different superscript letters are significantly different ($p < 0.05$). BM: barley microgreen.

Homeostatic model assessment

The β-cell function was significantly decreased in STZ and/or AF groups. The β-cell function was significantly regained partly in the AF-BM group, STZ-BM group, STZ-AF-BM group but was still significantly impaired compared to the control. On the other hand, insulin resistance was significantly increased in the AF group, STZ group, and AF-STZ group. BM diet decreased insulin resistance in the AF group, STZ group, and STZ-AF group recording insignificant differences with the control group. Insulin sensitivity was significantly decreased in the STZ group and was more obvious in the STZ-AF group. BM diet enhanced insulin sensitivity in the STZ group and STZ-AF group, which recorded no significant difference with the control group.

The deposition index was significantly decreased in the AF group, STZ group and greatly in the STZ-AF group. On the reverse, feeding BM diet significantly increased the deposition index in the AF-BM group, STZ-BM group, and STZ-AF-BM group, which, however, still recorded a significant decrease compared to the control (Table 4).

Liver function parameters

The liver function enzymes and bilirubin were significantly elevated in the AF-STZ group followed by the AF group and STZ group. BM diet was able to decrease significantly the AST and ALP activity and bilirubin in the AF-BM group, AST in the STZ-BM group, and activities of AST, ALT, ALP, and bilirubin in the AF-STZ-BM group. Total protein was significantly decreased in rats of groups receiving AF and/or STZ with the highest decrease in G7 (AF-STZ). BM diet increased the concentration of total protein in G4 (AF-BM group) recording an insignificant difference with the control. However, the BM diet did not improve the total protein significantly in G8 (AF-STZ-BM) and G6 (STZ-BM) (Table 5).

Kidney function parameters and LDH activity

The highest increase in urea and creatinine concentration and LDH was recorded in G7 (STZ and AF) followed by G3 (AF) and G5 (STZ). BM diet decreased LDH activity significantly in G4 (AF-BM), G6 (STZ- BM), G8 (AF-STZ-BM) compared to untreated groups. The urea and creatinine concentration was increased significantly in the STZ-BM group by BM diet and was not affected in the AF-BM group and AF-STZ-BM group compared to untreated groups (Table 6).

Histopathological and immunohistochemical findings in pancreas

Microscopy of the pancreas of rats in the control group and BM group showed normal islets of Langerhans and pancreatic acini (Figure 1(a) and (b)). The pancreas of the AF group showed moderate-sized islets of Langerhans with irregular boundaries and degeneration of islet cells (Figure 1(c)). On the other hand, the pancreas in the AF-BM group revealed a larger islet of Langerhans and less degenerated cells (Figure 1(d)). The pancreas of rats

Table 4. Homeostatic model assessment of β-cell function (HOMA-B), homeostatic model assessment insulin resistance (HOMA-IR), homeostatic model assessment insulin sensitivity (HOMA-% S), and disposition index (DI) of the experimental rats.

Groups	Treatment	HOMA-B value	HOMA-IR value	HOMA-%S value	Disposition index value
G1	Control	71.55 ± 6.61a	1.82 ± 0.12c	54.95 ± 4.41a	0.393 ± 0.031a
G2	BM	75.30 ± 5.99a	1.84 ± 0.17c	54.35 ± 4.63a	0.409 ± 0.040a
G3	aflatoxin	35.29 ± 3.12c	2.16 ± 0.20b	47.30 ± 4.18ab	0.167 ± 0.011c
G4	aflatoxin BM	51.47 ± 4.21b	1.94 ± 0.15c	51.55 ± 5.00a	0.265 ± 0.025b
G5	STZ	25.67 ± 2.41d	2.30 ± 0.19b	43.48 ± 4.34b	0.112 ± 0.010d
G6	STZ-BM	37.17 ± 3.55c	1.93 ± 0.18c	51.81 ± 5.03a	0.193 ± 0.018c
G7	STZ-aflatoxin	18.15 ± 1.12e	2.48 ± 0.21a	40.32 ± 3.98b	0.073 ± 0.006e
G8	STZ-aflatoxin-BM	24.84 ± 2.32d	2.22 ± 0.20b	45.05 ± 4.14ab	0.112 ± 0.010d

Note: all values are represented as mean ±S.D. Means with different superscript letters are significantly different ($p < 0.05$).
BM: barley microgreen.

Table 5. Liver function enzymes, total bilirubin, and total protein in different groups.

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin total (mg/dl)	T.protein (g/dl)
G1 control	124.83 ± 10.12^{e}	64.83 ± 5.77^{c}	722.50 ± 20.26^{d}	0.51 ± 0.07^{d}	6.86 ± 0.09^{a}
G2 BM	120.33 ± 8.01^{e}	48.50 ± 3.28^{d}	681.17 ± 55.34^{d}	0.56 ± 0.02^{c}	6.93 ± 0.40^{a}
G3 aflatoxin	247.67 ± 9.29^{ab}	93.17 ± 5.84^{b}	1268.67 ± 101.54^{a}	0.71 ± 0.09^{b}	6.43 ± 0.13^{b}
G4 aflatoxin BM	144.67 ± 3.01^{d}	77.67 ± 3.51^{b}	906.67 ± 72.90^{b}	0.66 ± 0.16^{c}	6.61 ± 0.23^{ab}
G5 STZ	189.50 ± 6.06^{b}	71.83 ± 2.84^{bc}	980.50 ± 17.50^{b}	0.77 ± 0.16^{ab}	6.13 ± 0.28^{b}
G6 STZ-barley	156.67 ± 5.51^{cd}	53.83 ± 4.54^{c}	908.17 ± 83.75^{b}	0.83 ± 0.02^{a}	6.07 ± 0.40^{b}
G7 STZ-aflatoxin	322.00 ± 12.12^{a}	125.00 ± 6.06^{a}	1303.67 ± 117.27^{a}	0.89 ± 0.02^{a}	5.86 ± 0.95^{b}
G8 STZ- aflatoxin BM	170.17 ± 9.44^{c}	81.00 ± 1.00^{b}	998.33 ± 25.53^{b}	0.75 ± 0.09^{b}	6.42 ± 0.38^{b}

Note: All values are represented as mean ±S.D. Means with different superscript letters are significantly different ($p < 0.05$).

Table 6. Kidneys function enzymes and lactate dehydrogenase (LDH) in different groups.

Treatment	LDH activity (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
G1 control	1388.50 ± 29.50^{e}	39.67 ± 1.44^{b}	0.61 ± 0.11^{c}
G2 BM	1411.17 ± 44.50^{d}	39.17 ± 2.85^{b}	0.77 ± 0.11^{b}
G3 aflatoxin	2136.67 ± 91.12^{b}	52.17 ± 9.17^{a}	0.81 ± 0.19^{a}
G4 aflatoxin-BM	1737.50 ± 34.60^{c}	46.17 ± 2.36^{ab}	0.90 ± 0.16^{a}
G5 STZ	1743.17 ± 58.31^{c}	40.67 ± 2.13^{b}	0.71 ± 0.08^{b}
G6 STZ- BM	1434.00 ± 59.69^{d}	52.83 ± 2.29^{a}	0.87 ± 0.06^{a}
G7 STZ-aflatoxin	2426.00 ± 77.74^{a}	41.00 ± 2.00^{b}	0.80 ± 0.06^{a}
G8 STZ-aflatoxin-BM	1408.50 ± 65.56^{d}	42.67 ± 3.69^{ab}	0.79 ± 0.29^{a}

Note: All values are represented as mean ±S.D. Means with different superscript letters are significantly different ($p < 0.05$).

in the STZ group (Figure 1(e)) revealed a few small islets of Langerhans with fibrosis and vacuolar degeneration of cells, whereas these lesions were alleviated in the pancreas of rats in the STZ-BM group (Figure 1(f)). The pancreas of rats in the STZ-AF group showed islets of Langerhans with irregular boundaries and degenerated cells (Figure 1(g)). These lesions were improved in the pancreas of rats STZ-AF-BM group (Figure 1(h)).

The pancreas in the control group (G1) and BM group (G2) had insulin-positive beta cells which were arranged in a regular continuous cord in the islets of Langerhans. In G3 (AF group), the area % of beta cells moderately decreased, and the cells were irregularly arranged compared to the control group. However, the area % of beta cells was restored and well arranged in the AF-BM group. In the STZ group, the area % of beta cells was almost diminished compared to control and recorded a significant difference. On the other hand, the area % of beta cells in the STZ-BM group was significantly higher compared to the STZ group and almost similar to the control group. The area % of insulin-positive beta cells in the STZ-AF group was significantly decreased compared to G1. On the reverse, the area % of insulin-positive beta cells in G8 was similar to the control group (Figure 2).

Microscopic examination of the liver revealed moderate hypertrophy of hepatocytes in addition to karyomegaly, binucleation, and mild solitary necrosis in the AF group compared to control and BM groups. These lesions were less severe in the AF-BM group. Rats injected with STZ in G5 and G7 showed hepatic lesions which were mainly periportal hepatocytes vacuolation. The hepatic lesions were alleviated in rats of the STZ-BM group and STZ-BM-AF group (Figure 3(a) to (h)).

Microscopic examination of the kidneys of rats in the AF group showed mild hypertrophy and vacuolar degeneration of tubular epithelium compared to control and BM. These lesions were mildly alleviated in the AF-BM group. The kidneys of rats injected with STZ had mesangial cells hyperplasia, increased glomerular matrix, and mild thickening of glomerular basement membrane beside degenerative and necrotic changes in epithelium and casts in the lumen of renal tubules. These lesions were more severe in the STZ-AF group. On the other hand, these lesions were lessened in the groups fed BM (G6, G8) (Figure 3(i) to (p)).

The microscopic examination of the brain of rats in the control group and BM group revealed normal histological structure, whereas the brain of rats in the AF group showed neuronal degeneration and neurophagia in cortical neurons

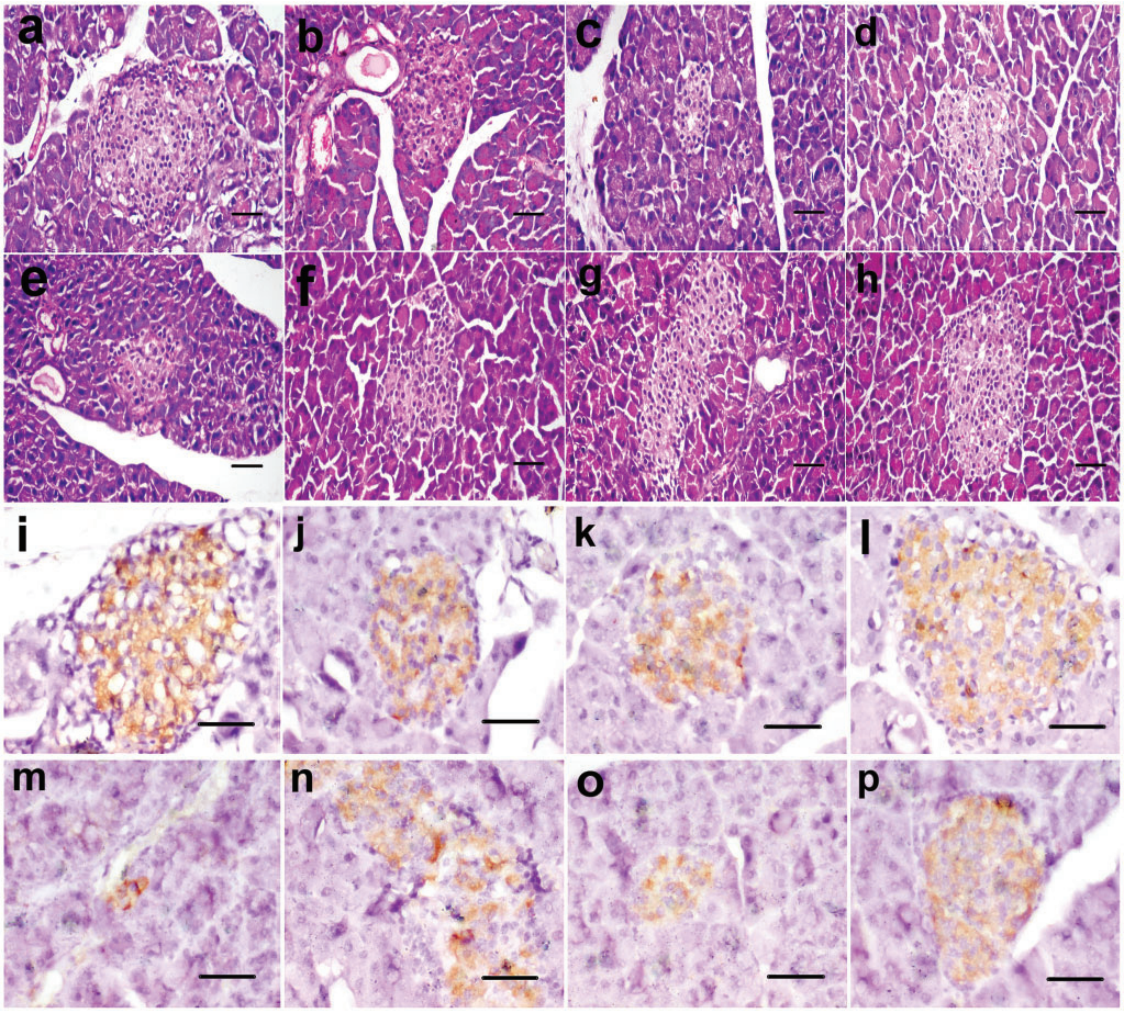

Figure 1. Pancreas of rats showing islets of Langerhans in (a) control group, (b) BM group, (c) aflatoxin group, (d) aflatoxin and BM group, (e) STZ group, (f) STZ and BM group, (g) STZ and aflatoxin group, (h) STZ, aflatoxin and BM. Hematoxylin and eosin stain (Scale bar= 100 μm). (i–p) Pancreas of rats showing insulin-positive Beta cells in islets of Langerhans in (i) control group, (j) BM group, (k) aflatoxin group, (l) aflatoxin and BM group, (m) STZ group, (n) STZ and BM group, (o) STZ and aflatoxin group, (p) STZ, aflatoxin, and BM. Immunoperoxidase stain (Scale bar= 50 μm). (A color version of this figure is available in the online journal.)

which appeared dark, shrunken, engulfed by glia cells, and with neurofibrillary tangles. Brain lesions in the AF-BM group were less severe compared to the AF group. The brain of rats in the STZ group had neuronal degeneration and decreased the number of pyramidal cells in the hippocampus and mild gliosis. These lesions were regressed in the STZ-BM group. The brain in the STZ-AF group showed severe diffuse neuronal degeneration and neurophagia which was moderately alleviated in the STZ-AF-BM group.

Oxidative stress biomarkers in liver

The MDA content was highly and significantly elevated in G7 (STZ-AF), G3 (AF), and G5 (STZ) sequentially, whereas the GSH content and activity of antioxidant enzymes were significantly impaired in these groups compared to control. In the AF-BM group and STZ-BM group, MDA content was significantly decreased, but GSH content and activity of SOD and GST were elevated. In the AF-STZ-BM group, MDA content was significantly decreased, while GSH content, and SOD, CAT, GST activities were significantly increased (Table 7).

Discussion

Exposure to environmental toxins can aggravate diabetes-causing severe complications which urge further investigation.[1] AF exposure was linked before with type 2 DM.[11] It can occur through the consumption of food, milk, and dairy products.[6] In the past decade, research is directed toward the discovery of natural functional food that would help reduce and control chronic diseases. Microgreens are rich in many nutrients, as they are rich in minerals and antioxidants, and recently, they gained high popularity in the prevention of chronic diseases.[38,39] A diabetes model in rats was developed using STZ injection and a HFD to evaluate the health effect of the BM diet. In the present study, BM was fed to diabetic rats with or without AF intoxication to elucidate its effect on hyperglycemia, insulinemia, and beta-cell function.

Injection of STZ in rats damages B-cells in the islets of Langerhans and causes hyperglycemia,[12] similar to the present study, which also showed decreased insulin-positive cells in the STZ group. AF seems to possess a synergistic cytotoxic effect with STZ on B cells. In the present study,

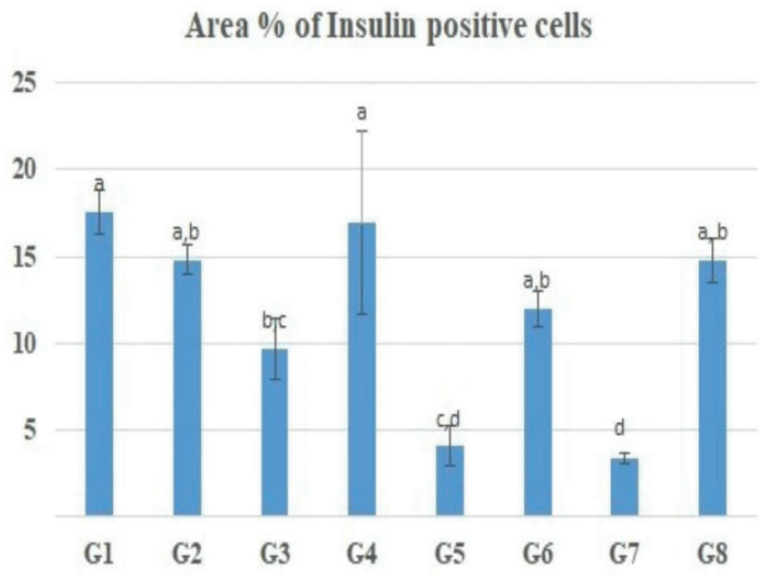

Figure 2. Area % of insulin-positive cells in the islets of Langerhans of the pancreas of different groups. G1: control group, G2: BM group, G3: aflatoxin group, G4: aflatoxin and BM group, G5: STZ group, G6: STZ and BM group, G7: STZ and aflatoxin group, G8: STZ, aflatoxin and BM. Columns bearing different superscripts are significant at $p < 0.05$. (A color version of this figure is available in the online journal.)

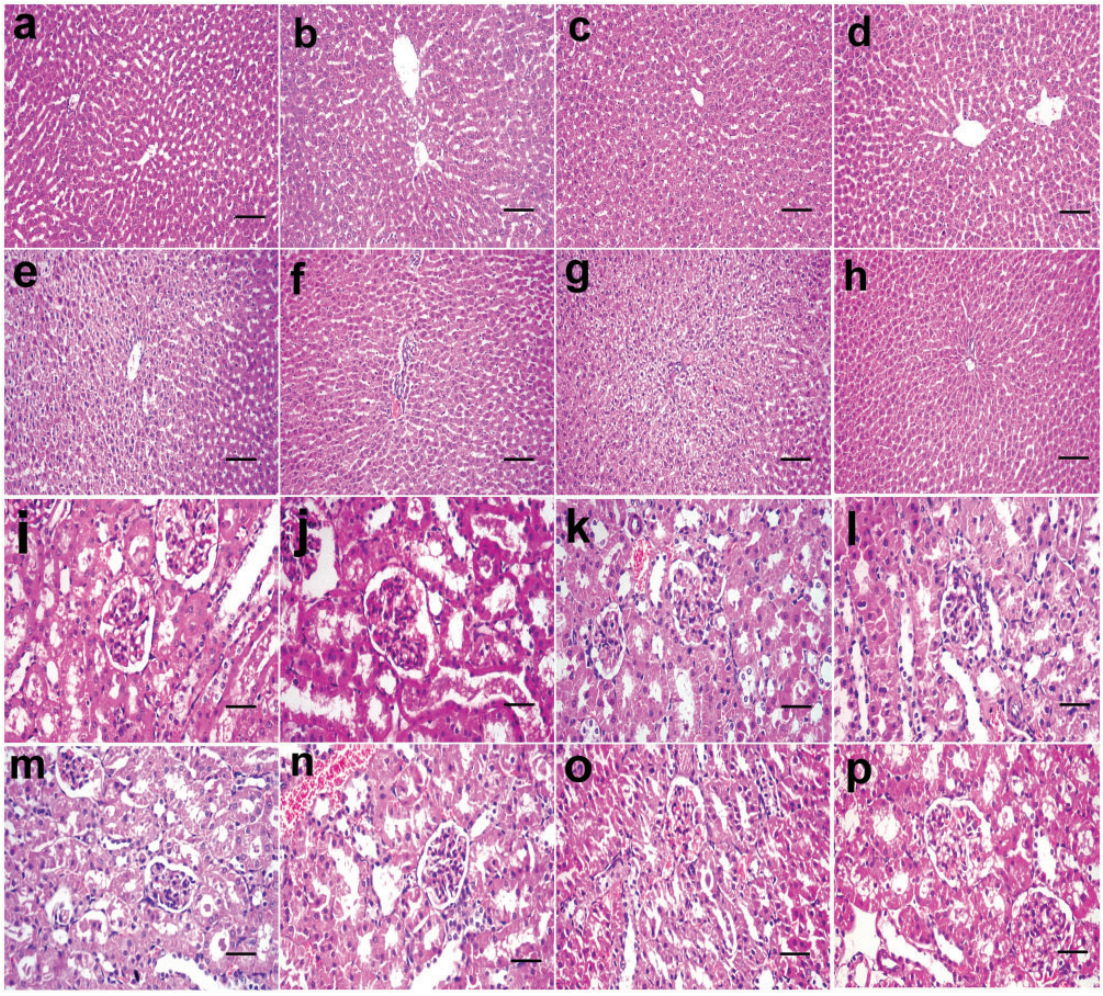

Figure 3. (a–h) histopathological structure of liver of rats in (a) control group, (b) BM group, (c) aflatoxin group, (d) aflatoxin and BM group, (e) STZ group, (f) STZ and BM group, (g) STZ and aflatoxin group, (h) STZ, aflatoxin and BM. Fig (i–p) kidney of rats in different groups (i) control group, (j) BM group, (k) aflatoxin group, (l) aflatoxin and BM group, (m) STZ group, (n) STZ and BM group, (o) STZ and aflatoxin group, (p) STZ, aflatoxin, and BM. Hematoxylin and eosin stain (Scale bar = 100 μm). (A color version of this figure is available in the online journal.)

Table 7. Effect of barley microgreen on oxidative stress biomarkers in liver of rat.

Treatment	MDA content (Nmol/g)	Liver oxidation system			
		GSH content (μmol/ml)	SOD activity (U/g)	CAT activity (U/g)	GST activity (μmol/gt)
G1 control	6.361 ± 0.421^{de}	0.411 ± 0.032^{a}	77.10 ± 8.88^{a}	158.11 ± 7.81^{a}	6.13 ± 0.43^{a}
G2 barley	6.072 ± 0.414^{e}	0.419 ± 0.022^{a}	82.21 ± 5.11^{a}	159.13 ± 9.17^{a}	6.19 ± 0.51^{a}
G3 aflatoxin	10.112 ± 0.712^{b}	0.222 ± 0.013^{d}	47.04 ± 3.71^{d}	99.12 ± 6.16^{cd}	4.11 ± 0.21^{d}
G4 aflatoxin BM	8.210 ± 0.611^{c}	0.289 ± 0.014^{c}	58.21 ± 4.01^{c}	113.20 ± 6.17^{c}	4.79 ± 0.26^{c}
G5 STZ	8.221 ± 0.521^{c}	0.301 ± 0.020^{c}	60.09 ± 4.02^{c}	120.12 ± 8.27^{bc}	5.07 ± 0.32^{b}
G6 STZ- BM	6.792 ± 0.420^{d}	0.396 ± 0.021^{b}	70.09 ± 4.32^{b}	140.19 ± 8.01^{b}	5.91 ± 0.49^{a}
G7 STZ-aflatoxin	12.781 ± 0.941^{a}	0.154 ± 0.010^{e}	35.03 ± 2.12^{e}	84.20 ± 5.14^{e}	3.45 ± 0.21^{e}
G8 STZ-aflatoxin BM	9.993 ± 0.555^{b}	0.209 ± 0.014^{d}	50.13 ± 3.13^{cd}	98.08 ± 6.06^{d}	3.87 ± 0.19^{d}

Note: All values are represented as mean ±S.D. Means with different superscript letters are significantly different ($p < 0.05$).

BM incorporation in the diet was able to decrease hyperglycemia, improve beta-cell function, decrease insulin resistance, increase insulin sensitivity, and alleviate histopathological alteration in the islets of Langerhans similar to a previous study.[39] In addition, BM protected the islet cells against damage and resulted in a high area % of insulin-positive cells in the pancreas. BM was found to contain 4′,6-dimethoxyisoflavone-7-O-β-D-glucopyranoside (7.33%) also known as wistin and phytanic acid. The wistin can activate peroxisome proliferator-activated receptors PPARγ which is crucial in adipogenesis regulation, energy balance, and insulin sensitivity.[40] Phytanic acid, a methyl-branched fatty acid, was also reported to prevent type 2 diabetes.[41]

In the current study, AF induced oxidative stress and histopathological alterations in different organs. STZ injection also caused oxidative stress, which, however, was less than that of AF. The combination of both STZ injection and AF injection worsened the oxidative stress, prompting elevated liver enzymes and increased severity of hepatocytes degeneration similar to a previous study.[11] BM diet, however, decreased oxidative stress induced by AF STZ. The analysis of BM showed that it contained a high percent of 3′-benzyloxy-5,6,7,4′-tetramethoxyflavone (48.8%) which is a type of flavonoid. Flavonoids are potent antioxidants that have strong free radical scavenging abilities.[42] Therefore, BM may have protected the islet cells against cytotoxic effects and oxidative stress induced by STZ and AF.

The metabolic disorder associated with diabetes also initiates a nephropathic and neuropathic complication[43] likewise to our findings which were further complicated with aflatoxicosis. AF and/or STZ groups fed BM diet showed a great improvement in liver and kidney functions, different organs histopathology, and oxidative stress biomarkers. 5β,7βH,10α-Eudesm-11-en-1α-ol (Eudesmol), a hydroxylated sesquiterpene, was the second most abundant phytochemical in BM used in the current study. Eudesmol possesses multiple positive bioactivities such as antiangiogenic, anticancer, anti-inflammatory activities which might therefore have played a role in the health improvement of diabetic and/or aflatoxicated rats fed BM.[44]

LDH is a crucial enzyme in the anaerobic metabolic pathway which catalyzes the conversion of lactate to pyruvate-reducing NAD+ to NADH.[45] LDH overexpression in some forms of type 2 diabetes hinders normal glucose metabolism and insulin secretion from beta-cells in islets of Langerhans leading to insulin secretory defects.[46] This would therefore explain the high elevation of LDH in diabetic and intoxicated rats.[47] BM diet was able to decrease LDH serum level significantly in treated groups, although it was still significantly higher compared to the control.

In the present study, brain lesions were mainly observed in the hippocampus of diabetic rats, whereas degeneration of cortical neurons was the most prominent lesion in aflatoxicated rats. A previous study reported a significant upregulation of mRNA levels, protein levels, and enzymatic activity of LDH-A in the hippocampus of STZ-injected rats proposing increased glycolysis activity.[48] Subsequently, brain structural lesions and cognitive decline in DM were attributed to the progressive increase in lactate levels.[48] The brain lesions were regressed in diabetic and/or aflatoxicated rats fed BM which might be due to its ability to lower serum LDH activity.

In conclusion, incorporation of BM in the diet was able to control diabetes type 2, counteracted the oxidative stress induced by AF and/or STZ, and decreased the nephropathy and neuropathy associated with diabetes.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies and analysis of the data, and review of the manuscript; SM, EAA, TAAA, AMN, MSK. SM conducted the experiment. MSK wrote the article.

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SUPPLEMENTAL MATERIAL

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REFERENCES

1. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. *Oman Med J* 2012;**27**:269–73
2. Maitra A, Abbas AK. Endocrine system. In: Kumar V, Fausto N, Abbas AK (eds) *Robbins and Cotran pathologic basis of disease*. 7th ed. Philadelphia: Saunders, 2005, pp.1156–226.
3. Seong J, Kang JY, Sun JS, Kim KW. Hypothalamic inflammation and obesity: a mechanistic review. *Arch Pharm Res* 2019;**42**:383–92
4. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. *Front Endocrinol (Lausanne)* 2019;**10**:80
5. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 2001;**345**:790–7
6. Mahato DK, Lee KE, Kamle M, Devi S, Dewangan KN, Kumar P, Kang SG. Aflatoxins in food and feed: an overview on prevalence, detection and control strategies. *Front Microbiol* 2019;**10**:2266
7. Shen HM, Shi CY, Lee HP, Ong CN. Aflatoxin B1-induced lipid peroxidation in rat liver. *Toxicol Appl Pharmacol* 1994;**127**:145–50
8. Abdel-Wahhab MA, Aly SE. Antioxidants and radical scavenging properties of vegetable extracts in rats fed aflatoxin-contaminated diet. *J Agric Food Chem* 2003;**51**:2409–14
9. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: a global concern for food safety, human health and their management. *Front Microbiol* 2016;**7**:2170
10. Tsai FJ, Chen SY, Liu YC, Liao HY, Chen CJ. The comparison of CHCA solvent compositions for improving LC-MALDI performance and its application to study the impact of aflatoxin B1 on the liver proteome of diabetes mellitus type 1 mice. *PLoS One* 2017;**12**:e0181423
11. Aleissa MS, Alkahtani S, Abd Eldaim MA, Ahmed AM, Bungău SG, Almutairi B, Bin-Jumah M, AlKahtane AA, Alyousif MS, Abdel-Daim MM. Fucoidan ameliorates oxidative stress, inflammation, DNA damage, and hepatorenal injuries in diabetic rats intoxicated with aflatoxin b1. *Oxid Med Cell Longev* 2020;**2020**:9316751
12. Abdel-Mobdy AE, Khattab MS, Mahmoud EA, Mohamed ER, Abdel-Rahim EA. Semi-modified okara whey diet increased insulin secretion in diabetic rats fed a basal or high-fat diet. *Food Sci Biotechnol* 2021;**30**:107–16
13. Minaiyan M, Ghannadi A, Movahedian A, Hakim-Elahi I. Effect of *Hordeum vulgare* L. (barley) on blood glucose levels of normal and STZ-induced diabetic rats. *Res Pharm Sci* 2014;**9**:173–8
14. Lee NY, Kim YK, Choi I, Cho SK, Hyun JN, Choi JS, Park KH, Kim KJ, Lee MJ. Biological activity of barley (*Hordeum vulgare* L.) and barley byproduct extracts. *Food Sci Biotechnol* 2010;**19**:785–91
15. Zeng Y, Pu X, Yang J, Du J, Yang X, Li X, Li L, Zhou Y, Yang T. Preventive and therapeutic role of functional ingredients of barley grass for chronic diseases in human beings. *Oxid Med Cell Longev* 2018;**2018**:3232080
16. Panthi M, Subba RK, Raut B, Khanal DP, Koirala N. Bioactivity evaluations of leaf extract fractions from young barley grass and correlation with their phytochemical profiles. *BMC Complement Med Ther* 2020;**20**:64
17. Abdallah MMF. Seed sprouts, a Pharaoh's heritage to improve food quality. *Arab Universities Journal of Agriculture Science* 2008;**16**:469–78
18. Dzowela BH, Hove L, Mafongoya PL. Effect of drying method on chemical composition and in vitro digestibility of multi-purpose tree and shrub fodders. *Tropical Grasslands* 1995;**29**:263–9
19. Santana PM, Miranda M, Payrol JA, Silva M, Hernández V, Peralta EP. Gas chromatography-mass spectrometry study from the leaves fractions obtained of *Vernonanthura patens* (kunth) H. Rob. *Ijoc* 2013;**3**:105–9
20. Davis ND, Diener UL, Eldridge DW. Production of aflatoxins B1 and G1 by *Aspergillus flavus* in a semisynthetic medium. *Appl Microbiol* 1966;**14**:378–80
21. Trucksess MW, Stack ME, Nesheim S, Page SW, Albert RH, Hansen TJ. Immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization for determination of aflatoxins in corn, peanuts, peanut butter: collaborative study. *J Assoc off Anal Chem* 1991;**74**:81–8
22. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp Diabetes Res* 2008;**2008**:704045
23. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* 1969;**22**:158–61
24. Temple TC, Clark PM, Hales NNC. Measurement of insulin secretion in type 2-diabetes problems and pitfalls. *Diabet Med* 1992;**9**:503–12
25. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957;**28**:56–63
26. Belfield A, Goldberg D. Colorimetric determination of alkaline phosphatase activity. *Enzyme* 1971;**12**:561–6
27. Walter M, Gerarde H. Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. *Microchem J* 1970;**15**:231–6
28. Gornnall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 1949;**177**:751–66
29. Naithani M, Singh P. Teitz textbook of clinical chemistry & molecular diagnostics. *Med J Armed Forces India* 2006;**62**:204
30. Fawcett JK, Scott JE. Determination of urease modified Berthelot reaction. *J Clin Pathol* 1960;**13**:156–9
31. Moore JF, Sharer JD. Methods for quantitative creatinine determination. *Curr Protoc Hum Genet* 2017;**93**:A.3O.1–.3O.7.
32. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963;**61**:882–90
33. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 1972;**46**:849–54
34. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–8
35. Aebi H. Catalase in vitro. *Methods Enzymol* 1984;**105**:121–6
36. Kinnear PR, Gray CD. *SPSS for windows made simple*. 3rd ed. Hove: Psychology Press/Taylor & Francis (UK), 1999.
37. Waller RA, Duncan DB. A Bayes rule for the symmetric multiple comparisons problem. *J Am Stat Assoc* 1969;**64**:1484–503
38. Turner ER, Luo Y, Buchanan RL. Microgreen nutrition, food safety, and shelf life: a review. *J Food Sci* 2020;**85**:870–82
39. Azam A, Itrat N, Ahmed U. Hypoglycemic effect of barley (*Hordeum vulgare*) in diabetics. *Int J Innov Sci Res Technol* 2019;**4**:515–9
40. Sanada M, Hayashi R, Imai Y, Nakamura F, Inoue T, Ohta S, Kawachi H. 4′,6-dimethoxyisoflavone-7-O-*β*-D-glucopyranoside (wistin) is a peroxisome proliferator-activated receptor *γ* (PPAR*γ*) agonist that stimulates adipocyte differentiation. *Anim Sci J* 2016;**87**:1347–51
41. Roca-Saavedra P, Mariño-Lorenzo P, Miranda JM, Porto-Arias JJ, Lamas A, Vazquez BI, Franco CM, Cepeda A. Phytanic acid consumption and human health, risks, benefits and future trends: a review. *Food Chem* 2017;**21**:237–47
42. Karak P. Biological activities of flavonoids: an overview. *Int J Pharm Sci Res* 2019;**10**:1567–74
43. Lim AK. Diabetic nephropathy – complications and treatment. *Int J Nephrol Renovasc Dis* 2014;**7**:361–81
44. Acharya B, Chaijaroenkul W, Na-Bangchang K. Therapeutic potential and pharmacological activities of *β*-eudesmol. *Chem Biol Drug Des* 2021;**97**:984–96

45. Farhana A, Lappin SL. *Biochemistry, lactate dehydrogenase*. Treasure Island, FL: StatPearls Publishing, 2021.
46. Ainscow EK, Zhao C, Rutter GA. Acute overexpression of lactate dehydrogenase – a perturbs beta-cell mitochondrial metabolism and insulin secretion. *Diabetes* 2000;**49**:1149–55
47. Rastogi R, Srivastava AK, Srivastava M, Rastogi AK. Hepatocurative effect of picroliv and silymarin against aflatoxin b1 induced hepatotoxicity in rats. *Planta Med* 2000;**66**:709–13
48. Zhao L, Dong M, Ren M, Li C, Zheng H, Gao H. Metabolomic analysis identifies lactate as an important pathogenic factor in diabetes-associated cognitive decline rats. *Mol Cell Proteomics* 2018;**17**:2335–46

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