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Study of the Hypoglycemic Effect of Mango Leaves Powder Fortified Balady Bread on Diabetic Rats Induced by Alloxan

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Abstract: The mango is a rich source of various polyphenolic compounds which has been reported to have antioxidant properties but not much is known about its hypoglycemic effects. This study aims to demonstrate the hypoglycemic potentials of different levels of mango leaves powder fortified balady bread on alloxan-induced diabetic rats. Thirty-two male albino rats of weights between 150±10g were used for the study and divided into four groups of eight rats each. Eight rats were kept a normal control group, while the rest of rats were induced with diabetes using alloxan by a single dose administration of 150 mg/kg body weight (BW), then those diabetic rats were reclassified to three groups, one kept as positive group, 5% treated with mango leaves powder fortified balady bread and 10% treated with mango leaves powder fortified balady bread for thirty days. Blood samples were collected and lipid profile, brain antioxidant enzymes activity, insulin, HbA1C% and glucose levels were assayed. The results showed that both of mango leaves powder groups reverse the impact of alloxan as they showed increase in insulin levels and decrease in HbA1C% and glucose levels.

Key words: Mango Leaves · Alloxan · HbA1C% · Hypoglycemic · Phenolic Compounds

INTRODUCTION

Diabetes mellitus is a systemic disease characterized by abnormal metabolic regulation of glucose, resulting in hyperglycemia. The standard method of inducing diabetes in animal models is with alloxan, a toxic glucose analogue, which selectively destroys insulin-producing (beta) cells in the pancreas when administered to rodents this causes an insulin-dependent diabetes mellitus called “alloxandiabetes” in these animals [1]. Mango or "the king of the fruits" is one of the most popular delicious seasonal fruits grown in the tropics, this exotic fruit belongs to the genus *Mangifera*, consisting of numerous tropical fruiting trees which they all mango belong within the family of Anacardiaceae, a family that also includes numerous species of tropical-fruiting trees. Often labeled as “super fruits” because its beneficial uses [2, 3]. Mango leaves are alternately arranged, lanceolate (long and narrow) shaped, 6 to 16 inches in length and leathery in texture. The mango leaves are reddish or purplish when tender and new and grows into dark green colour and pale underside. These leaves are rich in vitamin C, B and A. They are also rich in various other nutrients which, the energy value per 100 g (3.5 oz) serving of the common mango is 250 kJ (60 kcal) and that of the apple mango is slightly higher (79 kcal per 100g) [4, 5]. The mango leaves have powerful antioxidant properties as it has high flavonoids and phenol contents. Nowadays, most of researches and investigations have been carried out to find out the benefits of mango leaves as soon as it contains valuable polyphenolic compounds. The amounts of the different polyphenolic compounds in the mango vary from part to part (pulp, peel, seed, bark, leaf and flower) with most polyphenols being found in all the parts [6, 7].

Berardini *et al.* [8] and Pandit *et al.* [9] in the past few years, there has been increasing interest in the study of mango phenolics from mango fruits, peels, seeds, leaves, flowers and stem bark due to their antioxidative and health promoting properties that make consumption of mangoes and derived products a healthy habit. Bioactive compounds found in the mangos, among other plants and herbs have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic and angiogenesis inhibitor activities [10]. Interestingly, mango polyphenols,

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like other polyphenolic compounds, work mainly as antioxidants, a property that enables them to protect human cells against damage due to oxidative stress leading to lipid peroxidation, DNA damage and many degenerative diseases. Leaves of mango are used for people who suffering from restlessness due to anxiety, the mango leaves provide a good home remedy. Adding two to three glasses of mango leaf tea to bathing water helps to treat uneasiness and refreshes the body. As Vitamin C, Vitamin A and carotenoids are found in mango juice so these constituents combine together to keep your immune system strong and healthy. Also Mango juice is well-known to promote the health of the digestive system by settling an upset stomach. Most of investigations now are trended for treating diabetes by mango leaves. The tender leaves of the mango tree contain tannins called anthocyanidins, which helps to treat early diabetes. The leaves are dried and powdered used as an infusion to treat the same. It also helps to treat angiopathy diabetes and diabetic retinopathy. Mango contains various classes of polyphenols, carotenoids and ascorbic acid, which demonstrate different health-promoting properties, mainly from their antioxidant activities [11, 12]. Therefore, the present study aims to investigate the antidiabetic activity of mango leaves powder by fortifying the bread with 5% and 10%.

MATERIALS AND METHODS

Material: Mango leaves (*Mangifera indica* L.): Mango leaves powder and wheat flour (72% extraction) was obtained from Agricultural Research Center Giza, Egypt. Casein, cholesterol, cellulose, all vitamins and minerals were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt. Corn oil and starch were obtained from the local market.

Rats: Thirty two albino rats (Sprague Dawley strain) weighting an average (150±10g) were obtained from Helwan Breeding Farm, Cairo, Egypt.

Methods: Chemical Analysis of Raw Materials and Balady Bread: Moisture, fiber, ash, protein and fat were determined according to the method outlined in AOAC [13]. Total carbohydrates were determined by difference as mentioned by Abd El-Latif [14]. Types and concentrations of polyphenolic compounds and flavonoids were estimated as recommended by Geissman [15]. Types of balady bread were classified into:

- Control Balady Bread: was made from 100% wheat flour without any fortification.
- Different Formulas: were made from a mixture of wheat flour and mango leaves powder at various concentrations (5% and 10%) were prepared at Agricultural Research Center according to the common method described by Khorshid *et al.* [16]. Then according to the panel test was detected the best two concentration.

Animals and Treatment: Adult male albino rats were selected for the study. The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for 1 week in order to adapt to the laboratory condition, fed with commercial diet and water ad libitum, obtained from the Experimental Animal House of Helwan, Egypt. The rats were housed in stainless steel cages with wire mesh bottoms and maintained in temperature and humidity control with 12 hrs light/dark cycle. The animals were kept under observation for five days before experiment and fed the standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamins mixture (20g/kg) and DL-methionine (3g/kg) according to Reeves *et al.* [17].

Induction of Diabetes: Diabetes was induced by intravenous injection of a freshly prepared aqueous solution of alloxan monohydrate (150mg/kg body weight) according to Malekinejad *et al.* [18]. Blood was extracted from the tail vein for glucose analysis and rats with fasting glucose ranging from 210-220 mg/dl, showing clear signs of polyuria, polyphagia and polydipsia were considered diabetic and were analyzed 48 hours after alloxan treatment. Animals with fasting blood glucose less than 200 mg/dl were rejected. The rats were divided into the following groups of 8 animals each:

- Group 1: negative controls groups fed standard diet only
- Group 2: positive controls groups fed standard diet only + alloxan monohydrate (150 mg/kg body weight).
- Group 3 and Group 4: (16 rats) obtained the same composition as positive diet, in replacement fortified balady bread with 5% and 10% mango leaves powder. For 30 days and were then rendered diabetic and decapitated 48 h after alloxan administration. During the experiment period, the quantities of diet, which were consumed and / or wasted, were recorded

every day. In addition, rat's weight was recorded weekly to determine food intake and body weight gain % according to Chapman and Pratt [19].

Biochemical Parameters: Serum glucosalated heamoglobin (Hb A1C %) and insulin were estimated according to Abraham *et al.* [20] and Wilson and Miles [21], respectively. Enzymatic colorimetric determination of triglycerides was carried out according to Fossati and Prencipe [22]. Total cholesterol was determined by colorimetric method according to Allian *et al.* [23]. Determination of HDL was carried out according to the method of Fnedewaid [24] and Gordon *et al.* [25]. The determination of VLDL (Very low density lipoproteins) and LDL (Low density lipoproteins) were carried out according to the method of Lee and Nieman [26] by calculation as follows:

-VLDL (mg/dl) = Triglycerides /5

- LDL (mg/dl) = Total cholesterol –HDL-VLDL

Determination of Brain Antioxidant Parameters: The brains were quickly removed and washed in ice-cold saline. Cerebral hemispheres were dissected out and carefully separated at 0°C with bent forceps and scalpel, weighed in an electric balance in mammalian Ringer solution and immediately used for biochemical analyses. Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid (TBA) reaction according to Uchiyama and Mihara [27]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of H_2O_2. Ten percent (w/v) tissue homogenate was prepared, centrifuged for 90 min and the resulting supernatant was used for determining CAT activity [28]. Agar gel electrophoresis was employed to study isoforms of CAT and the relative activity measured. Ten percent (w/v) brain tissue homogenate in 0.1M phosphate buffer pH 7.0 was prepared and centrifuged at 2000 rpm for 30 min. 20µl of the clear supernatant was spotted on the filter paper strip embedded in centrally made slots of the 2.5% solidified agar gel spread over 245x70mm glass plate with 3mm height frame. Constant current of 10mA (Electroselenium Ltd, Essex, England) was employed. Electrophoresis was carried out at 7°C for 16 hours. After the run, the gels were removed washed in phosphate buffer and soaked in 0.6M H_2O_2 in 0.2M phosphate buffer (pH 7). In few minutes at carried out at 7°C for 16 hours. After the run, the gels were removed washed in phosphate buffer and soaked in 0.6M H_2O_2 in 0.2M phosphate buffer (pH 7). In few minutes at the region where CAT has migrated, O_2 bubbles were liberated due to enzymatic hydrolysis of H_2O_2. As the bubbles accumulated a pearly granular region became visible on the gel representing activity bands. Cerebral protein carbonyl content (PrC) and glutathione (GSH) levels were determined according to the methods described by Levine *et al.* [29] and Tietze [30].

Statistical Analysis: The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups according to Snedecor and Cochran [31].

RESULTS AND DISCUSSION

Data represented in Table 1 indicated that chemical composition of the balady bread prepared from wheat flour without or with mango leave powder. The unfortified balady bread contains (13.95, 65.28 and 2.13g) of moisture, carbohydrates and fat, respectively. While, 5% mango leaves fortified balady bread contains (10.78, 67.71 and 2.74g) of moisture, carbohydrates and fat respectively and the level of 10% mango leaves fortified balady bread contains (9.25, 67.7 and 3.08g) of moisture, carbohydrates and fat, respectively. Data in Table 2 shows the percentages in ppm of the polyphenolic compounds in mango leaves as it contains high percentage of polyphenolic compounds as: mangiferin, mangiferin gallate, isomangiferin, isomangiferin gallate and quercetin by 1563.2, 311.5, 124.7, 72.6 and 65.3 ppm, respectively. The effect of feeding mango leaves on fortified balady bread at different ratios on feed intake and body weight gain of diabetic rats are presented in Table 3 which revealed that positive group showed significant decrease in feed intake and body weight gain compared to normal group (negative group). The other treated group reverses the effect of alloxan monohydrate as it showed significant increase in feed intake and body weight gain compared to positive control group. These results are in agreement with those obtained by Scalbert and Williamson [32] as the mango is one of fruits which contains a wide verity of polyphenolic compounds and human consumption

Table 1: Chemical composition of the balady bread prepared from wheat flour (72% extraction) without and with various levels of mango leaves powder (g/100g dry weight basis).

Sample	Moisture	Crude protein	Crude fat	Ash	Crude fiber	Carbohydrates
Un-fortified balady bread (control).	13.95	11.21	2.13	3.71	3.72	65.28
5% mango leaves fortified bread	10.78	10.23	2.74	4.95	3.60	67.70
10% mango leaves fortified bread	9.25	10.99	3.08	4.46	4.52	67.70

Each value represents the average of three determinations

Table 2: Types and concentrations of phenolic compounds (ppm) of mango leaves.

Phenolic compounds (ppm)	Mangiferin	Mangiferin gallate	Isomangiferin	Isomangiferin gallate	Quercetin
	1563.2	311.5	124.7	72.6	?65.3

Table 3: Effect of feeding mango leaves fortified balady bread at different ratios on feed intake and body weight gain of diabetic rats.

Parameters		
Groups	Feed intake (g/day)	Body weight gain %
Negative control	17.20±0.83a	20.60±0.89a
Positive control	11.80±1.09d	11.40±1.34d
5% mango leaves fortified balady bread	13.60±1.67c	16.00±2.91bc
10% mango leaves fortified balady bread	16.40±1.67b	16.40±1.67b

Values with the same letters indicate no significant different (p=0.05) and vice versa

Table 4: Effect of mango leaves fortified balady bread on glucose (HbA1C %) and insulin of diabetic rats.

Parameters			
Groups	Glucose (mg/dl)	HbA1C %	Insulin (µ/l)
Negative control	120.11±6.21c	5.11±0.67b	17.25±2.14a
Positive control	317.31±38.21a	8.14±0.88a	8.60±1.16c
5% mango leaves fortified balady bread	142.16±8.41b	4.60±0.33b	14.01±2.20b
10% mango leaves fortified balady bread	130.21±9.33b	4.17±0.44b	15.16±1.99b

Values with the same letters indicate no significant different (p=0.05) and vice versa.

HbA1C%: Serum glucosalated heamoglobin.

studies indicate 1g of total polyphenols is frequently consumed per day and it is not anticipated that any acute or lethal toxicity would be observed through the oral intake route and those compounds showed great effects on body weight also mango has a lot of vitamins and nutrients that help the body feel fuller. Also, the fibrous fruit boosts the digestive function of the body by burning additional calories, helping in weight loss. The results of the effect of mango leaves fortified balady bread on glucose, HbA1C% and insulin of diabetic rats were represented in Table 4 as the positive group showed a significant increase in glucose and HbA1C% levels while the level of insulin was decreased compared to normal control group. This is due to the toxic effect of alloxan which is consider as an oxygenated pyrimidine derivative toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents. This causes an insulin-dependent diabetes mellitus called "alloxan diabetes" in these animals, because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter [33].

The best result was for the 10% mango leaves fortified bread. The magic polyphenolic compound in mango fruit is mangiferin which is a xanthone and xanthones are some of the most potent antioxidants known, they are thought to be more potent than both vitamin C or vitamin E and it is a pharmacologically active phytochemical and a natural polyphenolic antioxidant present in the bark, fruits, roots and leaves of *Mangifera indica* Linn and a few other medicinal plants recommended in the Indian system of medicine for treatment of a number of immunodeficiency diseases [34]. Similar results were reported by Muruganandan *et al.* [35], who mentioned the effect of mangiferin in reducing atherogenicity in streptozotocin diabetic rats and to reduce the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. Many researchers have established mangiferin as the possible active principle of mango, the tender leaves of the mango tree contain tannins called anthocyanidins, which helps to treat early diabetes, helps to treat angiopathy diabetes and diabetic retinopathy. Mango tea leaves are helping in the

Table 5: Effect of mango leaves fortified balady bread on total lipids , total cholesterol (T.C), total triglyceride(T.G), low density lipoprotein(LDL-c), high density lipoprotein(HDL-c) and very low density lipoprotein(VLDL-c) of diabetic rats.

Parameters						
Groups	Total lipids (g/l)	T.C(mg/dl)	T.G(mg/dl)	LDL-c(mg/dl)	HDL-c(mg/dl)	VLDL-c(mg/dl)
Negative control	2.67±0.33c	96.73±0.83c	147.18±1.58c	19.39±0.46d	47.90±1.14a	29.43±0.31c
Positive control	4.25±0.52a	168.26±10.96a	216.00±11.40a	87.63±12.26a	37.43±3.53 c	43.20±2.28a
5% mango leaves fortified balady bread	2.95±0.19b	108.12±7.54b	173.95±3.55b	25.51±8.69c	47.85±1.95a	34.76±0.69b
10% mango leaves fortified balady bread	2.78±0.18b	104.13±5.36b	172.20±4.38b	28.89±6.78b	40.80±2.49b	34.44±0.87b

Values with the same letters indicate no significant different (p=0.05) and vice versa

treatment of hyperglycemia. As the leaves contain a compound called tarakserol-3beta and ethyl acetate extract which synergize with insulin to activate GLUT4 and stimulate the synthesis of glycogen [36].

From the various studies done on mangiferin and the extracts from mango leaves, it has been found to exhibit normalizing insulin levels effect in the blood. Studies show the mangiferin exhibits potent antidiabetic in additional to, the mango has a relatively low glycemic index (41-60) so moderate quantities do not spike the sugar level. In a study of drug induced diabetic animals, the results indicated that the aqueous extract of the leaves of *Mangifera indica* possess hypoglycaemic activity this action is may be due to an intestinal reduction of absorption of glucose [37]. Yoshikawa *et al.* [38] have been suggested that mangiferin reduces blood glucose levels by inhibiting the glucose absorption from the intestine. This hypothesis could be supported by the recent findings that mangiferin inhibits the glucosidase enzymes sucrase, isomaltase and maltase from rats which are involved in the digestion of carbohydrates into simple sugars in the gut leading to delay or inhibition of carbohydrate breakdown and subsequent slower glucose absorption from the intestine also they have been suggested that mangiferin possess both pancreatic and extrapancreatic mechanisms in its antidiabetic action.

Data presented in Table 5 shows the effect of mango leaves powder fortified balady bread on serum lipid profile as the positive control group showed significant increase in the levels of TL, TC, TG, V-LDL and LDL, while decrease in HDL compared to negative control group. Our present findings also indicated that the lipid profiles (TL, TC, TG, V-LDL and LDL) were significantly lower in diabetic rats treated with different levels of mango leaves powder fortified balady bread compared to the positive group. In contrast, the mean HDL level of the treated diabetic rats was significantly higher than the positive group. This finding are in agreement with those obtained by Muruganandan *et al.* [39], who reporting that mangiferin was found to significantly reduce plasma total cholesterol, triglycerides and LDL-C associated with a concomitant increase in HDL-C levels and a decrease in atherogenic index in diabetic rats indicating its potential antihyperlipidemic and antiatherogenic activity. Our finding concurs with the findings of Randle *et al.* [40] and Rocha Ribeiro *et al.* [41], showed that the triglyceride-lowering property of mangiferin could indirectly contribute to the overall antihyperglycemic activity through the glucose-fatty acid cycle mechanism. It is thought that this reduction in lipid profile levels may be due to the effect of pectin, quercetin and gallic acid which confers hypolipidimic ability on the mango. This finding supports the works of Berardini *et al.* [42] which demonstrated that mangoes high levels of fiber, pectin and vitamin C help lower serum cholesterol levels, specifically low-density lipoprotein or the bad cholesterol. Other studies indicated that *Mangifera indica* extracts might offer a natural key in hypolipidemic and hepatoprotective activity as flavonoids from mango effectively reduced lipid levels in serum and tissues of rats with induced-hyperlipidemia. Also, oral administration of flavonoids showed significant antioxidant action in cholesterol-fed experimental rats. Free radical-scavenging enzyme activity was significantly elevated and lipid peroxide content was reduced in flavonoids treated hypercholesterolemia rats [43].

Table 6 expresses the effect of mango leaves fortified balady bread on lipid peroxidation, catalase activity and glutathione (GSH) in the brain of diabetic rats where Our present findings indicated that the lipid peroxidation and catalase activity were significantly higher while lower GSH level in positive diabetic rats group compared to normal control group and treated with different levels of mango leaves powder fortified balady bread compared to the positive group. In contrast, the treated diabetic rats with different levels of mango leaves powder fortified balady bread show significant decrease in lipid peroxidation and catalase activity than the positive group.

Table 6: Effect of mango leaves fortified balady bread on lipid peroxidation (LPO), catalase (CAT) activity and glutathione (GSH) in the brain of diabetic rats.

Parameters			
Groups	LPO (nmol)	Catalase (nmol)	GSH (mg/g)
Negative control	180.4±8.24d	70.13±5.22a	16.04±1.40a
Positive control	333.5± 11.72a	21.25±3.47c	9.22± 0.60d
5% mango leaves fortified balady bread	301.67±9.34b	68.33±6.35b	11.21±0.20c
10% mango leaves fortified balady bread	291.35±8.12c	63.14±7.16b	13.23±0.20b

Values with the same letters indicate no significant different (p=0.05) and vice versa

Our results were matched with those reported by Andreu *et al.* [44], who cleared that mangiferin (MF) was found to protect mitochondrial membrane against lipid peroxidation hence preserving its integrity. They have been suggested that oxidation derivatives of mangiferin may sensitize mitochondria to calcium-induced permeability transition, a process often related to apoptotic/necrotic cell death. In this regard, it was proposed that the accumulation of such oxidation products would take place in such cells exposed to an overproduction of reactive oxygen species. Mango is a good source of Antioxidants substances like quercetin, isoquercitrin, gallic acid and methylgallat which protect neuroblastoma cells of the body against MPP+ induced cytotoxicity, to restore the glutathione (GSH) content and to downregulate both superoxide dismutase 1 (SOD1) and catalase (*cat* mRNA) expression all being mediated by oxidative stress [45]. Our finding was totally are in agreement with those reported by Rodriguez *et al.* [46], who found that the mangiferin has been shown to be able to scavenge reactive oxygen species, thus inhibiting all those processes leading to energy charge decrease, red blood cell damage and membrane destabilization. After human cell studies, they suggested that mangiferin protects erythrocytes and red blood cells from reactive oxygen species production thus contributing to integrity and functionality of these cells.

CONCLUSION

In conclusion and view of the above, mango leaves extract indeed reduced the glucose and HbA1C% levels in alloxan-induced diabetic rats while insulin level in the same group of rats. These findings suggest that the mango leaves extract has hypoglycemic and antioxidant effects. The mango is readily available supplier of dietary polyphenols with great antioxidative potential that will help reduce degenerative diseases such as diabetes.

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