Antidiabetic And Antioxidant Effects Of A Multigrain Diet

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Abstract

Purpose: To investigate the efficacy of a formulated multigrain diet on metabolic alterations in carbohydrate profiles and antioxidant status in diabetic animals **Methods:** Diabetes was induced by administration of alloxan monohydrate and diabetic status was confirmed by blood glucose levels over a period of two weeks. Diet was formulated using *Sorghum vulgare*, *Avena sativa*, *Pennisetum typhoideum*, *Oryza sativa*, *Eleusine coracana* and *Zea mays* and casein, corn starch and vegetable oil. Carbohydrate (plasma glucose, hepatic glycogen, G-6-Pase and hexokinase), hepatic and renal functions (Total protein, albumin, urea, creatinine, SGOT, SGPT) and antioxidant profiles (SOD, CAT, GSH, GPx, TAA) were determined. **Results and Conclusions:** The diabetic animals when fed formulated diet, exhibited significant decreases in serum glucose, SGPT, SGOT, urea, creatinine and hepatic G-6-pase levels along with increased serum protein and glycogen content and hepatic hexokinase activity as compared to those of commercial diet fed diabetic animals. Hepatic and renal enzymatic and non enzymatic antioxidant profiles were also found to be restored to their normal values in those diabetic group of animals fed formulated diet. This study thus indicated that a multigrain formulated diet has a positive effect on diabetic animals with respect to serum glucose levels, hepatic and renal functions and antioxidant activity.

Key words: Type-1 Diabetes, alloxan, multigrain diet, antioxidant status, hepatic and renal functions

Introduction

Diabetes mellitus (DM) is not a single disorder but it is a group of metabolic disorders characterised by chronic hyperglycemia, increased thirst, increased urine output, ketonemia and ketonuria [1]. Complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy, amputations and blindness are also related to DM apart from other complications like hypertension, hypercholesterolemia, hypertriglycerdemia and low density lipoprotein cholesterol (LDL-C) [2, 3]. In addition, the metabolic deregulation associated with diabetes mellitus also causes secondary pathophysiological changes in multiple organ systems that are associated with oxidative stress and tissue damage [4]. Various experimental and clinical investigative reports indicated that elevated blood glucose levels in diabetic individuals lead to oxidative stress with subsequent formation of advanced glycation end products (AGE) [5,6]. Oxidative stress due to increased ROS generation and an imbalance in oxidative/antioxidative equilibrium in hyperglycemia plays a major role in diabetic complications [7]. The free radical generation in diabetes exhausts the components of the antioxidant defence system including enzyme antioxidants i.e., superoxide dismutase (SOD; EC 1.15.1.1), Catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC 1.11.1.9) and non-enzymatic antioxidants- reduced glutathione (GSH), vitamin C and vitamin E [8].

Diet plays an important role in the overall well-being of an individual, and the utilization of wholegrain cereals in food formulations is increasing worldwide, since they are rich sources of phytochemicals, dietary fibres and minerals that offer several health benefits [9]. For instance, the use of whole grain based products or the extracts of *Sorghum vulgare*, *Avena sativa*, *Pennisetum typhoideum*, *Oryza sativa*, *Eleusine coracana* and *Zea mays* have been well documented for their antioxidant, hypocholesterolaemic, hypolipidemic, insulinemic activities and lower the plasma glucose levels in diabetic subjects [10-15]. Therefore, the present study was aimed at investigating the physiological role of multigrain diet composed of millets and cereals- bajra, ragi, jowar, oat, rice and maize in ameliorating diabetes and its associated metabolic alterations with special reference to plasma glucose, hepatic glycogen, G-6-Pase, hexokinase activities, lipid peroxidation and antioxidant status in laboratory rats.

Materials and methods

Animal maintenance

Adult albino female rats (*Charles Foster*) weighing 200-280 gm were used in the present study. The animals were fed on a commercial diet (Pranav Agro Ind. Ltd., Vadodara, India), lodged in polypropylene cages with *ad libitum* access to water and maintained in a well ventilated room in departmental animal house facility with constant temperature and humidity of 26 ± 2°C and 60% respectively. The care and procedures adopted for the present investigation were in accordance with the regulations of Institutional Animal Ethics Committee (MoEF/CPCSEA/Reg.337).

Experimental procedure

After a 10 day adaptation period, 24 animals were randomly segregated into four groups consisting of six animals in each: two groups fed commercial diet (CD) and two groups fed formulated multigrain diet (FD) and, the experiment was carried out for duration of eight weeks.

The CD groups were sub-grouped in to CCD (control group for commercial diet) and DCD (diabetic animals fed commercial diet). The FD groups were also sub-grouped as CFD (control group for formulated diet) and DFD (diabetic animals fed formulated diet). Diabetes was induced by a single intra peritoneal (i. p.) injection of alloxan monohydrate (Sigma Aldrich) (150 mg/ kg bw) dissolved in normal saline. Induction of diabetes was confirmed by blood glucose levels (> 140 mg/dl) over a period of two weeks.

The commercial diet (containing *Triticum aestivum*- 80g%; soybean meal-10g%; SMP-5g%; casein-3g%; vegetable oil- 1g%; minerals and vitamins-1g%) was procured from VRK Nutritional Solutions (Pune, Maharashtra, India). A multigrain diet was formulated based on NIN formulation [16] using 10 g % each of *Sorghum vulgare* (jowar), *Avena sativa* (oats), *Pennisetum typhoideum* (bajra), *Oryza sativa* (rice), 20 g% each of *Eleusine coracana* (ragi) and *Zea mays* (maize) and casein (12 g %), corn starch and vegetable oil (4 g% each) [17].

At the end of eight week period animals were fasted overnight and sacrificed under mild anaesthesia. Blood was collected by cardiac puncture in EDTA coated tubes; plasma was separated by centrifugation and stored at low temperature. Liver and kidneys were excised and kept frozen (-20 °C) until used for analyses.

Analytical Procedures

Plasma glucose levels were measured by standard kit (Eve's Inn Diagnostics, India). Hepatic glycogen was extracted with 30 % KOH, and the yield was estimated by anthrone-sulfuric acid method [18]. Hepatic glucose-6-phosphatase (EC 3.1.3.9) and hexokinase (EC 2.7.1.1) activities were determined following methods of Baginsky *et al.,* and Brandstup *et al.,* [19, 20].

Total protein, albumin fraction, urea and creatinine levels and serum glutamate oxaloacetate and pyruvate transaminase (SGOT, SGPT) activities were determined using the standard kits (Eve's Inn Diagnostics, Baroda, India). Plasma FRAP values were measured by Benzie and Strain's method [21].

The hepatic and renal total ascorbic acid and reduced glutathione contents were estimated using methods of Schaffert and Kingsley and Jollow *et al.,* [22, 23]. Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC 1.11.1.9) activities were measured in both hepatic and renal tissues following the standard methods [24-26]. The hepatic and renal lipid peroxidation was determined by the thiobarbituric acid (TBA) assay [27].

Data Analysis

Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's significant difference post hoc test was used to compare differences among groups. Data were statistically analyzed using Graph Pad Prism 3.0 statistical software. P values <0.05 were considered statistically significant.

Results

Plasma and hepatic carbohydrate profiles

Formulated diet fed group of animals registered a significant decline in their plasma glucose levels (17.04%) and glucose-6-phosphatase activity (15.47%) with an increase in hepatic hexokinase (17.52%) and glycogen (7.22%) activity as compared to CCD group. A significant increase both in plasma glucose level and hepatic glucose-6-phosphatase activity with a decrease in hepatic glycogen content and hexokinase activity were observed in alloxan induced diabetic groups fed either commercial or formulated diet as compared to their respective controls. In addition, when DCD and DFD groups were compared, the plasma glucose content and hepatic G-6-Pase activity were found to decline by 18.73 and 39.16 % respectively, while both hepatic glycogen content and hexokinase activity were found to increased by 36.84 and 41.55 % in formulated diet fed diabetic group (Table-1).

Plasma protein, albumin, FRAP, SGPT, SGOT urea and creatinine indices

A significant increase in the levels of plasma protein, albumin and FRAP with a decline in urea, creatinine, SGPT and SGOT were registered with multigrain diet fed groups as compared to CCD. Diabetic groups fed either commercial or formulated diet exhibited a significant reduction in plasma protein (23.47 & 12.02 %), albumin (37.67 & 26.24 %) and FRAP (50.07 & 23.04 %) with an increase in urea (77.22 & 52.33 %), creatinine (26.53 & 32.25 %), SGPT (17.21 & 54.99 %) and SGOT (126.58 & 77.05 %) levels compared to their respective control groups. However, formulated diet fed diabetic animals showed a significant recovery in plasma protein (28.07%), albumin (25.42%) and FRAP (59.93%) contents with a simultaneous decreases in urea, creatinine, SGPT and SGOT (35.04%, 33.87%, 37.17% and 25.41% respectively) levels (Tables-2 & 3).

Oxidative stress markers in hepatic and renal tissues

In both DCD and DFD groups, the hepatic and renal enzymatic and non-enzymatic antioxidant profiles (TAA, GSH, GPx, SOD and CAT activity) declined significantly and the lipid peroxidation increased. However, the DFD group showed a clear improvement in TAA (44.09 & 12.24 %), GSH (33.0 & 51.30 %), GPx (33.66 & 23.33 %), SOD (33.62 & 29.16 %), CAT (27.27 & 55.97 %) and lipid peroxidation (16.89 & 16.39 %) over DCD (Table -4).

DISCUSSION

The present investigation clearly demonstrates the beneficial effects of dietary ingredients used in formulation of diet in maintaining the carbohydrate and antioxidant profiles which were altered by induction of diabetes. The cereals and millets used are the major source of energy in Indian diets as they are the important sources of several nutrients such as protein, calcium, iron, vitamin B-complex various antioxidants (alkaloids, flavonoids, saponins, tannins etc.) and fibres [28].

Administration of formulated diet for 8 weeks caused substantial decline in plasma glucose and hepatic glucose-6-phosphatase activity with an elevation in hepatic glycogen and hexokinase activity in CFD group as compared to CCD group. Induction of diabetes caused significant elevation in blood glucose levels, G-6-Pase activity and reduction in hepatic glycogen content and hexokinase activity in DCD group. On the other hand, with formulated diet, the diabetic animals exhibited lowered glucose levels and G-6-Pase activity with improved hepatic glycogen content and hexokinase activity. These observations suggest that the diet formulated is effective in controlling the carbohydrate metabolism in diabetic animals confirming the earlier findings. The components of the cereals and millets used in the present investigation are rich in dietary fibres that slow down gastric emptying delaying the absorption of glucose from the gut lumen thus preventing hyperglycemia [12, 14, 29, 30]. Additionally, polyphenols present in the formulated diet may also have contributed to lowering the levels of plasma glucose and hepatic G-6-pase while improving hepatic hexokinase and glycogen content [31, 32].

The diabetic animals fed the formulated diet showed clear improvements in hepatic and renal functions with reference to total protein, albumin, urea, creatinine and SGOT, SGPT, FRAP index over the animals fed commercial diet, indicating the positive effects of formulated diet. Insulin deficiency is known to increase protein catabolism releasing the amino acids that are used in gluconeogenesis [33] and further increase in the non-enzymatic glycation of proteins in diabetic states are thought to contribute to long term complications of diabetes [34]. Diabetes is also responsible for an increase in transaminase activities through damaged tissues [35] and the transaminases are believed to be responsible for gluconeogenesis and ketogenesis [36].

Chronically elevated blood glucose levels lead to oxidative stress subsequently resulting in formation of advanced glycation end products (AGE) [5, 6]. Due to oxidative stress excessive amounts of free radicals are formed that damage the cellular proteins, membrane lipids and nucleic acids and eventually causes cell death. The oxidative stress (decline in GSH, GPx, CAT, SOD, TAA and FRAP; elevation in lipid peroxidation) noted in the experimental groups confirms that induction of diabetes causes decreases in antioxidant enzyme activities in organs/ tissues [37, 38] and with dietary formulation, a reduction in the oxidative stress clearly indicates the role of the phytochemicals such as phenols, flavonoids, ferulic acid and saponin (present in the cereals and millets) in improving the GSH, GPx, CAT, SOD activities and TAA content while decreasing the lipid peroxidation [10, 39, 40].

The observed overall beneficial effects in terms of carbohydrate and antioxidant metabolism in formulated diet fed diabetic animals as compared to those fed commercial diet can be attributed to the phytochemical constitution of the grains used in the formulation of diet. For instance, cereals and millets are known to be rich sources of dietary fibers, antioxidants such as polyphenols and flavonoids etc., besides minerals and thus it is probable that the phytoconstituents may bring about their effects synergistically and reduce the impact of diabetes induced physiological alterations.

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Table 1: Plasma glucose and hepatic carbohydrate profiles

Groups→ Parameters↓	**CCD**	**CFD**	**DCD**	**DFD**	**% Recovery DCD *Vs* DFD**
Plasma					
Glucose mg/dl	83.39±4.85	69.18±1.84[a] (-17.04)	131.7±3.58[a] (+57.93)	107.03±1.62[b] (+54.71)	-18.73
Hepatic tissue					
Glycogen mg/gm	19.4±0.92	22.8±0.71[a] (+7.22)	13.3±0.96[a] (-59.29)	18.2±0.53[b] (-46.25)	+41.55
G-6-Pase U/mg protein	0.168±0.02	0.142±0.04[a] (-15.47)	0.503±0.02[a] (+199.40)	0.306±0.008[b] (+115.49)	-39.16
Hexokinase U/mg protein	28.67±0.28	30.74±0.38[a] (+17.52)	11.67±1.42[a] (-31.44)	16.52±0.32[b] (-20.17)	+36.84

CCD- Control group for commercial diet; CFD- Control group for formulated diet; DCD-Diabetic animals fed commercial diet; DFD- Diabetic animals fed formulated diet
Values are Means ± SEM (n=6); $p<0.05$ were considered statistically significant: a-compared with CCD; b-compared with DCD

Table 2: Plasma total protein, albumin and FRAP indices

Groups→ Parameters↓	**CCD**	**CFD**	**DCD**	**DFD**	**% Recovery DCD *Vs* DFD**
Total Protein mg/dl	7.54±0.15	8.4±0.13[a] (+11.40)	5.77±0.14[a] (-23.47)	7.39±0.27[b] (-12.02)	+28.07
Albumin mg/dl	2.84±0.15	3.01±0.11[a] (+5.98)	1.77±0.04[a] (-37.67)	2.22±0.09[b] (-26.24)	+25.42
FRAP µmole/ml	19.15±0.45	19.87±0.64[a] (+3.75)	9.56±0.69[a] (-50.07)	15.29±1.18[b] (-23.04)	+59.93

CCD- Control group for commercial diet; CFD- Control group for formulated diet; DCD-Diabetic animals fed commercial diet; DFD- Diabetic animals fed formulated diet
Values are Means ± SEM (n=6); $p<0.05$ were considered statistically significant: a-compared with CCD; b-compared with DCD

Table 3: Plasma SGOT, SGPT, Creatinine and Urea profiles

Groups→ Parameters↓	**CCD**	**CFD**	**DCD**	**DFD**	**% Recovery DCD *Vs* DFD**
SGOT U/L	23.06±2.1	22.01±1.26[a] (-4.55)	52.25±1.92[a] (126.58)	38.97±2.05[b] (77.05)	-25.41
SGPT U/L	47.0±2.19	22.33±1.31[a] (-52.48)	55.09±1.58[a] (17.21)	34.61±0.68[b] (54.99)	-37.17
Creatinine mg/dl	0.98±0.03	0.62±0.02[a] (-36.73)	1.24±0.2[a] (26.53)	0.82±0.01[b] (32.25)	-33.87
Urea mg/dl	43.29±2.98	32.71±2.89[a] (-24.43)	76.72±7.36[a] (77.22)	49.83±2.54[b] (52.33)	-35.04

CCD- Control group for commercial diet; CFD- Control group for formulated diet; DCD-Diabetic animals fed commercial diet; DFD- Diabetic animals fed formulated diet

Values are Means ± SEM (n=6); p<0.05 were considered statistically significant: a-compared with CCD; b-compared with DCD

Table 4: Hepatic and renal tissue lipid peroxidation and antioxidant profiles

Groups→ Parameters↓	**Tissue**	**CCD**	**CFD**	**DCD**	**DFD**	**% Recovery DCD *Vs* DFD**
TBARS nM MDA/100gm	Hepatic	257.7±8.81	271.7±7.97[a] (+5.43)	409.5±7.06[a] (+58.90)	340.3±4.44[b] (+25.24)	-16.89
	Renal	142.2±1.45	130.3±1.57[a] (-8.36)	404.5±2.41[a] (+184.45)	338.2±2.42[b] (+159.55)	-16.39
GSH U/mg protein	Hepatic	67.86±6.4	70.86±4.22[a] (+4.42)	49.9±5.95[a] (-26.46)	66.37±6.67[b] (-6.33)	+33.00
	Renal	105.8±4.06	125.2±3.89[a] (+18.33)	64.97±4.98[a] (-38.59)	98.3±2.56[b] (-21.48)	+51.30
TAA µgm/gm	Hepatic	68.93±1.64	88.19±1.28[a] (+27.94)	63.91±0.73[a] (-7.28)	92.09±1.10[b] (+4.42)	+44.09
	Renal	203.3±2.1	225.3±11.84[a] (+10.82)	130.7±1.33[a] (-35.71)	146.7±2.12[b] (-34.88)	+12.24
GPx U/mg protein	Hepatic	16.69±0.24	15.87±0.22 (-4.91)	13.01±0.16[a] (-22.04)	17.39±0.38[b] (+9.57)	+33.66
	Renal	14.31±0.36	12.69±0.53[a] (-11.32)	10.84±1.08[a] (-24.24)	13.37±1.06[b] (+5.35)	+23.33
SOD U/mg protein	Hepatic	0.594±0.03	0.662±0.04[a] (11.44)	0.464±0.02[a] (-21.88)	0.62±0.01[b] (-6.34)	+33.62
	Renal	1.64±0.17	1.89±0.37[a] (15.24)	0.48±0.01[a] (-70.73)	0.62±0.01[b] (-67.19)	+29.16
CAT nM of H_2O_2 decomposed/ sec/gm	Hepatic	109.0±3.89	115.2±2.91[a] (+5.68)	68.24±2.89[a] (-37.39)	86.85±2.74[b] (-24.60)	+27.27
	Renal	21.05±0.36	20.97±0.67 (-0.38)	17.99±0.47[a] (-14.53)	28.06±0.77[b] (+33.81)	+55.97

CCD- Control group for commercial diet; CFD- Control group for formulated diet; DCD-Diabetic animals fed commercial diet; DFD- Diabetic animals fed formulated diet

Values are Means ± SEM (n=6); p<0.05 were considered statistically significant: a-compared with CCD; b-compared with DCD