RESEARCH PAPER | Biochemistry | Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

Anti-Diabetic Activity of Dried Moringa Oleifera Leaves in Normal and Streptozotocin (Stz)-Induced Diabetic Male Rats

KEYWORDS: Antidiabetic, Moringa oleifera, Streptozotocin, glibenclamide

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ABSTRACT *The prevalence rate of diabetes mellitus continues to increase all over the world. Medicinal plants constitute an important source of potential therapeutic agents for diabetes. Moringa oleifera has anti-cancer, anti-inflammatory and some researchers reported its hypoglycaemic potential. This study aimed to determine the antihyperglycemic effect of dried Moringa oleifera leaves powder or its ethanolic extract in STZ-induced diabetic male rats and on normal rats as well. One hundred and forty rats were included and divided into 7 groups (20/group). The active ingredients of Moringa oleifera were determined through HPLC analysis. A significant increase in body weight was found in normal groups treated with M. oleifera leaves powder. A significant increase in blood glucose, plasma and liver; and a significant ($P < 0.001$) reduction in insulin, liver glycogen, protein, super oxide dismutase and total antioxidant capacity level were observed in the STZ-induced diabetic rats. These changes were reversed by treatment with dried leaves of M. oleifera or its ethanolic extract. The administration of the M. oleifera leaves powder also caused a reduction in blood glucose level in normal rats. Moringa olifera leaves or its ethanolic extract can reduce reactive free radicals that might lessen oxidative damage and this might be due to rich presence of flavonoids which have antioxidant property.*

Although M. oleifera leaves powder or its ethanolic extract has hypoglycaemic activity in STZ-induced diabetic rats but hypoglycaemic properties of leaves in normal rats needs more study and elucidation.

Introduction

Diabetes is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion and disturbances in carbohydrate, protein and lipid metabolism (1). The International Diabetes Federation has predicted that the number of individuals with diabetes will increase from 240 million in 2007 to 380 million in 2025 with 80% of the disease burden in low and middle-income countries (2). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low cost (3). Most of the plants prescribed for Diabetes Mellitus are not edible and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease. It is the purpose of this experiment is to evaluate the effect of dried leaves powder or the ethanolic extract of Moringa Oleifera on blood glucose levels of Streptozotocin-Induced diabetics' rats.

Moringa oleifera belongs to the family of Moringacaea, a fast growing drought resistant tree but now distributed world wide in the tropic and sub tropics and is cultivated extensively in Central and South America, Africa, Indonesia, Mexico, Malaysia, the Philippines, and India (4). Moringa oleifera is an edible plant. Different parts of Moringa plant contain important minerals as K, Ca, P, Fe, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics as zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol (5) and high concentrations of natural dietary antioxidants: Vitamins A, C and E. Moringa provides high concentrations of four natural dietary antioxidants: Vitamins A, C, E and phenolics (6-8). Moringa contains 46 antioxidants which help cells to neutralize free radicals. It is traditionally used for relieving spasm, for treatment of diarrhea, diuretic and stimulant in paralytic affliction, epilepsy and hysteria (9) and treatment of diabetes mellitus (10); hepatotoxicity (11), rheumatism, venomous bites and also for cardiac stimulation (12). Moringa oleifera is very useful in regulating the thyroid hormone status in adult Swiss rats (13). Its leaves are also used as nutritional supplement and growth promoters (14, 15).

Materials and methods

This study was approved by the high society of scientific ethic committee of NNI (National Nutrition Institute) & GOTHI (General Organization for Teaching Hospitals and Institutes).

Chemicals Used

All chemicals used were of analytical grade.

Plant Extract Preparation

The leaves of Moringa oleifera were dried under shade (at room temperature) and ground into powder. The powder was macerated in 70% ethanol and placed in a shaker at room temperature for 24 hours. The mixture was then filtered using Whatmann filter paper. The process was repeated 3 times and the filtrate was collected then evaporated to dryness using rotatory evaporator at low temperature. A brownish residue weighing 80 gm/kg dried leaves powder (8 %) was obtained. The extract was kept in air tight brown bottle in a refrigerator until used.

Nutritional value and composition determination

Dried powdered Moringa leaves were assessed for protein, fat, ash, fibre, calcium (Ca), potassium (K), phosphorus (P), iron (Fe), selenium (Se) and vitamins as A, C, E and β-carotene according to Association of Official Agricultural Chemists (16) procedures. They were analysed for their fatty acid profile using GC mass. Lipid extraction was conducted using method of AOAC, 2000 (17). Separation of fatty acids (saponification, preparation of diazomethane, then methylation) was carried out using method of Vogel, 1975 (18). Identification and determination of saturated and unsaturated fatty acids was conducted using gas liquid chromatography (GLC, GC trace GC ULTRA) according to Farag et al., 1986 (19).

Phytochemical screening

A preliminary phytochemical screening of the leaves extract of Moringa oleifera was also done using the standard phytochemical reagents and procedures as described by (20-21) and standard methods of analysis (22). Also they were analyzed by HPLC according to Iswaldi et al., 2013 (23).

Animals used

One hundred and fourty (140) Sprague Dawly male rats weighing between (200-240 g) of 3 months age were used. All rats were housed individually in wire meshed cages. The animals were fed on a standard rat diet for 10 days for ac-

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

climatization and water was ad libitum. Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ, Sigma, St. Louis, Missouri, USA) at a dose of 50 mg/ kg body weight. STZ was dissolved immediately before use in 0.05 mol/ L sodium citrate (pH 4.5). STZ-injected animals exhibited massive glycosuria and hyperglycemia within 2-3 days.

Blood was drawn from the tail vein and blood glucose was measured using Bionime, Rightest, GM 300 instruments. Rats were considered diabetic only if their blood glucose levels exceeded 250 mg /dl (24). Rat diet and body weights were also recorded on a weekly basis.

The standard rat chow diet (AIN-93 M diet formulated for adult rodents) was prepared according to 25-26.

Experimental design:
Rats were divided into seven groups as follows taking into consideration that rats group (GII-GV) were made diabetic:

1 Group I: Control rats received standard normal diet.
2 Group II: Diabetic rats (Diabetes was induced by a single intraperitoneal injection of streptozotocin, 50 mg/kg body weight).
3 Group III: Diabetic rats treated with glibenclamide (5 mg/ kg body weight in aqueous solution).
4 Group IV: Diabetic rats treated with Moringa oleifera leaves powder (15gm/kg BW/day, 16% in diet).
5 Group V: Diabetic rats treated with ethanolic extract of Moringa oleifera leaves powder (1 g/kg BW/day, I. P.).
6 Group VI: Normal rats treated with Moringa oleifera leaves powder (15gm/kg BW/day, 16% in diet).
7 Group VII: Normal rats treated with ethanolic extract of Moringa oleifera leaves powder (1 g/kg BW/day, I. P.).

Blood Sampling
At the end of the experiment (45 days) fasting blood samples were drawn and collected in 3 tubes, 2 of them with anticoagulant then centrifuged. They were kept at -70 °C if not analyzed immediately.

Assay of Biochemical Parameters
Glucose was determined using Randox kit (27). HbA1C was determined using Human kits (Human Gessellschaft Für Biochemica und Diagnostica, mbh, Wiesbaden, Germany) according to (28). The protein was estimated by the method of Peters 1968 (29); Total antioxidant capacity was measured using Bio-diagnostic kits (Cairo, Egypt) according to (30). Malondialdehyde was determined in liver and plasma according to the method of (31). Superoxide dismutase (SOD) was determined according to (32). Liver glycogen was determined according to the method described by (33). Insulin was determined using rat insulin ELISA kit EIA 2018 (DRG International Inc, USA) according to (34). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance was calculated from the product of the fasting concentrations of plasma insulin (microunits per milliliter) and plasma glucose (millimoles / liter) divided by 22.5 according to (35).

Toxicity and LD $_{50}$ experiment
Toxicity of the extract was also studied by LD 50 experiment to determine the range of the lethal dose and the safe range for the extract. Six groups of six rats each of both sexes (3 females and 3 males) weighing about 200 g were orally administered a dose of (100, 500, 1000, 3000, 5000 mg/kg) of the ethanolic extract of M. oleifera. Rats were then observed continuously for their gross behavioural, death rate and toxic effects up to 24 h. Doses up to 10 gm/kg body weight were observed to be safe (with no recorded deaths), i.e. LD 50 > 5 gm%. The dose used in this study was carefully chosen to exclude the lethal range.

Statistical analysis
Data are expressed as Mean ±SEM. All statistical data and significance tests (T Test for comparison between individual groups and control group; and post hoc Duncan test analysis for comparison between groups) were performed using the Statistical Package for the Social Sciences version 11 (SPSS Inc, Chicago, IL, USA). Statistical significance was accepted at $P < 0.05$.

RESULTS

Toxicity and LD $_{50}$
No toxic effect was observed on treatment with doses up to 5 gm/kg of the ethanolic extract as the behaviour of the treated rats appeared normal and no death occurred in any of these groups.

Nutritive value and chemical composition
Moringa olifera has high nutritional value and is a good source of protein, vitamins, β-carotene, amino acids and various phenolics. M. oleifera leaves contain (g/100 g dry weight) large amounts of calcium (2.79) and significant amounts of selenium (2.65 mg/100g dry weight) and phosphorus (0.32). The leaves also contain high amount of protein (28.34 g)

Table (1): Nutritive value and chemical composition of dried leaves of Moringa oleifera L/100 gm.

Nutritive value (qm %)			Minerals (gm)		
Protein	28.34	9X the protein of yogurt (3.2 qm%)	Ca	2.79 g	17X Ca than milk (0.12 gm%)
Fat	7.3		P	0.32	
Ash	10.5		Fe	0.04	25X Fe than spinach (0.00114
Moisture	7.9		Se	0.39	
Fiber	16.78		K	1.28	15X the K of bananas (0.088 qm%)
Carbohydrate	45.96				
Fatty acids (% Fat)			Vitamins (mg)		
Total saturated fatty acids (SFA)	44.11		Vitamin E	87	
Total unsaturated fatty acids (USFA)	55.89		β-carotene	17.65	
Total Omega-3 fatty acids (n-6)	43.87		Vitamin C	50	50% Vitamin C than oranges (30 mg%)
Total Omega-6 fatty acids (n-6)	7.94		Vitamin A	15.82	10X Vitamin A than carrots

Phytochemical screening
Result of the preliminary phytochemical screening of Moirnga oleifera extract revealed the presence of flavinoids, tannin, anthraquinone, cardiac glycosides alkaloids, triterpenoids, saponins, reducing sugars and phenolic acids. Quercetin and kaempferol, as 3'-O-glycoside forms, are the predominant flavonols in Moringa oleifera leaves. Chlorogenic acid (caffeic acid) and quinic acid, is a major phenolic acid in M. oleifera leaves. The flavonol quercetin is found at high concentrations

Table(2-a):Phytochemical Screening of Leaves Extracts of Moirnga oleifera			Table (2-b): HPLC analysis of Leaves Extracts of Moirnga oleifera	
		qm%	mg/100g	
Cyanogenic glycosides	ND			
Cardiac glycosides	++			
Steroid glycoside	++		Gallic	12.34
Saponins	++	0.11	Pyrogallol	100.0
Tannins	++	0.35	Catachin	25.45
Alkaloids	++		Catechol	74.89
Flavonoids	++		Coumaric	0.99
Anthroquinones	+		Caffeic	18.11
Terpenoids	++		Quercetin	22.42

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

Polyphenol	++	2.93	Kaempferol	17.45
+: Relative abundance of compounds; ND: Not Detected				

Body weight (BW) and body weight gain (BWG)

At the beginning of the experiment, the groups were matched for age and weight with no significant differences between them. In the present study, STZ-induced diabetes induced significant weight loss. Administration of glibenclamide, Moringa oleifera leaves powder or its ethanolic extract to diabetic rats minimized body weight loss.

In the present study, treatment of normal rats with Moirnga oleifera leaves showed a significant increase in body weight when compared with their respective initial body weight respectively (the % increase reach 30.67, 33.36 % for G1, G6 when compared with their respective initial body weight respectively) and also a significant increase was observed when comparing with the normal control group, while no change was observed when normal rats was treated with ethanolic Moirnga oleifera leaves extract (Table 3). Normal rats treated with Moirnga oleifera leaves showed the highest body weight gain (75.8±1.59 gm).

Table (3): The mean values of body weight in normal, diabetic and treated groups with glibenclamide, Moringa oliferea leaves powder or its ethanolic extract.

	Mean initial Bodyweight (IBW, g)	Mean final bodyweight (FBW, g)	Mean body weight gain (BWG, g)
G 1	222.202.41	290.35±3.69	68.15±2.20
G 2	223.251.87	204.50±1.96 [a]	-18.75±0.59 [a]
G 3	225.951.40	277.60±1.81 [a,b]	51.65±1.89 [a,b]
G 4	225.601.23	281.95±1.48 [a,b]	56.35±1.93 [a,b]
G 5	225.35±1.95	280.70±2.13 [a,b]	55.35±3.29 [a,b]
G 6	225.401.37	301.20±1.31 [a,b,c,d,e]	75.80±1.59 [a,b,c,d,e]
G 7	224.10±1.41	288.90±1.18 [b,c,d,e]	64.80±2.09 [b,c,d,e]

n = 20, Values are expressed as mean ± SEM; P< 0.001, G 1: Control; G 2: Diabetic Control; G 3: diabetic treated with Glibenclamide; G 4: diabetic treated with Moringa olifera leaves; G 5: diabetic treated with ethanolic extract of Moringa olifera leaves; G 6: Normal treated with Moringa olifera leaves; G 7: normal treated with ethanolic extract of Moringa olifera leaves: normal treated with ethanolic extract of Moringa olifera leaves.

Changes in blood glucose, HbA1c Insulin, HOMA-IR and liver glycogen

Table 4 showed the results of the effect Moirnga oleifera leave or its ethanolic extract on glucose, HbA1c, insulin and HOMA-IR level. Serum levels of glucose and HbA1C of streptozotocin-induced diabetic group were significantly higher (P< 0.001) than control group and decreased significantly (P< 0.001) in all treated diabetic groups compared to streptozotocin-induced diabetic group. The decrease reaches about 43% but still significantly higher than normal control group. The levels of glucose in normal rats treated with Moringa leaves or its extract showed a significant decrease (13, 11 % respectively, P< 0.01). The levels of HbA1C in normal rats treated with Moringa leaves or its extract showed non significant decrease.

The insulin level was significantly decreased in diabetic group when compared with normal group. Treatment with ethanolic extract of Moringa oleifera and glibenclamide significantly increased the level of insulin when compared with diabetic group.

The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance was significantly increased in diabetic group compared to control group and decreased significantly in all treated diabetic groups (treated with glibenclamide, Moringa leaves or its extract) compared to diabetic group.

Table (4): Glucose, Insulin, HOMA-IR and HbA1c level in STZ-induced diabetic and normal rats treated with Moringa olifera dried leaves or its ethanolic extract.

	Glucose mg/dl	Glucose (mmole/l)	Insulin (µU/ml)	HOMA-IR	HbA1c (g/dl)
G 1	92.80±2.02	5.16±0.11	12.32±0.14	2.82±0.06	5.41±0.10
G 2	256.35±2.32 [a]	14.24±0.13 [a]	8.75±0.18 [a]	5.54±0.13 [a]	8.19±0.16 [a]
G 3	128.73±3.23 [a,b]	7.150.18 [a,b]	10.55±0.21 [a,b]	3.37±0.13 [a,b]	6.230.12 [a,b]
G 4	144.60±2.11 [a,b,c]	8.030.12 [a,b,c]	11.09±0.24 [a,b]	3.96±0.11 [a,b,c]	6.540.09 [a,b,c]
G 5	146.10±1.60 [a,b,c]	8.12±0.09 [a,b,c]	10.68±0.10 [a,b]	3.85±0.06 [a,b,c]	6.58±0.09 [a,b]
G 6	81.64±1.29 [a,b,c,d,e]	4.560.07 [a,b,c,d,e]	12.74±0.13 [a,b,c,d,e]	2.58±0.04 [a,b,c,d,e]	6.28±0.09 [a,b]
G 7	83.27±0.80 [a,b,c,d,e]	4.63±0.04 [a,b,c,d,e]	12.66±0.17 [b,c,d,e]	2.60±0.04 [a,b,c,d,e]	6.16±0.09 [a,b,d,e]

G1: Control; G 2: Diabetic Control; G 3: diabetic treated with Glibenclamide; G 4: diabetic treated with Moringa olifera leaves; G 5: diabetic treated with ethanolic extract of Moringa olifera leaves; G 6: Normal treated with Moringa olifera leaves; G G 7: normal treated with ethanolic extract of Moringa olifera leaves: normal treated with ethanolic extract of Moringa olifera leaves

Liver glycogen decreased significantly in diabetic group compared to control group and increased significantly in all treated diabetic groups (treated with glibenclamide, Moringa leaves or its extract) compared to diabetic group (Table 5) but significantly lower than normal control group. Also treatment of normal rats with Moringa oleifera leaves or its ethanolic extract showed significant decrease in glycogen content.

Protein

Protein level decreased significantly in diabetic group compared to control group and a significant improvement in total protein levels on treatment with M. oleifera leaves or its ethanolic extract. No significant change was observed in normal group treated with M. oleifera leaves or its ethanolic extract.

Lipid Peroxidation and Antioxidant activity

Liver or plasma MDA is found to be significantly increased in STZ-induced diabetic rats. Treatment with ethanolic extract of Moringa oleifera and glibenclamide significantly decreased the level of liver or plasma MDA when compared with diabetic group. No significant change was observed in normal group treated with M. oleifera leaves or its ethanolic extract.

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

SOD and total antoxidant capacity were found to be significantly decreased in STZ-induced diabetic rats. Treatment with ethanolic extract of Moringa oleifera and glibenclamide significantly decreased SOD and total antoxidant capacity when compared with diabetic group. No significant change was observed in normal group treated with M. oleifera leaves or its ethanolic extract.

Table (5): Glycogen, protein, P & L MDA, SOD levels and total antioxidant capacity in STZ-induced diabetic and normal rats treated with Moringa olifera dried leaves or its ethanolic extract.

	Glucogen (mg/g tissue)	Protein (g/100 ml)	P. MDA (nmol/L)	L. MDA (nmol/g)	SOD	Total antioxidant Capacity (TAC, mmol/l)
G 1	23.16±0.53	6.61±0.07	62.95±0.95	61.57±0.81	80.24±1.29	2.31±0.02
G 2	12.70±0.14 [a]	5.38±0.06 [a]	134.96±2.31 [a]	124.75±2.31 [a]	41.81±0.82 [a]	1.82±0.02 [a]
G 3	18.83±0.48 [a.b]	5.88±0.05 [a.b]	93.18±1.46 [a.b]	87.48±1.31 [a.b]	65.41±1.10 [a.b]	2.12±0.05 [a,b]
G 4	17.26±0.31 [a.b,c]	5.97±0.07 [a.b]	89.73±0.87 [a.b]	73.16±0.85 [a.b,c]	68.49±1.18 [a.b]	2.14±0.01 [a,b]
G 5	15.65±0.14 [a.b,c,d]	5.860.07 [a.b]	94.33±0.79 [a.b,d]	72.80±0.40 [a.b,c]	68.40±0.65 [a.b,c]	1.99±0.02 [a,b,c,d]
G 6	19.25±0.36[a.b,c,d,f]	6.56±0.06 [b,c,d,e]	64.89±0.98[a.b,c,d,f]	63.50±1.62 [b,c,d,e]	81.16±1.35 [b,c]	2.31±0.04 [b,c,d,e]
G 7	18.32±0.29[a,b,d,e]	6.60±0.06 [b,c,d,e]	64.96±1.20[a,b,c,d,e]	64.202.12[b,c,d,e]	80.87±0.74 [b,c]	2.27±0.02 [b,c,d,e]

G 1: Control; G 2: Diabetic Control; G 3: diabetic treated with Glibenclamide; G 4: diabetic treated with Moringa olifera leaves; G 5: diabetic treated with ethanolic extract of Moringa olifera leaves; G 6: Normal treated with Moringa olifera leaves; G G 7: normal treated with ethanolic extract of Moringa olifera leaves: normal treated with ethanolic extract of Moringa olifera leaves

DISCUSSION

Toxicity and LD $_{50}$

Treatment with different doses (up to 5gm/Kg BW) of Moirnga oleifera leave or its ethanolic extract was well tolerated by all the animals, as there were no toxic effects observed by direct visual observation of the animals throughout the experiment. There was no death and apparent behavioural changes recorded during the period of the experiment in all treatment groups as compared to the control group. This might suggest the non-toxic effect of the plant leaves or its ethanolic extract at these levels.

According to 36, LD 50 of the ethanol extract was 39.6 g/kg, while aqueous extract of Moringa oleifera leaves showed LD 50 of 16.1g/kg. High LD 50 of the extract indicates its high margin of safety. This meant that in order for someone to die from acute Moringa oleifera leaves toxicity, one needed to eat more than 1 Kg in a single dose (36). Also using rats, Adedapo et al., 2009 (37) examined the safety of an aqueous extract of M. oleifera taken orally and they stated that nutritional and therapeutic consumption of M. oleifera leaves at doses below 2g/kg BW is safe without any sign of toxicity or mortality. Also the toxicity studies revealed nontoxic nature of the Moringa oleifera leaves at a concentration of 2 and 5gm/kg of body weight/day for a period of 14 days (38).

Mechanism

Streptozotocin is a naturally occurring nitrosamide extracted from Streptomyces acromogenes (39), used to develop animal models of diabetes by exerting cytotoxic effect on pancreatic β-cells possibly by generating lipid peroxides and excess reactive oxygen species (ROS), interfering with glucose transporter GLUT-2 and causing DNA damage either by alkylation or peroxynitrite formation (40). The DNA strand breakage by streptozotocin activates poly ADP-ribose polymerase (PARP) and causes ATP depletion leading to cell death and drop in insulin level (41).

To assess therapeutic efficacy of Moringa oleifera leaves or its ethanolic extract we chose glibenclamide, a member of sulfonylurea drugs used in treatment of type II diabetes. The mechanism of action of glibenclamide was reported to be inhibition of a K ATP channel leading to depolarization of pancreatic β cells and stimulation of insulin release (39, 42).

Nutritive value and chemical composition

There are considerable variations among the nutritional values of Moringa, which depend on factors like location, genetic background, environment and cultivation methods (43, 44). As such, it necessitates determination of the nutritive value of Moringa of Egypt. Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (45).

Moringa olifera has high nutritional value and is a good source of protein, vitamins, β-carotene, amino acids and various phenolics which agree with (5). M. oleifera leaves contain (g/100 g dry weight) large amounts of calcium (2.79) and significant amounts of selenium (2.65 mg/100g dry weight) and phosphorus (0.32). The leaves also contain high amount of protein (28.34 g) (Table 1).

Just 100 grams of fresh Moringa leaves will provide a child ages 1-3 years with all his daily requirements for calcium, about 75% of his iron and about half of his protein needs, as well as important supplies of K, B vitamins, and all the essential amino acids. For a pregnant or breast-feeding woman, 10 grams of fresh Moringa leaves can supply over a third of her daily Ca requirements as well as provide necessary quantities of Fe, protein and B vitamins. Indeed, for children under three, 30g of dry leaf powder can cover one third of the daily allowance for proteins, 75% of the calcium needs, more than half of the iron necessary, the totality of the recommended dietary allowance for vitamin A, and almost one third of the needs in vitamin C. The leaf powder is also an interesting dietary supplement for pregnant and lactating women (46). Moringa leaves are natural sources of calcium and multivitamins with high bioavailability.

Phytochemical screening

Result of the preliminary phytochemical screening of Moirnga oleifera leaves or its ethanolic extract revealed the presence of flavinoids, tannin, anthraquinone, cardiac glycosides alkaloids, triterpenoids, saponins, reducing sugars and phenolic acids which agree with (7, 47). Quercetin and kaempferol, as 3′-O-glycoside forms, are the predominant flavonols in Moringa oleifera leaves. Biologically, flavonoids are best known for their antioxidant properties (48). Chlorogenic acid (caffeic acid) and quinic acid, is a major phenolic acid in M. oleifera leaves which agree with (49, 7). The flavonol quercetin is found at high concentrations which agree with (50).

Body weight

The rate of food intake of the rats given Moirnga oleifera leave or its ethanolic extract (1g/kg BW) was comparable

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

with those of the control group.

STZ- induced diabetes is characterized by severe loss in body weight (51) and this may be because of damaged insulin-secreting cells in pancreatic islets (52). Hence, the weight gain after administration of the extract in diabetic rats is simply due to the ability of the extract to reduce hyperglycemia.

In the present study, diabetes induced significant weight loss which agree with the findings of Torres et al., (1999 (53) due to excessive breakdown of tissue proteins (54) as well as muscle wasting, dehydration and catabolism of fats (55). Administration of glibenclamide, Moringa oleifera leaves or its ethanolic extract to diabetic rats minimized body weight loss which suggests interruption, at least partially, of the previously mentioned metabolic derangements.

In the present study, treatment of normal rats with Moirnga oleifera leave or its ethanolic extract had no effect on body weight although the plant leaves is known to be a good source of nutrition since it contain important nutrients such as vitamins, proteins, minerals, carbohydrates and fats. It also contains calcium which is essential at all ages irrespective of physiological status of individuals and for the normal development of skeletal system (56). This result disagree with (57) where they found a significant increase in body weight of mice treated with Moringa stenopetala, another species of Moringa.

Blood glucose, HbA1c, HOMA-IR and glycogen levels of Streptozotocin-induced diabetic male rats

Data of table (2) reveal that dry Moringa oleifera leaves powder (DMOLP) or its ethanolic extract reduces the blood glucose, HbA1c and glycogen level in STZ induced-diabetic rats. Glibenclamide was used as reference drug in diabetic models for positive control. It is interesting to note that dried leaves or its ethanolic extract was more effective than reference drug and this may be because leaves might have some direct effect by increasing the tissue utilization of glucose (58), by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues (59) or through stimulating the β-cells due to presence of terpenoids, or due to its insulin-like activity. Also leaves have quercetin which is considered as a potent antioxidant (60) with multiple therapeutic properties (61). It has anti-diabetic effects in the obese Zucker rat model of metabolic syndrome (62). It can protect insulin-producing pancreatic β cells from STZ-induced oxidative stress (63). Also leaves have chlorogenic acid which has an effect on glucose metabolism through inhibition of glucose-6-phosphate translocase in rat liver, reducing hepatic gluconeogenesis and glycogenolysis (64).

In the present study, treatment of normal rats with Moirnga oleifera leave or its ethanolic extract showed a significant decrease (-13, -11% respectively) on glucose level when compared with normal control group and this might be due to its insulin-like activity or presence of terpenoids (stimulate β-cells). This result agrees with (65-66) where they found a significant decrease in blood glucose level of rats and rabbits (respectively) treated with aqueous extract of Moringa olifera leaves.

The decreased level of glycogen agrees with (67) where they use alloxan to induce diabetes mellitus.

Protein

Significant improvement in total protein levels on treatment with extract for 45 days indicated that it has favourable effect in bringing down the severity of diabetes that the extract may have a mixture of biomolecules with hydroxyl groups that prevent the abstraction of hydrogen atom from the double bond of lipid bilayer thereby avoiding the damage of lipid membrane.

Lipid Peroxidation and Antioxidant activity

MDA is considered as an important indicator of lipid peroxidation which is found to be increased in STZ-induced diabetic rats. This might be due to lipid peroxidation. Rat treatment with Moringa olifera showed protection against lipid peroxidation characterised by significant decrease in MDA level.

The antioxidant enzyme superoxide dismutase (SOD) is considered the first line of defensive enzymes against free radicals. In the present study, there is a significant decrease in activity of superoxide dismutase (SOD) with a significant increase in malondialdehyde (MDA) concentration in blood and plasma of STZ-induced diabetic rats and it was observed that treatment with Moringa olifera leaves or its ethanolic extract significantly increases SOD in STZ-induced diabetic rats and significantly decreased MDA levels. This shows that the Moringa olifera leaves or its ethanolic extract can reduce reactive free radicals that might lessen oxidative damage and this might be due to rich presence of flavonoids which have antioxidant property. Moringa oleifera is a rich source of antioxidant (68, 69) such as quercetin and kaempferol (major bioactive compounds of phenolics) and are responsible for antioxidant activity (70-71). Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation (72).The antioxidant property also can be due to the presence of carotenoids, alkaloids, proanthocyanidins in this plant (72) or to the high content of flavonoids such as kaempferol, presence of other polyphenols, carotenoids and cinnamic acid derivatives (70-71).

According to Siddhuraju & Becker, 2003 (70), all leaf extracts of Moringa olifera were capable of scavenging peroxyl and superoxyl radicals. Overall, both methanol (80%) and ethanol (70%) extract were found to be the best solvents for the extraction of antioxidant compounds from Moringa leaves (70).

The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that leads to the formation of MDA, a biomarker used for studying the oxidation of lipids under different conditions (73).

In the present study, there is a no change in the activity of superoxide dismutase (SOD) or malondialdehyde (MDA) concentration in blood and plasma of normal rats treated with Moringa olifera leaves or its ethanolic extract which disagree with (74) where they found a significant increase in SOD activity and a non significant increase of MDA.

CONCLUSION

The present study showed that leaves or ethanolic leaves extract of Moringa oleifera possessed anti hyperglycaemic properties in streptozocin diabetic male rats, which suggest the presence of biologically active components which may be worth further investigation and elucidation. The hypoglycaemic properties of leaves or ethanolic leaves extract of Moringa oleifera in normal rats needs more study and elucidation.

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

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