

Potential Therapeutic Activity of Mango (*Mangifera indica L.*) Leaves on Excess Consumption of Fructose in Rats

Abstract

Background and Objective: High fructose consumption has increased worldwide. It causes various metabolic, genetic and histologic alterations. Alternative medicine, essentially herbal plants, has been projecting to ameliorate high fructose consumption consequences. The goal of this study was to investigate the efficacy of supplementation or treatment with mango leaves against high fructose induced alterations in male rats. **Methodology:** Mango leaves nutritional and active components were determined. A total of sixty adult male rats were used in this study. Fifteen rats were kept as healthy (negative control group; rats fed on balanced diet) while in others metabolic alterations were induced by consumption of high fructose diet *ad libitum*. Rats fed on high fructose diet were divided into 3 groups (15 rats/each), one group set as positive control group; rats fed on high fructose diet only and the other 2 groups; mango treated group; rats fed on high fructose diet until induction of hyperglycemia (one month and half) then fed on high fructose diet with replacement of fiber with 5% mango leaves and mango supplemented group; rats fed on high fructose diet with 5% mango leaves replacing fiber. **Results:** Mango leaves contain significant amounts of crude protein, crude fat, carbohydrates, crude fiber, ash, total flavonoids and polyphenols that controlled and corrected the following high fructose consumption results. Consumption of high fructose diet significantly ($p \leq 0.05$) increased final body weight (FBW), body weight gain (BWG), abdominal circumference (AC), Lee index and body mass index (BMI). High fructose also significantly ($p \leq 0.05$) increased levels of systolic blood pressure (SBP), serum triacylglycerol (TAG), total cholesterol (TC), fasting blood glucose, insulin, homeostasis model assessment-insulin resistance (HOMA-IR), serum tumor necrosis factor- α (TNF- α), leptin, malondialdehyde (MDA), advanced glycation end products (AGEs) and adipocyte size as well as blood histone deacetylase (HDAC) enzyme activity. High fructose consumption contrarily caused significant decrease ($p \leq 0.05$) in levels of quantitative insulin check index of insulin sensitivity (QUICKI), adiponectin, muscular insulin receptor substrate-1 (IRS-1) and glucose transporter-4 (GLUT-4) gene expression as well as blood reduced glutathione (GSH). Moreover, microscopic observations of pancreas and adipose tissues were consistent with the biochemical results. **Conclusion:** Mango leaves is a cheap source of macro and micronutrients as well as active constituents. Either mango leaves supplementation or treatment improved and ameliorated all the biochemical and microscopic observations by restricting metabolic and genetic alterations induced by high fructose consumption. The best improvement was observed in the mango leaves supplemented group.

Keywords Mango leaves, High fructose, Metabolic, Genetic, Alterations.

1. INTRODUCTION

Fructose is a simple carbohydrate that is found naturally in fruits and honey. It is used as artificial sweetener. Sugar-sweetened beverages and processed foods are the main source of

fructose in diet. Consumption of these products became higher especially in children and adolescents [1].

Chronic consumption of high fructose in diet was found to be associated with increased risk of different diseases [2]. A recent epidemiological analysis in humans also found an association between metabolic syndrome (MS) prevalence and sugar availability. MS is a cluster of hyperglycemia, dyslipidemia, elevated blood pressure, insulin resistance and abdominal obesity [3].

Metabolic abnormalities due to high fructose consumption for a long time results in hyperglycemia that explained by the defect in glucose uptake, insulin receptors, signaling and action that may be due to genetic and epigenetic dysregulation [4,5]

In the last few years there has been an exponential growth in the field of herbal medicine. The usage of these natural drugs increased worldwide because of their low cost with minimal side effects .Mango (*Mangifera indica L.*) is a juicy stone fruit belongs to the family of *Anacardiaceae*. Mango is grown in many parts of the world, particularly in tropical countries. It has been well documented that mango leaves are an important source of both macro and micro nutrients [6].

Mango leaves provide energy from macronutrients including carbohydrates, proteins and fats. Leaves also contain dietary fiber, phenolic compounds, vitamins and other phytochemicals that are vital to normal human growth, development and health. Mango leaves contain diverse health promoting chemical constituents including mangiferin. Mangiferin is a natural polyphenol of C-glycosylxanthone structure, with diverse pharmacological/physiological activities. Mangiferin promotes gut health and regularity. It also have antioxidant and anti-inflammatory benefits [7].

This study was designed to assess the ability of mango leaves consumption to compete with high fructose diet genetic, metabolic, oxidative and inflammatory changes in male rats.

2. MATERIALS and METHODS

2.1. Materials

2.1.1. Plant: The mango leaves were obtained from the Ministry of Agriculture in Giza, Egypt, and botanists verified their authenticity (Department of Botany, women faculty, Ain Shams University). The leaves were rinsed in water and dried entirely in well-ventilated stores under proper conditions before being processed into powder with an electric grinder [8].

2.1.2. Chemicals: Fructose and other chemicals were obtained from EL-Gomhouria Company for Trading Chemicals, Cairo, Egypt.

2.1.3. Animals: Sixty male adult Sprague-Dawley strains weighing (100 ± 5 g) were kept in wire cages in the well-ventilated animal house of the Faculty of Pharmacy, El-Azhar University for acclimatization (7 days) till the completion of the experimental period (12weeks). They were given a 12 hour light/dark cycle and a standard diet as well as unlimited tap water. The

Institutional Animal Ethics Committee approved all animal experiments and followed the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.1.4. Diet: Balanced diet was prepared according to American Institute of Nutrition AIN-93M and adjusted by [9]. High fructose diet was prepared according to [10].

2.2. Methods

2.2.1. Determination of mango leaves nutritional and active composition

Mango leaves crude protein, crude fat, carbohydrates, crude fiber and ash were determined following AOAC technique [11]. Also total flavonoids and polyphenols contents were determined by Folin–Ciocalteu reagent as described by Arnous et al., [12].

2.2.2. Experimental design

The rats were divided into four groups, each with 15 rats, and were treated as follows: Group I: healthy negative control group of rats that have been fed a well-balanced diet. Group II: Positive control group; rats fed on high fructose diet. Group III: Mango treated group; rats fed on high fructose diet until induction of hyperglycemia (one month and half) then fed on high fructose diet with replacement of fiber with 5% mango leaves [8]. Group IV: Mango supplemented group; rats fed on high fructose diet with 5% mango leaves replacing fiber.

2.2.3. Anthropometric measurements

Body weight gain (BWG), abdominal circumference (AC), Lee index and body mass index (BMI): Animals were weighed twice a week using electrical balance. All rats were fasted overnight at the end of the experiment (90 days), final body weight (FBW) were measured using electrical balance. The abdominal circumference and body length (nose-to-anus length) were measured using non-stretchable tape for calculation of lee index and body mass index (BMI) under deep anesthesia. Lee index is calculated as cubic route of final body weight (g) divided by the length (cm); where > 0.30 considered obese. The BMI was calculated as final body weight (g) divided by the square of the anal-nasal length (cm); where > 0.68 considered obese [13,14].

2.2.4. Systolic blood pressure (SBP)

Systolic blood pressure was measured under anesthesia the day of sacrifice, using a tail cuff blood pressure system (Model 29 pulse amplifier with tail cuff sensor and adapted rodent restrainer, iitc incorporated). Three readings were taken consecutively to calculate the average as a final reading for SBP [15].

2.2.5. Handling of blood and organs samples

Rats were anaesthetized before being sacrificed. Blood was drawn from the hepatic portal vein and separated into two tubes, one for blood and the other to separate serum for biochemical analysis. The pancreatic and adipose tissues were extracted to be analyzed microscopically, while thigh muscle tissues were separated to evaluate gene expression.

2.2.6. Biochemical determination

Blood fasting glucose concentration and insulin level were determined by glucometer and enzyme immunoassay test kit [16, 17]. Blood histone deacetylase (HDAC) enzyme activity was measured using a colorimetric assay kit (BioVision, CA, USA) following Strahl and Allis method [18] while blood reduced glutathione level was determined calorimetrically according to Beutler et al [19]. Homeostasis model assessment-insulin resistance (HOMA-IR), and the quantitative insulin check index of insulin sensitivity (QUICKI) were calculated according to the following equations [20]:

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/l}) \times \text{fasting glucose}(\text{mg/dl})]/405$$

$$\text{QUICKI} = 1/(\log \text{fasting glucose [mg/dL]} + \log \text{fasting insulin } [\mu\text{U/mL}])$$

Serum was analyzed for tumor necrosis factor- α (TNF- α), leptin and adiponectin levels using Elisa kits purchased from MyBioSource, (San Diego, CA, USA). Serum malondialdehyde (MDA) and advanced glycation end products (AGEs) levels were determined using standard determination methods [21,22]. Serum total cholesterol (TC) and total triacylglycerol (TAG) were measured using *Biodignostic Kits* (Giza, Egypt).

2.2.7. Determination of insulin receptor substrate-1 (IRS-1) and glucose transporter-4 (GLUT-4) genes expressions using real- time quantitative polymerase chain reaction (qRT-PCR)

Total RNA was extracted by Trisol reagent (Invitrogen, CA) from anterior thigh muscle tissue using total RNA purification Kit. RNA and cDNA concentration were quantified using Nanodrop method to make sure they are pure and suitable to conduct real time PCR [23]. IRS-1 and GLUT-4 genes expressions were measured in three replicates using 18S rRNA expression level as an internal reference. Data were normalized using the $2^{-\Delta\Delta C_t}$ method Livak and Schmittgen, [24]. Table (1) shows the primers sequences.

Gene	Forward primer	Reverse primer
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IRS-1	'5-TGCACTGTGACACCAGAATAAT-3 '	'5-TTGAATGAACAATGTGGGCG -3 '
GLUT-4	'5-TCATTGTCGGCATGGGTTTC-3 '	'5-CGGCAAATAGAAGGAAGACGTA-3 '
18S rRNA	'5-CGGCTACCACATCCAAGGAA-3 '	'5-GCTGGAATTACCGCGGCT-3 '

Table (1): Studied genes forward and reverse primers sequence

2.2.8. Microscopic examination

Pancreas and white adipose tissues from all rats were dissected out and fixed instantaneously in 10% formalin solution for 24 h. Paraffin blocks were prepared and 5µm thick sections were subjected to microscopic examination. Hematoxylin and Eosin stains (H & E) were used with magnification power 200 and 400 [25]. Image-Pro plus Version 5.0 (Media Cybernetics) was used to measure adipocyte average diameters as described by Chen and Farese [26] at the Veterinary Medical Laboratory- Cairo University, Egypt.

2.2.9. Statistical analysis

The SPSS software was used to analyze the results (version 16). The findings were expressed as mean ± standard deviation (ANOVA; F-test) and the least significant difference (L.S.D) was calculated according to Levesque [27].

3. RESULTS

3.1. Mango leaves nutritional and active composition

From analysis data of mango leaves fig. (1), it was found that leaves contain significant amounts of crude protein, crude fat, carbohydrates, crude fiber and ash. This explain the high nutritive value of mango leaves with low caloric content on comparison. Leaves also were found to contain valuable amounts of polyphenols and flavonoids with many health benefits.

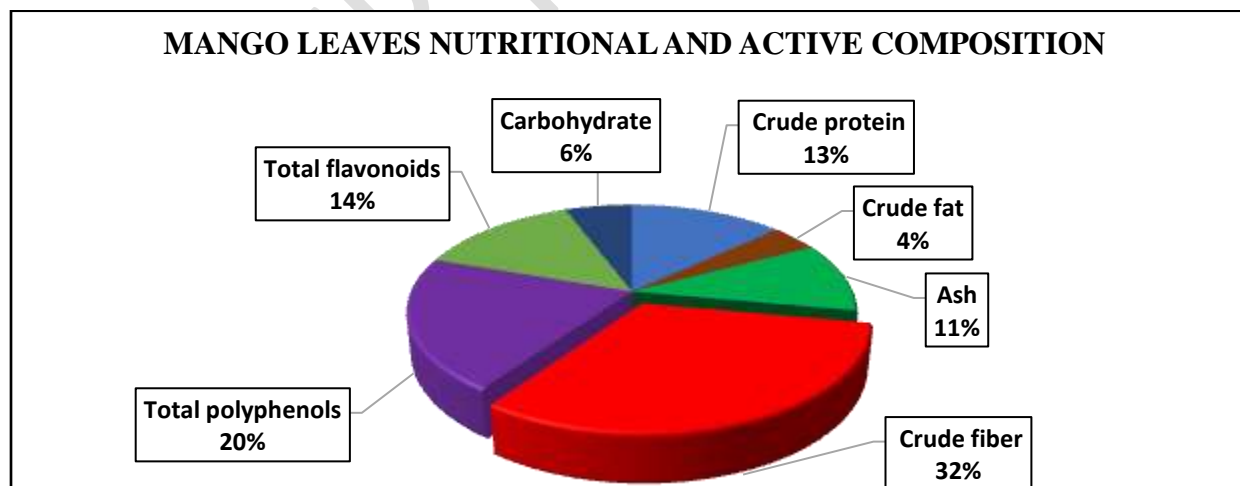


Fig (1): Mango leaves nutritional and active composition

3.2. Effect of mango leaves on the anthropometric measurements of male rats fed on high fructose diet:

Current research results revealed that there was significant increase ($p \leq 0.05$) in the final body weight (FBW), body weight gain (BWG), abdominal circumference (AC), Lee index, and body mass index (BMI) in positive control group with respect to the healthy negative control group leading to obesity in rats fed with high fructose diet. On the other hand, there was significant decrease ($p \leq 0.05$) in FBW, BWG, AC, Lee index, and BMI in mango supplemented and treated groups and that mango leaves oral supplementation to rats fed on high fructose diet from the beginning prevented and improved these values with respect to the positive control group as presented in table (2).

Table(2): Effect of mango leaves on final body weight (FBW), body weight gain (BWG), abdominal circumference (AC), naso- anal length, Lee index and body mass index (BMI) in male rats fed on high fructose diet

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
FBW (g)	290.36±3.50 ^c	476.81±7.64 ^a	346.46±5.36 ^b	271.21±2.30 ^d
BWG (g/day)	2.14±0.31 ^c	4.75±0.61 ^a	2.72±0.13 ^b	1.891±0.09 ^d
AC (cm)	11.2±0.5 ^c	16.8±1.03 ^a	14.1±0.6 ^b	10.7±0.3 ^d
Naso-anal length (cm)	21.30±1.17 ^a	21.40±1.20 ^a	21.54±1.25 ^a	21.65±1.32 ^a
Lee Index (g/cm)	0.31±0.05 ^c	0.37±0.04 ^a	0.33±0.03 ^b	0.30±0.01 ^d
BMI (g/cm ²)	0.63±0.12 ^c	1.03±0.34 ^a	0.75±0.28 ^b	0.58±0.07 ^d

All values are expressed as mean ±SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.3. Effect of mango leaves on systolic blood pressure and serum lipids fractions levels in male rats fed on high fructose diet

It is obvious from current results that, high fructose intake significantly increased ($p \leq 0.05$) systolic blood pressure (SBP), serum triacylglycerol (TAG) and total cholesterol (TC) levels in comparison with healthy negative control group as shown in table (3) leading to hypertension and hyperlipidemia. The increase in these parameters was inhibited by oral mango leaves administration. The mango supplemented group showed the most significant improvements.

Table (3): Effect of mango leaves on systolic blood pressure (SBP), serum triacylglycerol (TAG) and total cholesterol (TC) levels in male rats fed on high fructose diet

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
SBP (mmHg)	117.25±0.92 ^c	140.86±4.15 ^a	126.46±2.08 ^b	110.12±0.42 ^d
TAG(mg/dl)	103.14±2.67 ^c	267.39±6.98 ^a	116.66±2.05 ^b	100.1±1.94 ^d
TC (mg/dl)	142.18±3.45 ^c	265.22±5.27 ^a	164.15±2.66 ^b	114.18±1.83 ^d

All values are expressed as mean ±SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.4. Effect of mango leaves on fasting blood glucose level, insulin level, resistance and sensitivity in male rats fed on high fructose diet

Compared with the healthy negative control rats, high fructose intake caused significant ($P \leq 0.05$) increase in the blood levels of fasting glucose, insulin, homeostasis model assessment-insulin resistance (HOMA-IR), with significant decrement in the quantitative insulin check index of insulin sensitivity (QUICKI) index. As compared to the positive control group, mango leaves treatment of high fructose fed rats resulted in a significant ($P \leq 0.05$) reduction in insulin, fasting blood glucose, HOMA-IR, and an increase in the QUICKI index, lowering the likelihood of developing diabetes mellitus (Table 4). Oral supplementation with mango leaves prevented diabetes mellitus and improved all of the tested parameters.

Table (4): Effect of mango leaves oral administration on fasting blood glucose, insulin levels, HOMA-IR and QUICKI index in male rats fed on high fructose diet

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
Glucose (mg/dl)	92.51±3.5 ^c	208.70±19.7 ^a	125.37±6.1 ^b	89.24±5.8 ^d
Insulin (µU/ml)	26.03±0.09 ^c	41.4±0.25 ^a	30.20±0.13 ^b	22.6±0.02 ^d
HOMA-IR	5.96±1.32 ^c	21.34±1.20 ^a	9.35±1.25 ^b	4.97±1.17 ^d
QUICKI index	0.295±0.001 ^c	0.254±0.014 ^a	0.279±0.003 ^b	0.302±0.002 ^d

All values are expressed as mean ±SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.5. Effect of mango leaves on inflammation, appetite and insulin resistance related hormones levels in male rats fed on high fructose diet

In comparison to healthy negative control rats fed a balanced diet, serum levels of tumour necrosis factor- α (TNF- α) and leptin hormone were found to be significantly ($P \leq 0.05$) increased in positive control rats fed a high fructose diet, accompanied by a significant reduction in serum adiponectin hormone level ($P \leq 0.05$). When compared to the positive control group, oral administration of mango leaves demonstrated a significant ($P \leq 0.05$) improvement in TNF- α , leptin resistance status, with a significant ($P \leq 0.05$) decrease in serum leptin levels and an increase in serum adiponectin levels (Table 5). The mango supplemented group showed the greatest improvement.

Table (5): Effect of mango leaves on serum tumor necrosis factor- α (TNF- α), leptin and adiponectin levels in male rats fed on high fructose diet

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
TNF- α (pg/ml)	46.20 \pm 0.81 ^c	76.65 \pm 3.61 ^a	53.17 \pm 1.38 ^b	40.98 \pm 0.54 ^d
Leptin (ng/ml)	11.74 \pm 0.28 ^c	24.01 \pm 2.01 ^a	13.94 \pm 1.06 ^b	9.93 \pm 0.04 ^d
Adiponectin (ng/ml)	30.67 \pm 1.04 ^b	16.23 \pm 0.51 ^d	22.35 \pm 0.39 ^c	34.95 \pm 2.06 ^a

All values are expressed as mean \pm SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.6. Effect of mango leaves on blood histone deacetylase (HDAC) enzyme activity, muscular insulin receptor substrate-1 (IRS-1) and glucose transporter-4 (GLUT-4) gene expression in male rats fed on high fructose diet

Histone deacetylase enzyme activity in blood (HDAC) was significantly ($P \leq 0.05$) increased accompanied with significant ($P \leq 0.05$) decrease in gene expression of IRS-1 and GLUT-4 in positive control rats fed with high fructose diet in comparison with the healthy negative control rats fed on the balanced diet. Whereas oral administration of mango leaves showed significant ($P \leq 0.05$) amelioration in the tested parameters as compared with positive control group (Table 6). The most improvements were recorded in mango supplemented group.

Table (6): Effect of mango leaves on blood histone deacetylase (HDAC) enzyme activity, muscular insulin receptor substrate-1 (IRS-1) and glucose transporter-4 (GLUT-4) gene expression in male rats fed on high fructose diet

All values are expressed as mean \pm SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.7. Effect of mango leaves on oxidative status in male rats fed on high fructose diet

Oxidative biomarkers measured in serum that illustrated in table (7) showed that MDA and AGEs contents were significantly increased ($p \leq 0.05$) associated with significant reduction in blood antioxidant GSH level in positive control rats fed with high fructose diet as compared to healthy negative control rats composing a state of oxidative stress. Remarkably, mango leaves treatment and supplementation was accompanied with a significant increase in antioxidant GSH level and decrease in oxidative stress indicators MDA as well as AGEs content in comparison

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
HDAC (μ mol/ ml)	83.31 \pm 1.46 ^c	129.79 \pm 2.30 ^a	105.07 \pm 1.85 ^b	76.10 \pm 0.84 ^d
IRS-1 (IRS-1 /18S rRNA)	1.84 \pm 0.06 ^b	0.34 \pm 0.012 ^d	0.79 \pm 0.09 ^c	2.19 \pm 0.029 ^a
GLUT-4 (GLUT-4/ 18S rRNA)	1.29 \pm 0.04 ^b	0.18 \pm 0.02 ^d	0.59 \pm 0.015 ^c	1.95 \pm 0.031 ^a

with positive control group.

Table (7): Effect of mango leaves on serum malondialdehyde (MDA), advanced glycation end products (AGEs) and blood reduced glutathione (GSH) levels in male rats fed on high fructose diet

Group Parameter	Healthy negative control group	Positive control group	Mango treated group	Mango supplemented group
MDA($\mu\text{mol/L}$)	2.57 \pm 0.61 ^c	34.17 \pm 1.93 ^a	20.76 \pm 1.04 ^b	1.92 \pm 0.09 ^d
AGEs (mg/ml)	0.74 \pm 0.05 ^c	7.41 \pm 1.27 ^a	4.61 \pm 0.18 ^b	0.51 \pm 0.02 ^d
GSH (mg/dl)	57.18 \pm 1.04 ^b	21.08 \pm 0.31 ^d	38.94 \pm 0.95 ^c	69.15 \pm 2.31 ^a

All values are expressed as mean \pm SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.8. Effect of mango leaves on adipocyte size in male rats fed on high fructose diet

The size of adipocytes as presented in table (8) is significantly increased ($p \leq 0.05$) in positive control group on comparison with healthy negative control group, while mango treated and supplemented groups showed significant decrease ($p \leq 0.05$) in visceral fat thickness and mean adipocyte diameter as compared to positive control group. Protective effect of mango leaves against expansion of adipose tissue by high fructose diet was observed. The results of biochemical analysis were supported by microscopic examination of adipose tissue.

Table (8): Effect of mango leaves on adipocyte size in male rats fed on high fructose diet

Group	Adipocyte size (mm)
Healthy negative control group	36.40 \pm 3.12 ^c
Positive control group	151.32 \pm 3.56 ^a
Mango treated group	68.20 \pm 2.57 ^b
Mango supplemented group	32.19 \pm 2.84 ^d

All values are expressed as mean \pm SD, n=10, there was no significant difference between means with the same alphabetical superscripts in the same column, significant difference ($p \leq 0.05$).

3.9. Effect of mango leaves on microscopic examination of pancreatic and white adipose tissues in male rats fed on high fructose diet

Microscopic examination of pancreatic and white adipose tissues (**fig. 2 and 3**) showed the degenerative changes caused by high fructose consumption and the corrective role of mango leaves provided supportive evidence for biochemical analysis. Microscopic examination of the negative control group pancreas showed normal parenchyma with normal pancreatic acini, duct, and islets (**Fig. 2a**), while that of positive control group revealed acute pancreatitis with congested blood vessels and mononuclear cells infiltrations (**Fig. 2b**). Mango treated group reported normal pancreatic tissue with slight congestion in the blood vessels (**Fig. 2c**) while mango supplemented group denoted normal histological pancreatic tissues the same as control negative group (**Fig. 2d**), The adverse effect of high fructose intake has been ameliorated in mango treated group and counteracted in mango supplemented group.

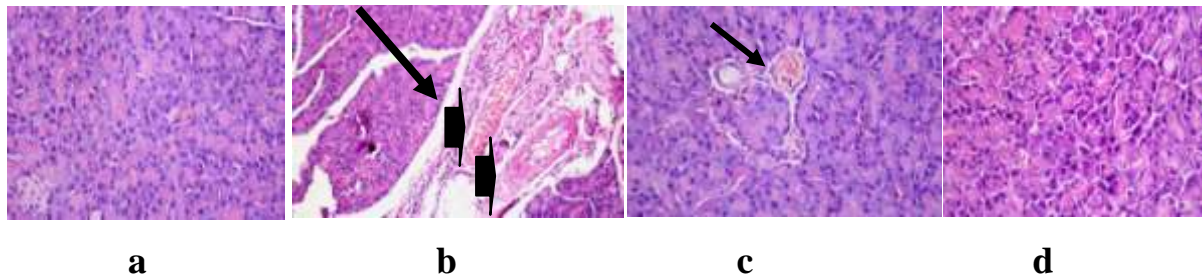


Fig (2): Microscopic examination of pancreatic tissues in all experimental groups

a: Pancreatic tissue of negative control group showing normal parenchyma; pancreatic acini, duct, and islets (H&E X 400)

b: Pancreatic tissue of positive control group showing acute pancreatitis; congested blood vessels (arrow heads) and the mononuclear cells infiltrations (arrow), (H&E X 200)

c: Pancreatic tissue of mango treated group showing slight congestion in the blood vessels (arrow) with normal pancreatic acini, (H&E X 400).

d: Pancreatic tissue of mango supplemented group showing normal parenchyma; normal pancreatic acini, duct, and islets (H&E X 400).

Microscopic examination of the negative control group adipose tissues showed normal adipocytes distribution with regular sizes (**Fig. 3a**), while that of positive control group revealed increased size with abnormal distribution (**Fig. 3b**). Mango treated group reported slight increased size with normal distribution of white adipose tissues (**Fig. 3c**) while mango supplemented group denoted normal histological adipose tissues the same as control negative group (**Fig. 3d**). The adverse effect of high fructose intake has been ameliorated in mango treated group and counteracted in mango supplemented group.

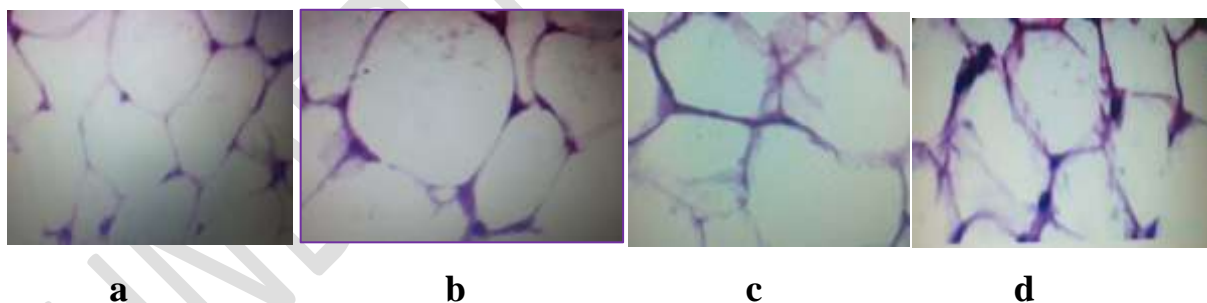


Fig (3): Microscopic examination of White adipose tissues in all experimental groups

a: White adipose tissue of negative control group showing normal adipocytes distribution with regular sizes (H&E X 200)

b: White adipose tissue of positive control group showing increased size with abnormal distribution (H&E X 200)

c: White adipose tissue of mango treated group showing slight increased size with normal distribution (H&E X 400).

d: White adipose tissue of mango supplemented group showing normal size and distribution (H&E X 400).

4.DISCUSSION

Mangifera indica L. is an important fruit cultivated in various countries all over the world. Mango leaves have been studied for their beneficial health effects. Mango leaves contain significant amounts of crude protein, crude fat, carbohydrates, crude fiber and ash as well as a plethora of phytochemicals including polyphenols and flavonoids. Phytochemicals include mangiferin, benzophenones and phenolic acids. Mango leaves also contain antioxidants such as ascorbic acid, flavonoids, tocopherols and carotenoids. Mango leaves extracts have been studied for their different biological activities and various bioactivities. Mango leaves can be used as a potential ingredient for the development of functional foods and pharmaceutical drugs considering their nutritional and phytochemical profile and beneficial effects [28,29]. Through this study the role of feeding mango leaves in association with high fructose diet was evaluated.

Fructose has a chemical formula as glucose ($C_6 H_{12} O_6$). Fructose metabolism differs markedly from that of glucose. Fructose is completely extracted by hepatic tissues and rapidly converted into glucose, glycogen, lactate, and fat [30]. A high-fructose diet leads to the development of metabolic syndrome, which includes symptoms such as hypertension, a high body mass index (BMI), hyperglycemia, dyslipidemia and insulin resistance. Obesity and type 2 diabetes can result from this combination of factors [31].

It was found that rats fed on high fructose diet gained weight and became obese with high BMI, Lee index associated with accumulation of fat in the abdomen as indicated by increased abdominal circumference. These observations were accompanied by increased systolic blood pressure and increased fat synthesis as indicated by increased triacylglycerol and cholesterol levels in serum. These metabolic effects is due to the nature of fructose metabolism. Although fructose is a monosaccharide, it is not directly metabolized to provide energy in most metabolic activities (ATP). Instead, fructose aids liver synthesis of glycogen through a metabolic cascade that overlaps with gluconeogenesis. Fructose promotes the creation of triacylglycerol (TAG) from glycerol and the synthesis of fatty acids. TAG is then stored as fat until the deposited glucose is depleted, triggering a negative feedback loop. However, because glucose levels are rarely reduced, digested fructose is considered excessive and is primarily stored as fat. In this setting, increased fructose consumption is linked to the occurrence of metabolic syndrome criteria [32].

On the other hand mango leaves supplementation and treating effects were obvious on experimental rats affected by consumption of high fructose diet. Mango leaves active constituents regulated fat metabolic pathways through controlling of different genes expression in rats causing an amelioration in fat deposition in the rat's body and correction of obesity markers and body weight. This is due to mango leaves fiber and polyphenolic active components that modulate the expression of transcriptional factors and enzymes related to adipogenesis [33-35].

High fructose consumption for a time causes a state of hyperglycemia, hyperinsulinemia, insulin resistance and finally induction of type-2 diabetes. Fructose consumption causes a low

glycemic index and does not need insulin to be transported into cells. While glucose needs insulin. After fructose absorption, it is transported to the liver. In the liver, fructose can be oxidized to be converted to glucose (and glycogen) or converted to lactic acid, or enter de novo lipogenesis. After an overnight fast, approximately 50% of fructose eaten as an oral dose of approximately 30-70g is converted to glucose via gluconeogenesis. The other metabolic fate of fructose to form lactic acid only happens at high fructose intakes. Lactic acid can cause muscle pain and soreness. Excessive intake of fructose also has been shown to increase lipogenesis. Conversion of fructose to glucose thus increases risk of type 2 diabetes. Furthermore, fructose may also contribute negatively to blood glucose homeostasis by causing insulin resistance in the liver and muscles. In human studies, fructose has been shown to cause insulin resistance [36, 37]. On contrary administration of mango leaves to rats normalized blood glucose level, insulin level and sensitivity. These results was confirmed by previous studies that examined the effect of leaves powder, water extract and ethanolic extract on diabetic rats. The main reason that mango leaves have anti-diabetic properties is their active constituents principally mangiferin in addition to other constituents as anthocyanidins including delphinidin, cyanidin and peonidin, leucoanthocyanins, catechin, gallic tannins and flavonois as quercetin [38-41].

Study results revealed that high fructose administration was accompanied by an inflammatory status increasing levels of inflammatory markers including TNF- α and leptin with decreasing anti-inflammatory adiponectin level. Excess adipogenesis and lipogenesis would occur as a result of high fructose intake, resulting in elevated adipocytokines like leptin. Despite the fact that leptin maintains energy homeostasis by balancing hunger, but excessive production leads to leptin resistance. Hyperphagia, obesity, and an increased percentage of fat mass occur as a consequence [42]. Because it produces and secretes various immune-modulatory substances known as adipokines, adipose tissue may be considered an endocrine organ. Proinflammatory adipokines (such as TNF- α) and anti-inflammatory adipokines (i.e., adiponectin) are two types of cytokines. Adipocyte function is disrupted in obesity, with increased expression of pro-inflammatory adipokines and decreased expression of anti-inflammatory adipokines, resulting in a chronic, low-grade inflammatory state [35].

The fluctuating production of these metabolites can lead to inflammation, insulin resistance, and hypertension [43-45]. Individually, each of these adipo-secretomes exerts mild effects, but may collectively result in one or more systemic complications (Chan et al., 2021). Mango leaves supplemented and treated rats showed decreased levels of pro-inflammatory cytokines including TNF- α and leptin with increasing anti-inflammatory adiponectin. This is attributed to anti-inflammatory characteristics of mango leaves active components including polyphenols and flavonoids making mango leaves as a good functional food for obese and metabolic syndrome individuals [35, 29].

Mango leaves addition to rat's diet attenuated the genetic and epigenetic modifications caused by high fructose consumption. It was noticed that high fructose intake is associated with initiation of type 2 diabetes in rats with increased HDAC activity and decreased IRS-1 and GLUT-4 gene expression. Diabetes mellitus is characterized by persistent hyperglycemia. Hyperglycemia is due to the metabolic abnormalities. These abnormalities are explained by the

defects in glucose uptake and insulin action. Defective regulation of glucose transporter-4 (GLUT-4) protein takes place due to the inhibition of tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) followed by initiation of downstream signaling events which inhibits downstream signaling and insulin action [46].

The role of epigenetic histone modifications in diabetes is under investigation. HDACs are group of enzymes that remove the acetyl group from histone proteins on DNA, making DNA less accessible to transcription factors. HDACs have been revealed to play a significant role in the regulation of several key genes linked to diabetes by regulating the acetylation of histone and transcriptional factors that re-control glucose homeostasis. Consequently HDACs play a central role in the regulation of glucose metabolism and physiological insulin signaling. Thus, HDAC inhibition increase GLUT-4 translocation and insulin-induced glucose uptake in skeletal muscle [47].

High fructose diet detrimental effects are associated with the oxidative stress and that was confirmed by increased oxidative stress parameters in positive control rats. High fructose diet caused increased MDA and AGEs levels and decreased GSH level. High fructose diet increased lipid peroxidation leading to more formation of MDA. It also caused a state of hyperglycemia that initiate the formation of AGEs in tested rats. Opposing to this state of oxidative stress; antioxidants were depleted including GSH level [32, 48, 49]. Mango leaves attenuated oxidative stress due to their antioxidant capacity as they contain flavonoids and polyphenols [50].

The microscopic examination of pancreatic and adipose tissue supported the biochemical analysis results. Pancreas tissue examination of rats fed on high fructose diet revealed acute pancreatitis with congested blood vessels and mononuclear cells infiltrations thus may affect the function of β cells badly over time causing progression of type2 diabetes. While mango leaves active components protected pancreatic tissues from inflammation and consequent degeneration in supplemented and protected groups. Also, the microscopic examination of adipose tissue revealed increased size with abnormal distribution [49]. This is due to obesity caused by high fructose intake and confirmed by adipocyte size measurement. On the other hand addition of mango leaves powder to rat's diet controlled obesity and abnormalities in adipose tissues [32].

5. CONCLUSION

Mango leaves contain beneficial nutrients and active constituents with health benefits. Either mango leaves supplementation or treatment improved and ameliorated all the biochemical and microscopic observations by restricting metabolic and genetic alterations induced by high fructose consumption. The best improvement was observed in the mango leaves supplemented group. It is advised to add mango leaves to human diet as a seasoning to improve the health.

ETHECAL ISSUE

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All animal experiments were performed under a protocol approved by the Local Institutional Animal Ethics Committee of Ain Shams University.

NOTE:

The study highlights the efficacy of "herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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