THERAPEUTIC BENEFITS OF GLIBENCLAMIDE IN FLUORIDE INTOXICATED DIABETIC RATS

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SUMMARY: To examine its antidiabetic potential in fluoride (F) intoxicated rats, the anti-diabetic drug glibenclamide was administered for 4 weeks to diabetic rats and to diabetic rats exposed to 100 mg NaF/L in the drinking water. In the F treated rats there was a significant reduction in plasma glucose, plasma and hepatic total lipids, cholesterol, triglycerides and plasma low-density lipoproteins (LDL), VLDL-cholesterol, and atherogenic index accompanied by significant increases in HDL-cholesterol, FRAP (ferric reducing ability of plasma) and protein content. Furthermore, significant decreases in SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase), alkaline and acid phosphatase (ALP and ACP), and glucose-6-phosphatase (G-6-Pase) were observed in these F-treated animals. In addition, administration of the drug decreased hepatic and renal lipid peroxidation with a concomitant increase in total ascorbic acid (TAA), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPX), and FRAP levels in the F-treated animals. It is proposed that glibenclamide acts at two levels: (i) at pancreatic islets for increased release of insulin from surviving β cells, (ii) at the target sites, e.g., hepatic tissue to improve glucose uptake leading to an improvement in the activities of hepatic and renal TAA, SOD, GSH, GPX, and reduction in lipid peroxidation. Glibenclamide may therefore be useful for treatment of diabetes in F endemic areas.

Keywords: Antioxidants; Diabetic rats; Fluorotic rats; Glibenclamide; Lipid peroxidation; Liver enzymes; Plasma glucose.

INTRODUCTION

Fluorosis is a metabolic disease caused by ingestion of excessive amounts of fluoride (F), mainly through drinking water and food in endemic areas.[1] F concentrations as low as 0.10 ppm or below to as high as 177 ppm have been reported in natural water resources.[2,3] In general, the severity of fluorosis varies widely, even with similar F concentrations in different geographical locations owing to factors such as nutritional status, climate and altitude, individual susceptibility and biological response, duration of fluoride exposure, and dissolved salts in drinking water.[4] Generation of free radicals, lipid peroxidation, and altered antioxidant defense systems are considered to play an important role in bringing about the toxic effects of F.[5-12] Chronic exposure to F also results in hyperglycemia besides the development of classical symptoms of fluorosis,[13-16] indicating the diabetogenic effect of F. As the incidence of diabetes is projected to increase by 4.4% worldwide from 2.8% in 2000, and the number of diabetics in India to reach 74 million by 2025,[17] it is conceivable that populations living in F endemic areas could also become part of a global diabetic population. The present study deals with the effect of F intake in diabetic conditions and administration of the standard antidiabetic drug glibenclamide on carbohydrate, lipid, and antioxidant profiles of diabetic laboratory animals exposed to F in drinking water.

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MATERIALS AND METHODS

Adult female Albino rats (Charles Foster strain) weighing 230–300 g used for this investigation were housed in the Animal House Facility of the Department and maintained at 26±2ºC with a 12-hr light/dark cycle and 60% relative humidity. The care and procedures had the approval of Institutional Animal Ethics Committee. After a 10-day adaptation period, 36 animals were divided into six equal groups, and four of them were each given a single intraperitoneal injection of alloxan monohydrate (120 mg/kg bw). The diabetic status induced was confirmed over a period of two weeks. Rats with blood glucose >140 mg/dL were considered diabetic and used. Group I (control: C), Group II (alloxan administered diabetic control: DC), and Group V (diabetic animals administered glibenclamide orally, 50 µg/kg bw/day: DG) were given normal water and *ad libitum* access to standard pelleted diet (Pranav Agro Ind. Ltd., Pune, India. Group III (fluoride control: FC), IV (diabetic animals with exposure to F: DFC), and VI (diabetic animals with exposure to F and treated with glibenclamide orally, 50 µg/kg bw/day: DFG) were given 100 ppm NaF in their drinking water for a period of 30 days and *ad libitum* access to the same pelleted diet. After 30 days the rats were fasted overnight and sacrificed under mild anesthesia. Blood was drawn from the heart, and liver and kidney tissues were removed immediately and stored at low temperatures for further biochemical analyses.

Plasma glucose levels were measured by the *o*-toluidine method,[18] plasma total lipid (TL) content was estimated by the sulphophosphovanillin method.[19] Plasma total cholesterol (TC) and triglyceride (TG) contents were estimated by ferric perchlorate-sulphuric acid and GPO methods, respectively.[20,21] HDL-C was extracted from plasma using phosphotungstate-magnesium chloride reagent[22] and estimated according to the method of Wybenga et al.[20] LDL-C, VLDL-C, and atherogenic index (AI) were calculated according to Friedewald's formula.[23] Plasma FRAP (total antioxidant potential) activity was estimated as described by Benzie and Strain.[24] Total protein content of the plasma was determined by the method of Lowry et al.[25]

Hepatic glycogen was extracted with 30% KOH, and the amount was determined by the anthrone-sulfuric acid method.[26] The hepatic lipids were extracted in a 2:1 mixture of chloroform and methanol[27] and estimated by gravimetric analysis. The same extract was used for the estimation of TC and TG (total cholesterol and triglycerides) contents by standard kits (Eve's Inn Diagnostics, Baroda).

Serum glutamate oxalocetate (SGOT) and pyruvate (SGPT) transaminase, alkaline and acid phosphatases (ALP, ACP) levels were determined using standard kits (Eve's Inn Diagnostics, Baroda). Hepatic glucose-6-phosphatase (EC 3.1.3.9) activity was determined using the method developed by Baginsky et al.[28]

The hepatic and renal lipid peroxidation (malondialdehyde concentration) was determined by the thiobarbituric acid (TBA) assay.[29] Total ascorbic acid was estimated using 2,4-dinitrophenylhydrazine reagent.[30] Superoxide dismutase (SOD; EC 1.15.1.1) was measured using the nitro blue tetrazolium reduction

method.[31] Reduced glutathione (GSH) and glutathione peroxidase (GPx; EC 1.11.1.9) were measured by reduction of DTNB and GSH consumption.[32,33] All the chemicals used were of analytical grade (SISCO Research Laboratories, Mumbai, India).

Data are presented as means ± 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 handled by Graph Pad Prism 3.0 statistical software. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Diabetes mellitus (DM) confers an increased risk of many devastating complications such as cardiovascular disease, peripheral vascular disease, coronary artery disease, stroke, nephropathy, retinopathy, and blindness.[34,35] Increased hepatic glucose output in DM is evidently due to increased glycogenolysis or gluconeogenesis or both.[36,37] Exposure of diabetic laboratory animals to various concentrations of F in their drinking water (5–50 ppm for 3 weeks to 6 months) is reported to further elevate blood glucose levels aggravating hyperglycemia.[38-40] The present study also confirms earlier findings that, when the diabetic animals are exposed to F, a further increase in plasma glucose levels and a decrease in hepatic glycogen content occur. Treatment with glibenclamide decreased the blood glucose levels and increased the glycogen content in both the F treated and the non-F treated rats.

Elevated levels of serum lipids in DM are a high risk factor for coronary heart disease.[41] In the present study, both the alloxan administered diabetic animals and those exposed to NaF exhibited significantly reduced plasma and hepatic lipid profiles when given glibenclamide. Reduced insulin secretion and a deficit in insulin function are known to enhance the metabolism of lipids from adipose tissue. Moreover, impairment in insulin sensitivity due to high concentrations of lipids in the cells is also known to elevate cardiovascular risk in DM.[42,43] It is well documented that while low levels of HDL-C are indicative of a high risk for coronary heart disease, an increase in HDL-C level is considered beneficial.[44] As found here, administration of glibenclamide to diabetic animals and diabetic subjects exposed to F in drinking water decreased LDL-C levels and elevated the levels of HDL-C. A deficiency in lipoprotein lipase activity in diabetics is reported to contribute to significant elevation of triglycerides.[45] Glibenclamide administration almost reversed these increases in plasma and hepatic triglycerides. In diabetic conditions various proteins are subjected to non-enzymatic glycosylation, and this is thought to contribute to the long-term complications of the disease.[46] In the present investigation, both the non-F exposed and the F-exposed animals registered a decrease in plasma protein content. Treatment with

glibenclamide, however, increased the protein content in both the cases (Tables 1 and 2).

Table 1. Plasma glucose, lipid profiles, FRAP, and total protein of experimental rats

Parameter	C	DC	FC	DFC	DG	DFG
Glucose mg/dL	96.87 ± 0.46	214.63 ± 1.23[a] (+121.56%)	195.92 ± 0.84[ab] (+102.25%)	218.73 ± 1.79[ac] (+125.80%)	111.45 ± 1.56[ab] (-48.07%)	109.08± 1.84[abc d] (-50.13%)
TL mg/dL	328.89± 1.59	449.45± 1.80[a] (+36.66%)	458.31 ± 2.11[ab] (+39.35%)	470.55 ± 1.76[abc] (+43.07%)	357.47± 2.47[ab] (-20.46%)	338.93± 1.76[abc d] (-27.97%)
TC mg/dL	109.83± 0.94	164.20±1.57[a] (+49.50%)	174.15 ± 1.65[ab] (+58.56%)	181.08 ± 0.75[abc] (+64.87%)	116.47 ± 1.16[ab] (-29.07%)	123.36± 1.16[abc d] (-31.87%)
TG mg/dL	71.81 ± 0.59	102.00 ± 0.87[a] (+42.04%)	105.21 ± 0.54[a] (+46.51%)	106.68 ± 0.71[ab] (+48.56%)	80.32 ± 0.82[ab] (-21.25%)	79.95 ± 0.90[abcd] (-25.06%)
LDL-C mg/dL	28.39 ± 0.98	92.28 ± 1.33[a] (+225.04%)	101.86 ± 1.24[ab] (+258.79%)	108.10 ± 1.14[abc] (+280.76%)	37.65 ± 1.27[ab] (-59.20%)	45.22 ± 0.78[abcd] (-58.17%)
VLDL-C mg/dL	14.44 ± 0.12	20.40 ± 0.18[a] (+41.27%)	21.04 ± 0.11[a] (+45.70%)	21.33 ± 0.14[ab] (+47.71%)	16.06 ± 0.16[ab] (-21.27%)	15.99 ± 0.18[abcd] (-25.03%)
HDL-C mg/dL	66.99 ± 0.59	53.18 ± 0.38[a] (-20.61%)	51.26 ± 0.85[a] (-23.48%)	51.65 ± 0.56[a] (-22.90%)	62.76 ± 0.48[ab] (+18.01%)	62.16 ± 0.33[abcd] (+20.35%)
AI mg/dL	1.63 ± 0.01	3.08 ± 0.02[a] (+88.96%)	3.40 ± 0.04[ab] (+108.59%)	3.51 ± 0.05[ab] (+115.34%)	1.85 ± 0.02[ab] (-39.93%)	1.98 ± 0.01[abcd] (-43.54%)
FRAP mmole / L	1089.5 ± 4.69	667.33 ±7.85[a] (-38.75%)	617.17 ± 3.83[a] (-43.35%)	609.17 ± 7.47[ab] (-44.09%)	834.17 ± 11.17[ab] (+25.00%)	811.00± 7.52[abc d] (+33.13%)
Total protein mg/dL	7.32 ± 0.36	4.26 ± 0.14[a] (-41.80%)	4.06 ± 0.09[a] (-44.53%)	3.98 ± 0.08[a] (-45.63%)	6.18 ± 0.11[ab] (+45.07%)	6.23 ± 0.17[abcd] (+56.53%)

Values are Means ± SEM (n=6); $p < 0.05$ were considered statistically significant; [a] compared with C; [b] compared with DC; [c] compared with FC; [d] compared with DFC

Increased activities of serum transaminases (SGOT and SGPT) in diabetic subjects (owing to the absence/low levels of insulin) are believed to be responsible for increased gluconeogenesis and ketogenesis.[47] In the present context, these enzymes registered high levels of activities in both diabetic rats and diabetic animals exposed to NaF compared to the controls. Glibenclamide administration to these animals resulted in a significant decline in the activities of these enzymes compared to the control groups. Chronic hyperglycemic and hyperlipidemic conditions are reported to damage the membrane architecture resulting in increased activities of ACP and ALP.[48] Here both groups of diabetic animals

demonstrated increased activities of ACP and ALP; glibenclamide substantially decreased the activities of these enzymes. Also to be noted is the role of glucose-6-phosphatase (G-6-Pase) for releasing glucose molecules into the blood.[49] Whereas the activity of G-6-Pase was high in both groups of diabetic rats, glibenclamide treatment resulted in significant decline in G-6-Pase activity (Table 3).

Table 2. Hepatic glycogen and lipid profiles of experimental rats

Parameter	C	DC	FC	DFC	DG	DFG
Glycogen mg/gm	21.65 ± 0.51	9.69 ±0.24[a] (-55.24%)	10.86 ± 0.15[a] (-49.84%)	9.78 ± 0.30[a] (-54.83%)	19.18 ± 0.33[ab] (+97.94%)	19.51 ± 0.29[abcd] (+99.49%)
TL mg/gm	32.18 ± 0.49	50.89 ± 0.58[a] (+58.14%)	52.84 ± 0.65[a] (+64.20%)	55.18 ± 0.37[a] (+71.47%)	30.88 ± 0.29[b] (-39.32%)	26.69 ± 4.74[bcd] (-51.63%)
TC mg/gm	1.83 ± 0.02	3.84 ± 0.03[a] (+109.84%)	3.85 ± 0.12[a] (+110.38%)	3.97 ± 0.04[a] (+116.94%)	2.72 ± 0.07[ab] (-29.17%)	2.35 ± 0.03[abcd] (-34.55%)
TG mg/gm	12.21 ± 0.22	22.92 ± 0.13[a] (+87.71%)	21.51 ± 0.31[ab] (+76.17%)	23.63 ± 0.28[ac] (+93.53%)	15.00 ± 0.10[ab] (-34.55%)	14.50 ± 0.21[abcd] (-38.64%)

Values are Means ± SEM (n=6); p< 0.05 were considered statistically significant; [a] compared with C; [b] compared with DC; [c] compared with FC; [d] compared with DFC

Table 3. Levels of phosphatases in the experimental animals

Parameter	C	DC	FC	DFC	DG	DFG
SGOT U/L	38.18 ± 1.13	71.09 ± 0.79[a] (+86.20%)	74.06 ± 1.07[a] (+93.97%)	75.70 ± 1.02[ab] (+98.27%)	45.44 ± 0.95[ab] (-36.08%)	46.97± 0.41[abcd] (-37.95%)
SGPT U/L	38.51 ± 0.68	80.25 ± 0.50[a] (+108.39%)	83.07 ± 0.81[a] (+115.71%)	86.62 ± 0.73[ab] (+124.93%)	54.79 ± 1.25[ab] (-31.72%)	60.18± 0.90[abcd] (-30.64%)
ALP K.A. Units	10.91 ± 0.27	47.93 ± 0.94[a] (+339.32%)	54.20 ± 0.51[ab] (+396.79%)	57.33 ± 0.78[abc] (+425.48%)	24.59 ± 0.61[ab] (-48.70%)	35.03 ± 0.97[abcd] (-38.90%)
ACP K.A. Units	5.72 ± 0.20	18.18 ± 0.38[a] (+217.83%)	21.37 ± 0.47[ab] (+273.60%)	23.92 ± 0.43[abc] (+318.18%)	8.74 ± 0.25[ab] (-51.92%)	9.35 ± 0.49[abcd] (-60.91%)
G-6-Pase U/mg/ protein/min	0.193± 0.005	0.527 ±0.004[a] (+173.06%)	0.558±0.006[ab] (+189.12%)	0.582±0.003[abc] (+201.55%)	0.342±0.002[ab] (-35.10%)	0.377±0.005[abcd] (-35.22%)

Values are Means ± SEM (n=6) ; p< 0.05 were considered statistically significant; [a] compared with C; [b] compared with DC; [c] compared with FC; [d] compared with DFC

The increased lipid peroxidation in the diabetic animals is reported to be due to the heightened concentrations of TBARS and hydroperoxides in both liver and kidneys.[50] In the present context, TBARS levels in liver and kidney were significantly higher in the DC, FC and DFC groups compared to the nondiabetic control group, and the total antioxidant potential of plasma and hepatic tissue (FRAP) was significantly lower. Administration of glibenclamide to the experimental animals resulted not only in lowering lipid peroxidation but also significantly increased the FRAP. Ascorbic acid is a well-known antioxidant and reportedly reduces F levels in the body.[51] A significant elevation in the levels of ascorbic acid was found in the animals treated with glibenclamide. It has been reported that the decrease in tissue GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetic and/or fluorotic conditions.[52,53] In the present study, the decreased levels of GSH due to induction of diabetes and exposure to F were overcome with the administration of glibenclamide. Glutathione peroxidase (GPx) is a selenium-containing enzyme that uses glutathione in decomposing H_2O_2 or other organic hydroperoxides to non-toxic products. [54] The reduced activities of GSH and GPx in liver and kidneys of both groups of diabetic animals significantly improved upon administration of glibenclamide. Superoxide dismutase (SOD) is an enzyme that is responsible for the conversion of superoxide radicals into less harmful products like hydrogen peroxide. It also eliminates secondary toxicity of OH radicals and H_2O_2 by decreasing the concentration of superoxide radicals.[55] In the present context, the levels of SOD decreased in the diabetic animals and diabetic animals exposed to F as compared to controls. When glibenclamide was administered both groups registered a significant increase in SOD activity (Tables 4 and 5).

Table 4. Hepatic lipid peroxidation and antioxidants of experimental animals

Parameter	C	DC	FC	DFC	DG	DFG
TBARS nM MDA / gm	63.86 ± 0.21	63.43 ± 0.60[a] (+30.64%)	42.07 ± 0.26[ab] (+28.51%)	70.05 ± 0.44[abc] (+41.01%)	48.80 ± 0.29[ab] (-41.51%)	41.67 ± 0.34[abd] (-53.72%)
TAA µg/gm	118.98± 0.23	98.99 ± 0.70[a] (-16.80%)	94.00 ± 0.47[ab] (-20.99%)	90.46 ± 0.54[abc] (-23.97%)	108.16 ± 0.45[ab] (+9.26%)	107.26 ±0.49[abcd] (+18.57%)
SOD U / mg protein	5.22 ± 0.15	2.22 ± 0.09[a] (-57.47%)	3.20 ± 0.07[ab] (-38.70%)	3.07 ± 0.08[ab] (-41.88%)	4.31 ± 0.14[ab] (+94.14%)	4.51 ± 0.09[abc] (+46.90%)
GSH mg / 100gm	39.47 ± 0.31	31.21 ± 0.36[a] (-20.93%)	28.22 ± 0.21[ab] (-28.50%)	30.00 ± 0.39[ab] (-23.99%)	37.35 ± 0.40[ab] (+19.67%)	36.01 ± 0.18[abcd] (+20.03%)
GPX U / mg protein	8.20 ± 0.35	3.80 ± 0.07[a] (-53.66%)	3.03 ± 0.04[ab] (-63.05%)	3.36 ± 0.05[a] (-59.02%)	6.06 ±0.06[ab] (+59.47%)	6.90 ± 0.09[abcd] (+105.36%)
FRAP mmole / gm	43.07 ± 0.27	32.85 ± 0.21[a] (-23.73%)	26.56 ± 0.22[ab] (-38.33%)	28.86 ± 0.20[abc] (-32.99%)	38.62 ± 0.21[ab] (+17.56%)	38.58 0.30[abcd] (+33.68%)

Values are Means ± SEM (n=6); $p < 0.05$ were considered statistically significant; [a] compared with C; [b] compared with DC; [c] compared with FC; [d] compared with DFC

Table 5. Renal lipid peroxidation and antioxidants of experimental animals

Parameter	C	DC	FC	DFC	DG	DFG
TBARS nM MDA / gm	26.78 ± 0.53	40.40 ± 0.38[a] (+50.86%)	33.155 ± 0.39[ab] (+23.79%)	33.58 ± 0.42[ab] (+25.39%)	31.36 ± 0.35[ab] (-22.38%)	30.82 ± 0.49[abcd] (-8.22%)
TAA µg/gm	55.94 ± 0.32	42.18 ± 0.21[a] (-24.60%)	42.56 ± 0.31[a] (-23.92%)	37.91 ± 0.45[abc] (-32.23%)	48.27 ± 0.19[ab] (+14.43%)	44.87 ± 0.62[abcd] (+18.36%)
SOD U / mg protein	3.89 ± 0.03	2.69 ± 0.06[a] (-30.85%)	2.71 ± 0.11[a] (-30.33%)	2.83 ± 0.13[a] (-27.25%)	3.45 ± 0.12[ab] (+28.25%)	3.52 ± 0.07[bcd] (+24.38%)
GSH mg / 100gm	10.83 ± 0.11	7.95 ± 0.11[a] (-26.59%)	7.98 ± 0.09[a] (-26.31%)	7.05 ± 0.07[abc] (-34.90%)	8.99 ± 0.14[ab] (+13.08%)	8.90 ± 0.14[abcd] (+26.24%)
GPX U / mg protein	4.05 ± 0.06	2.52 ± 0.09[a] (-37.78%)	2.55 ± 0.15[a] (-37.04%)	2.18 ± 0.10[a] (-46.17%)	3.44 ± 0.08[ab] (+36.51%)	3.72 ± 0.08[bcd] (+70.64%)

Values are Means ± SEM (n=6); $p < 0.05$ were considered statistically significant; [a] compared with C; [b] compared with DC; [c] compared with FC; [d] compared with DFC

In conclusion, the present study demonstrated positive and beneficial effects of glibenclamide in diabetic rats exposed to NaF in their drinking water. Known for its effective antidiabetic activity, glibenclamide appeared to normalize the F induced changes in carbohydrate, lipid, and antioxidant metabolism in diabetic rats as it does in non-F exposed diabetic rats.

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