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Research Article

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Potential Antioxidant Effect of Bitter Melon against Fructose-Induced Metabolic Syndrome in Male Rats

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ABSTRACT

Metabolic syndrome (MetS) is a very complex disorder, that directly increases the risk of cardiovascular diseases, type 2 diabetes, and all-cause mortality. Bitter melon (BM) has possessed powerful antioxidant properties. The present study aimed to assess the effect of freeze-dried BM juice against fructose-induced MetS in male rats. Metabolic syndrome was induced in rats by feeding high fructose (HF) diet (60%). After 8 weeks, BM was administered daily by oral gavage at doses (5, 10 or 15mg/kg body weight (BW)) for 6 weeks. The efficacy of freeze-dried BM juice on biological and biochemical parameters, as well as histopathological changes of liver and pancreas tissues in MetS rats was examined. The results showed that feeding HF diet-induced significantly increased in BW, blood pressure, glucose, insulin, leptin hormone, uric acid, and hyperlipidemia, as well as significantly increased inmalondialdehyde level compared with control rats. In addition, rats fed HF diet showed many histopathological changes in liver and pancreatic tissues. Treatment with BM significantly ameliorated all the tested biochemical parameters and overcome the histopathological alterations in liver and pancreas tissues comparing with Mets untreated rats, especially the MetS treated group with medium dose (10 mg/kg BW). Since MetS is closely associated with systemic disorders, and there is no specific drug for all these dysfunctions, therefore BM could be more effective in ameliorated MetS disorders; this may be explained by its antioxidant action. Further studies are needed to examine this possibility.

Keywords: *Metabolic syndrome; Rats; Bitter melon; Antioxidant; Histopathology.*

INTRODUCTION

Metabolic syndrome defined by a bundle of interconnected factors including hypertension, dyslipidemia, insulin resistance and obesity that increase subjects' risk to develop cardiovascular diseases(CVD) and type 2 diabetes mellitus [1]. It confers greater than 5-fold increase in the risk of type 2 diabetes and 3-folds increase risk for cardiovascular mortality compared with those without the syndrome; therefore, it is considered a serious health problem [2]. Its prevalence has been increasing dramatically during recent years through rapid transitions toward excessive energy intake and sedentary lifestyle [3]. Overconsumption of fructose-containing products could have deleterious metabolic effects in humans. It has been climbed dramatically and coincided with the development of MetS and prevalence of obesity and diabetes [4].

In the management of MetS, some plants have the representative in alternative and complementary medicine [5]. Bitter melon (*Momordicacharantia* L. family Cucurbitaceae), is a powerful nutrients-dense plant composed of many beneficial bioactive phytochemicals compounds [6]. It showed powerful antiviral, antibacterial and anticancer properties and stimulated immune system [7][8]. It has a strong antioxidant, hypolipidemic and anti-obesity properties [9]. Therefore, this study aimed to assess the potential therapeutic role of freeze-driedBM juice against HFdiet-induced MetS in male rats.

MATERIALS AND METHODS

Chemicals and kits

Casein, cellulose, fructose, sucrose, choline chloride, vitamins, and minerals mixture were obtained from Sigma-Aldrich Company. Corn oil and corn starch were obtained from the local market, Jeddah, Saudi Arabia. Glucose kit was obtained from Biovision. Leptin, insulin and malondialdehyde (MDA) Elisa kits were purchased from Cayman Chemical Company, USA. Uric acid, total cholesterol, triglycerides, and high-density lipoprotein cholesterol kits were obtained from Spinreact, Girona, Spain.

Animals and care ethics

Male Wister albino rats (n=50 rats) (185-215g) were purchased from the animal experimental unit of King Fahd Center for Medical Research (KFCMR), KAU. Rats were housed in standard laboratory conditions. They were fed AIN-93 diet [10], and drinking water *ad libitum*. The experiment was conducted at KFCMR, KAU in compliance with ethical policies.

Preparation of freeze-driedBM Juice

Bitter melon (*Momordicacharantia* L., Cucurbitaceae family) was obtained from the local market from Jeddah, Saudi Arabia. The plant was identified by Prof. Dr. AlaaEldin M.S. Khedr, Faculty of Pharmacy, KAU, Jeddah, Saudi Arabia. Bitter melon was washed, cut open and seeds were removed. The Juice was extracted by an electric juicer (Braun MP80, USA), filtered, condensed in the rotary evaporator under vacuum, then lyophilized and stored at 4 °C until further use. Lyophilization was conducted by using Freeze-Dryer Lyophilizer, Virtis, USA in KFCMR. Freeze-dried BM juice was prepared by dissolving in distilled water at the room temperature, and administered to rats once daily at three doses (5, 10 or 15 mg/kg BW/day) by oral gavage. The dose of 10 mg/kg BW was selected based on a previous experimental study [8].

Experimental design

All rats (n=50) were allowed to one week acclimatize before being used for this study, then rats were classified to control group (n=10); rats fed standard AIN-93 diet, and MetS group (n=40) rats fed HF diet (60%).After 8 weeks blood samples were collected for biochemical analyses [2]. Rats in MetS group were classified to four subgroups (n=10/each) as follows; untreated group (MetS), BM treated groups (MetS +BM) received BM orally by gavage at levels (5, 10 or 15 mg/kg BW/day) for 6 weeks. Both control and MetS untreated rats were received orally by gavage distilled water. During the experimental period, rats were weighed twice weekly in all groups. Blood pressure (BP) was determined by using a tail-cuff sphygmomanometer (Visitech BP-2000, Visitech Systems, Apex, NC) [2], it consists of a rat platform, control unit and computer runs analysis software system, which automatically analyzes the pulse waveform, inflates the cuffs for BP measures.

Blood collection and serum separation

After 14 weeks the blood samples withdrawn by heparinized capillary tubes from the retro-orbital plexusof each rat under anesthesia with diethyl ether for serum separation. Immediately after blood sampling, liver and pancreas were dissected out and fixed in 10% formalin for histopathological examination.

Biochemical analysis

Serum glucose was measured by enzymatic kit [11], insulin by using enzyme-linked immunosorbentElisaassay kit [12], and leptin by Elisa kits (Active murine leptin DSL-10-24100) [13]. Total cholesterol (TC) [14], triglycerides (TG) [15], high-density lipoprotein cholesterol (HDL-C) [16], and uric acid (UA) [17] were measured by using enzymatic colorimetric kits. Malondialdehyde was measured [18].

Histopathological examination

The fixed tissues of liver and pancreas were prepared to examine microscopically.

Statistical analysis

Results were analyzed by SPSS version 22. Data were expressed as the mean ± SD. Comparisons between mean values after 14 weeks and the corresponding values after 8 weeks were carried out using the student t-test.

RESULTS

Body weight

Table (1) showed BW during experimental periods and body weight gain percent (BWG %) in MetS rats with/without treatment with different doses of BM. The results revealed that after 8 weeks of feeding HF diet, MetS groups induced significant increase (p<0.001) in BW compared with control rats. After 14 weeks MetS untreated group showed a significant increase (p<0.001) in final BW and BWG% with the percent (26.58 % and 80.23 %, respectively) as percent change from the control group. There was significant difference between low dose (5 mg/kg BW) and MetS untreated group (p<0.01and p<0.05 in final BW and BWG%, respectively), while both (10 and 15 mg/kg BW) BM treated groups showed significant differences in final BW and BWG% (p< 0.001) as compared with MetS untreated group. It noticed that MetS untreated rats and MetS treated with low dose showed significant (p<0.001) increase in final BW, while MetS treated rats with the medium and the high doses of BM showed significant (p<0.001) decrease in final BW after 14 weeks as compared with the corresponding values after 8 weeks.

Table (1): Body weight and BW gain percent (BWG %) in control and MetS rats with/without BM treatment

Experimental groups	Initial BW (g)	BW (g) After 8 weeks	Final BW (g) After 14 weeks	BWG %
Control	202.01 ± 7.70	264.85 ± 11.70	308.40 ±14.31 $^{e\#}$	52.87 ± 4.11
MetS	199.90 ± 7.28	337.25 ± 20.98 $^{a\#}$	390.38 ±16.23 $^{a\# e\#}$	95.29 ± 8.18 $^{a\#}$
MetS +BM (5 mg/kg)	196.59 ± 8.69	334.74 ± 22.49 $^{a\#}$	364.39 ±12.05 $^{a\# b+ e\#}$	85.36 ± 8.48$^{a\# b*}$
MetS + BM (10 mg/kg)	199.22 ± 9.31	341.71 ± 13.30 $^{a\#}$	336.09 ±14.13 $^{a+ b\# c+ e\#}$	68.69 ± 6.29 $^{a+ b\# c\#}$
MetS +BM (15 mg/kg)	201.20 ± 7.40	335.64 ± 14.67 $^{a\#}$	327.87 ±14.43 $^{a* b\# c\# e\#}$	62.96 ± 6.13 $^{a+ b\# c\#}$

Dataare presented as means ± SD (n=10).[a] Significant versus control group.[b]Significant *versus*MetS untreated group.[c]Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups.[d]Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups.[e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p$< 0.05, ^{+}p< 0.01 and $^{\#}p$< 0.001).

Blood pressure (BP)

There was significant increase (p< 0.001) in systolic and diastolic BP in MetS groups, with percentage (68.26 %, 67.07 %, 66.77% and 73.06 %) in systolic BP and (51.25%, 52.5%, 50% and 53.34%) in diastolic BP in MetS groups after 8 weeks, respectively as percent change from control group. All experimentally treated groups showed significantly reduced (p<0.001) in systolic and diastolic BP after 14 weeks as compared with MetS untreated group.

The medium dose was the most effective dose compared with the other two doses. There was a significant difference ($p< 0.05$) between MetS untreated group after 14 weeks and their corresponding values after 8 weeks on systolic and diastolic BP. However, treatment with BM induced significant improvement ($p< 0.001$) in systolic and diastolic BP after 14 weeks as compared with their corresponding values after 8 weeks (Table 2).

Table (2): Blood pressure (BP) (mm/Hg) in control and MetS rats with/without BM treatment

Experimental groups	**Systolic BP** (mm/Hg)		**Diastolic BP** (mm/Hg)	
	After 8 weeks	**After 14 weeks**	**After 8 weeks**	**After 14 weeks**
Control	111.33 ± 1.53	113.67 ± 2.08	80.0 ± 1.00	81.33 ± 1.53
MetS	187.33 ± 4.93 [a#]	198.33 ± 2.52 [a# e*]	121.00 ± 4.58 [a#]	128.33 ± 2.08 [a# e*]
MetS +BM (5 mg/kg)	186.00 ± 8.19 [a#]	133.33 ± 4.16 [a# b# e#]	122.00 ± 2.65[a#]	97.00 ± 3.00 [a# b# e#]
MetS + BM (10 mg/kg)	185.67 ± 7.77 [a#]	117.33 ± 3.51 [b# c# e#]	120.00 ± 2.65 [a#]	84.67 ± 1.53 [b# c# e#]
MetS +BM (15 mg/kg)	192.67 ± 9.29 [a#]	124.33 ±1.53 [a+ b# c+ d* e#]	122.67 ± 3.06 [a#]	90.00 ± 2.64 [a+ b# c+ d* e#]

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Glucose and insulin levels

The results showed that MetS groups showed a significant increase ($p< 0.001$) in glucose and insulin levels after 8 weeks compared with control rats. Moreover, the same trend was noticed in MetS untreated group after 14 weeks. There was a significant difference ($p<0.001$) between MetS untreated group after 14 weeks and their corresponding values after 8 weeks on glucose and insulin levels. However, treatment with BM induced significantly decreased ($p< 0.001$) in both glucose and insulinlevels in medium and high doses (10 and 15 mg/kg BW), with no significant difference in low dose after 14 weeks as compared with their corresponding values after 8 weeks (Table 3).

Table (3): Serum glucose and insulin concentrations in control and MetS rats with/without BM treatment

Experimental groups	**Glucose** (mg/dl)		**Insulin** (uU/ml)	
	After 8 weeks	**After 14 weeks**	**After 8 weeks**	**After 14 weeks**
Control	95.07 ± 7.83	105.29 ± 8.88	22.95 ± 1.91	24.77 ± 2.35
MetS	161.57 ± 7.24 [a#]	201.60 ± 14.84 [a# e#]	42.07 ± 3.48[a#]	59.32 ± 5.65 [a# e#]
MetS +BM (5 mg/kg)	158.12 ± 8.62 [a#]	153.39 ± 14.90 [a# b#]	41.71 ± 3.14 [a#]	39.26 ± 3.31[a# b#]
MetS + BM (10 mg/kg)	160.79 ± 6.58[a#]	119.97 ± 10.15[a* b# c# e#]	40.41 ± 2.06 [a#]	29.53 ± 2.86 [a* b# c# e#]
MetS +BM (15 mg/kg)	160.12 ± 6.43 [a#]	128.22 ± 11.89 [a+ b# c+ e#]	42.09 ± 1.82[a#]	32.48 ± 3.21[a+ b# c# e#]

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Leptin hormone

The results from Figure (1) showed that MetS groups recorded significant increase ($p< 0.001$) in leptin hormone level after 8 weeks, as well as untreated MetS group after 14 weeks compared with the corresponding values in control rats. A significant decrease ($p<0.001$) in leptin hormone level between all the experimental treated MetS groups as compared with MetS untreated group was shown. Treatment with BM induced significantly decreased ($p< 0.001$) in leptin hormone level between all the three used treatment doses (5, 10 or 15 mg/kg BW) after 14 weeks and their corresponding values after 8 weeks. The most effective dose was (10 mg/kg BW), there was a significant difference ($p<0.05$) between medium and high dose after treatment in leptin hormone level.

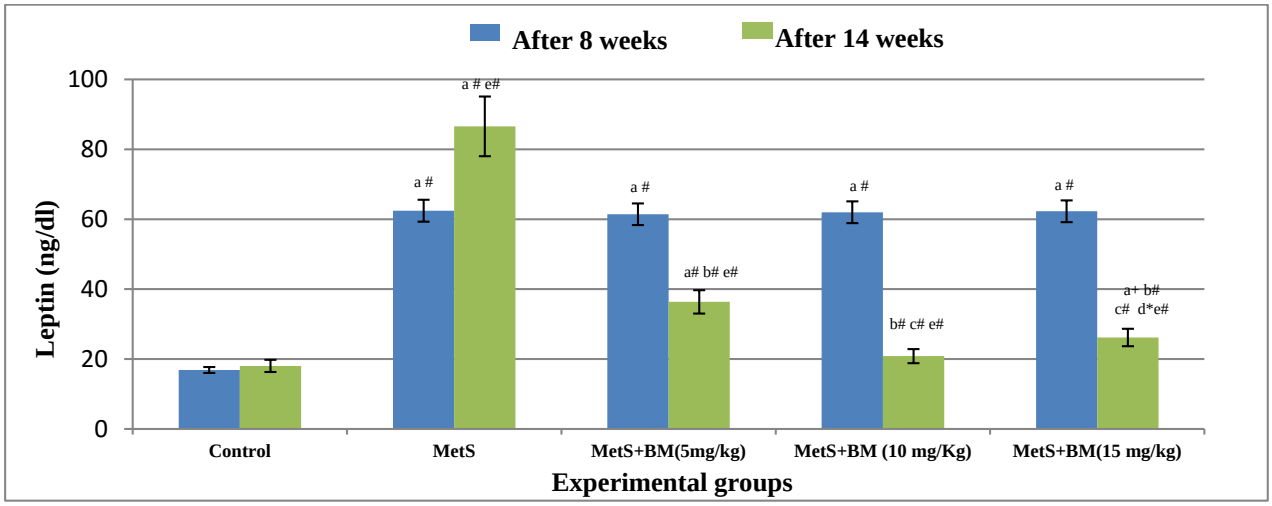

Figure 1: Serum leptin hormone (ng/dl) level in control and MetS rats with/without BM treatment

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Lipid profile

The data indicated that MetS groups recorded significant increase ($p< 0.001$) in TC, TG and LDL-C with a significant decrease ($p< 0.001$) in HDL-C levels after 8 weeks compared with their values in the control group. The same trend observed in MetS untreated group after 14 weeks when compared with control group. Oral administration of BM induced a significant decrease in TC, TG and LDL-C with a significant increase in HDL-C ($p<0.001$) levels between all the treated groups as compared with MetS untreated group, the medium dose was the most effective dose. In addition, there was no significant difference when compared between medium dose (10 mg) and high dose (15 mg/kg BW) of BM in all tested lipid profile parameters, expect there was a significant difference ($p< 0.05$) in LDL-C level. When compared with each group values after 14 weeks and the corresponding values after 8 weeks the results revealed that, there was no significant difference in control values, while significant change ($p<0.001$) between MetS untreated group and the corresponding values in all lipid tested parameters. Treatment with BM induced significant improvement ($p< 0.001$) in tested lipid parameters after 14 weeks and their corresponding values after 8 weeks (Tables 4 & 5).

Malondialdehyde (MDA)

The results revealed that MetS untreated groups recorded significant increase ($p< 0.001$) in MDA comparing with the control group after 8 weeks, as well as after 14 weeks for MetS untreated group. Treatment MetS with BM at the three doses used induced significantly improved ($p<0.001$) in MDA values compared with untreated MetS group. The medium dose was the most effective compared with the other two doses in reducing the MDA level (Figure 2).

Table (4): Serum total cholesterol (TC) and triglycerides (TG) levelsin control and MetS rats with/without BM treatment

Experimental groups	**TC** (mg/dl)		**TG** (mg/dl)	
	After 8 weeks	**After 14 weeks**	**After 8 weeks**	**After 14 weeks**
Control	88.30 ± 2.48	90.64 ± 5.44	78.39 ± 3.60	82.88 ± 7.43
MetS	170.86 ± 3.65 $^{a\#}$	195.28 ± 5.59 $^{a\# e\#}$	142.88 ± 5.61 $^{a\#}$	153.24 ± 6.94$^{a\# e\#}$
MetS +BM (5 mg/kg)	169.74 ± 4.74 $^{a\#}$	141.29 ± 5.09 $^{a\# b\# e\#}$	143.95 ± 4.34 $^{a\#}$	130.45 ± 8.85$^{a\# b\# e\#}$
MetS +BM (10 mg/kg)	170.53 ± 4.69 $^{a\#}$	98.46 ± 8.89 $^{b\# c\# e\#}$	142.47 ± 3.44 $^{a\#}$	89.10 ± 8.45 $^{b\# c\# e\#}$
MetS +BM (15 mg/kg)	171.33 ± 4.72$^{a\#}$	104.80 ± 7.65 $^{a+ b\# c\# e\#}$	145.11 ± 3.98 $^{a\#}$	95.85 ± 8.63$^{a+ b\# c\# e\#}$

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Table (5): Serum low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) (mg/dl) in control and MetS rats with/without BM treatment

Experimental groups	**LDL-C** (mg/dl)		**HDL-C** (mg/dl)	
	After 8 weeks	**After 14 weeks**	**After 8 weeks**	**After 14 weeks**
Control	7.91 ± 0.58	10.05 ± 0.87	64.71 ± 3.98	64.01 ± 4.06
MetS	103.77 ± 4.12$^{a\#}$	133.69 ± 5.99$^{a\# e\#}$	38.51± 3.49$^{a\#}$	30.94 ± 2.38 $^{a\# e\#}$
MetS +BM (5 mg/kg)	100.13 ± 5.16 $^{a\#}$	69.06 ± 4.04 $^{a\# b\# e\#}$	40.82 ± 3.58$^{a\#}$	46.14 ± 3.40$^{a\# b\# e\#}$
MetS + BM (10 mg/kg)	101.82 ± 3.43 $^{a\#}$	19.49 ± 1.32 $^{a* b\# c\# e\#}$	40.22 ± 2.15 $^{a\#}$	61.15 ± 4.34$^{a* b\# c\# e\#}$
MetS +BM (15 mg/kg)	102.93 ± 6.57 $^{a\#}$	27.34 ± 2.01$^{a\# b\# c\# d* e\#}$	39.38 ± 3.39 $^{a\#}$	58.29 ± 3.31$^{a+ b\# c\# e\#}$

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Uric acid (UA)

As illustrated in Figure (3), MetS groups recorded significantly increased ($p< 0.001$) in UA level after 8 weeks compared with control rats. In all MetS treated groups significantly decreased ($p< 0.001$) in UA level was shown compared with MetS untreated group. Comparing the effect of different doses of BM treatment it showed that, there was a significant difference ($p<0. 001$) in UA level between low and both medium and high doses after 14 weeks, while no significant difference when compared between the medium and the high dose of BM in UA level. There was a significant increase ($p< 0.001$) between MetS untreated group after 14 weeks and their corresponding values after 8 weeks in UA level. However, treatment with BM induced significant difference ($p< 0.001$) in UA level between all the three used doses (5, 10 or 15 mg/kg BW) after 14 weeks and their corresponding values after 8 weeks of feeding HF diet.

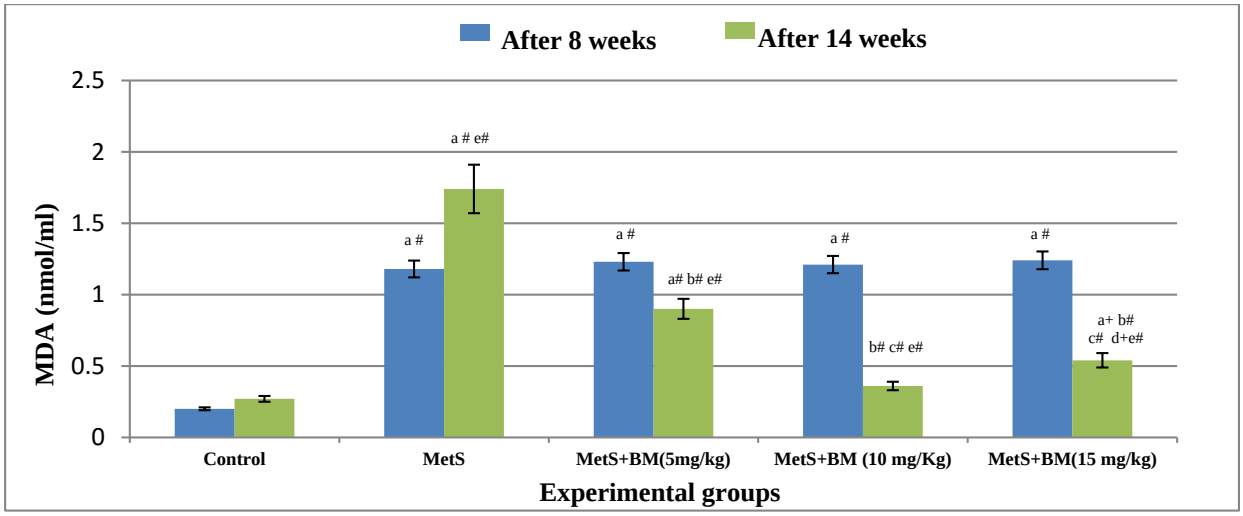

Figure 2: Serum malondialdehyde (MDA) (nmol/ml) level in control and MetS rats with/without BM treatment

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

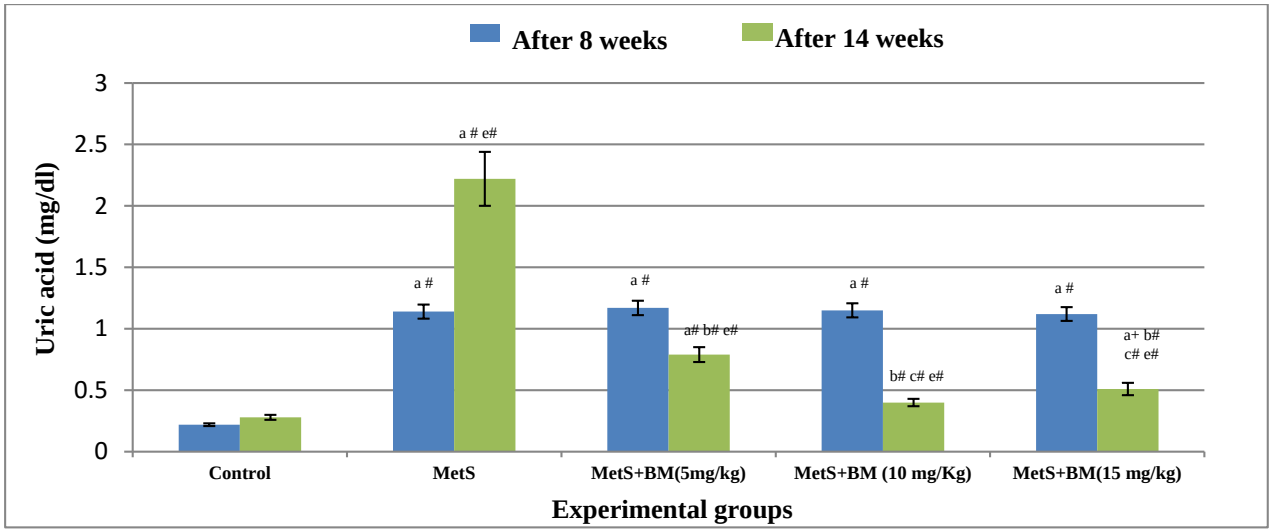

Figure 3: Serum uric acid (UA) (mg/dl) level in control and MetS rats with/without BM treatment

Data are presented as means ± SD (n=10).[a] Significant versus control group. [b] Significant *versus* MetS untreated group. [c] Significant between MetS + BM (5mg /Kg) and MetS + BM (10 and 15 mg/kg) groups. [d] Significant between MetS+ BM (10 mg /Kg) and MetS +BM (15 mg /Kg) groups. [e] Significant between mean values after 14 weeks and the corresponding values after 8 weeks. ($^{*}p< 0.05$, $^{+}p< 0.01$ and $^{\#}p< 0.001$).

Histopathological investigation

Liver sections from the control group showed no histological change (Figure 4(1)). Focal hepatic haemorrhage, necrosis of sporadic hepatocytes, hyperplasia of epithelial lining bile duct, inflammatory cells infiltration in portal triad, fibroblasts proliferation in the portal triad, and degenerated hepatocytes were shown in liver sections from the MetSuntreated rats (Figure 4(2.a-c)). The low dose of BM (5mg/kg BW) treated rats showed necrosis of sporadic hepatocytes, Kupffer cells activation, and congestion of hepatic sinusoids (Figure 4(3.a&b)). Sections from Mets + BM(10mg/kg BW) treated rats showed no histopathological changes, except some sections, showed slight congestion of hepatic sinusoids (Figure 4(4.a&b)). Liver sections of MetS rats treated with the high dose of BM (15 mg/kg BW) showed kupffer cells activation (Figure 4(5)).

Histological examination of pancreatic tissues of the control rats showed no structure change (Figure 5(1)). Pancreas sections of the MetSuntreated rats showed dilatation of pancreatic duct, inflammatory cells infiltration between pancreatic acini and necrosis of the islets of Langerhans (Figure 5(2.a-c)). Pancreatic sections from MetS + BM (5 mg/kg BW) showed vacuolation and necrosis of the islets of Langerhans (Figure 5(3)). In MetS rats treated with BM (10 mg/kg BW) pancreas sections showed no histopathological changes, expect slight congestion of blood capillaries (Figure 5(4.a&b). Meanwhile, pancreas sections from the MetS group treated with high dose (15 mg/kg BW) showed no histopathological changes, while some sections showed hyperplasia of the islets of Langerhans (Figure 5(5. a &b)).

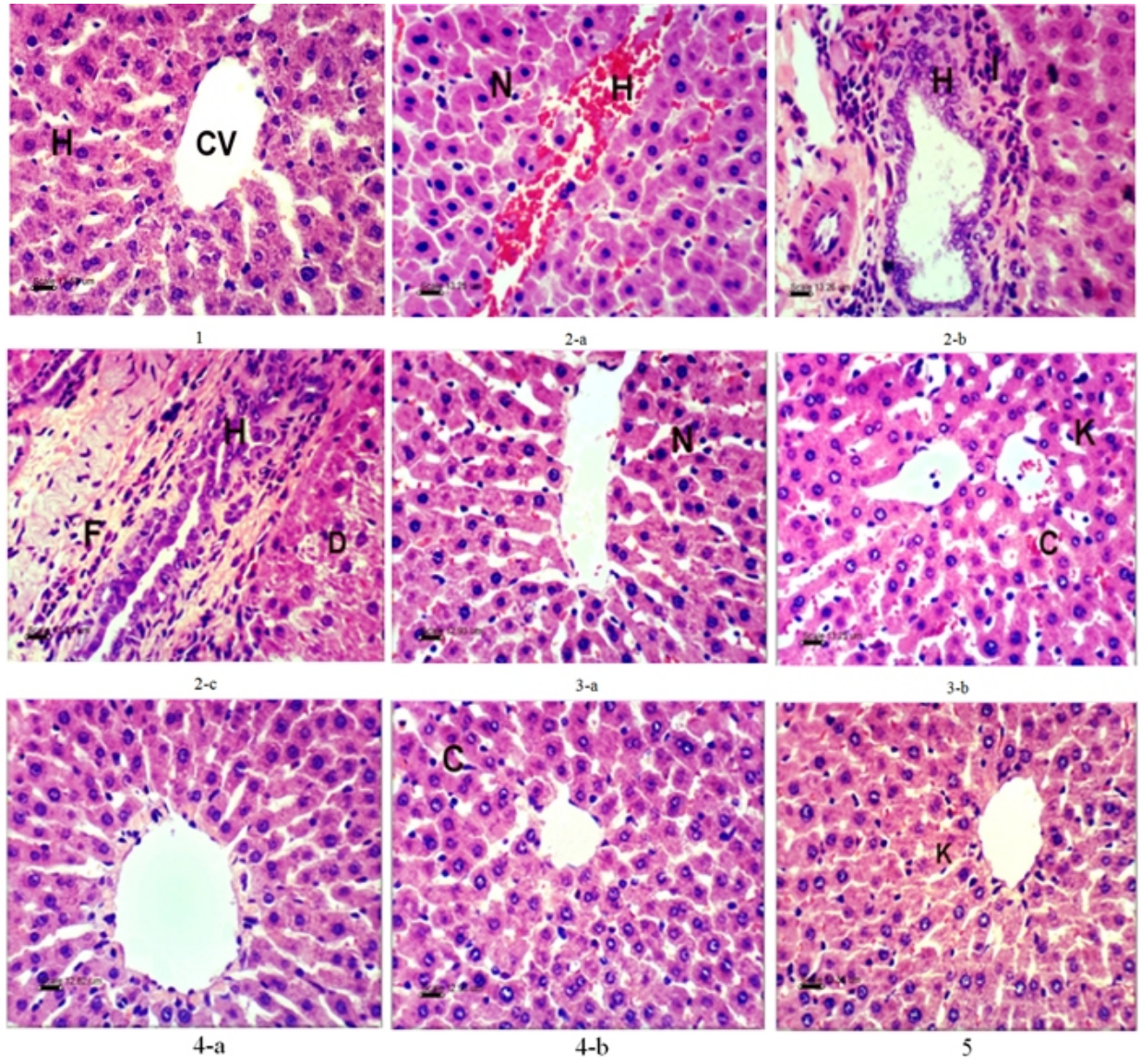

Figure (4): Liver sections from control rats showed no histological change of hepatic lobule from central vein (CV) and concentrically arranged hepatocytes (H) (Fig.1). Liver sections from MetS rats showed focal hepatic haemorrhage (H) and necrosis of sporadic hepatocytes (N) (Fig.2.a), hyperplasia of epithelial lining bile duct (H) and inflammatory cells infiltration in portal triad (I) (Fig 2.b), with fibroblasts proliferation (F) in portal triad and degenerated hepatocytes (D) (Fig 2.c). Low dose of BM (5mg/kg BW) treated rats showed necrosis of sporadic hepatocytes (N) (Fig 3.a), and Kupffer cells activation (K) with congestion of hepatic sinusoids (C) (Fig 3.b). Medium dose of BM (10 mg/kg BW) treated rats showed no histopathological changes (Fig 4.a), except some sections showed slight congestion of hepatic sinusoids (C) (Fig 4.b). Liver sections of rats treated with high dose of BM (15 mg/kg BW) showed Kupffer cells activation (K) (Fig. 5). (H & E X 400)

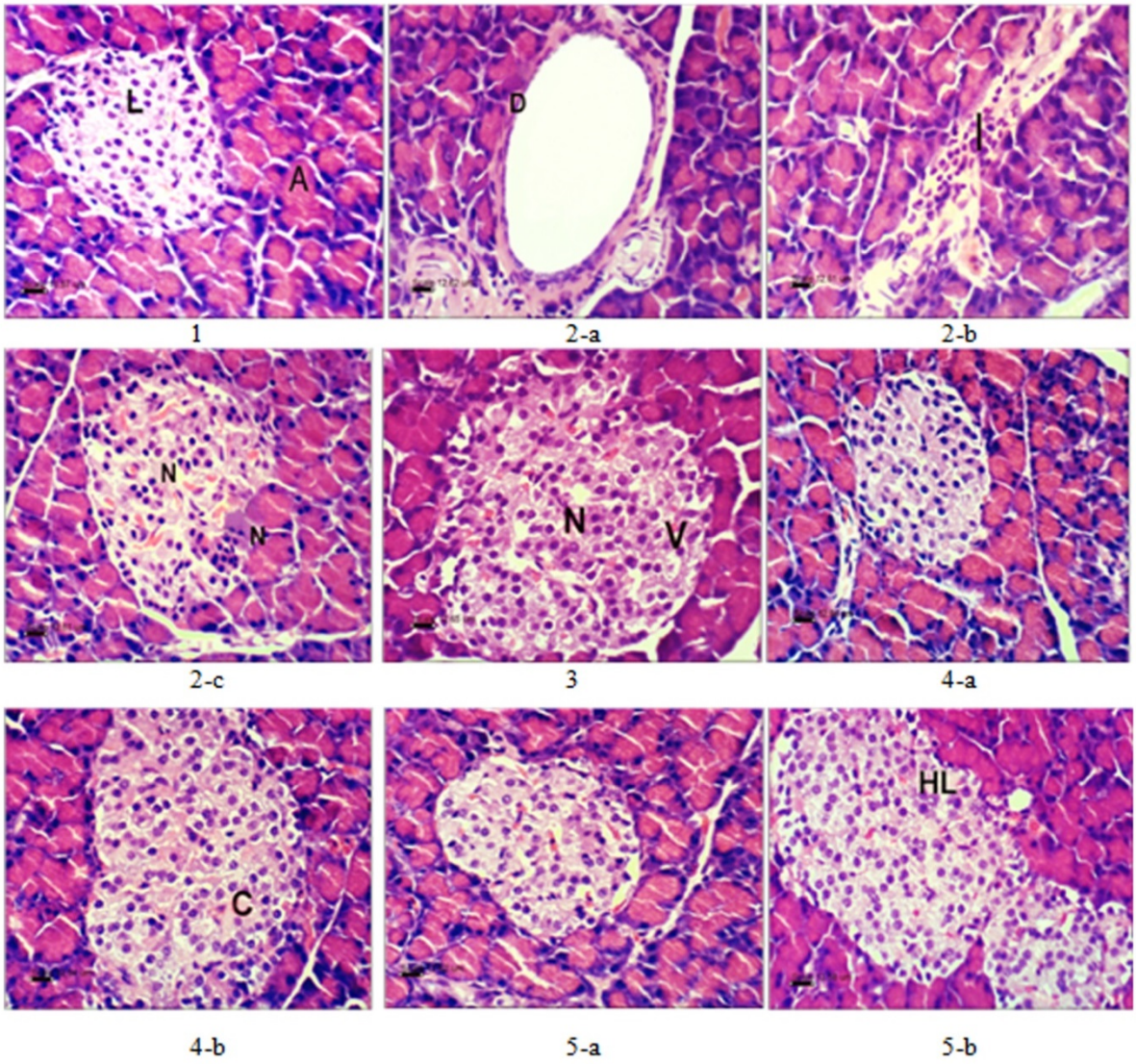

Figure (5): Pancreas sections from control rats showed normal islets of Langerhans (L) and normal exocrine pancreatic acini (A) (Fig.1). Pancreas sections from MetS rats showed dilatation of pancreatic duct (D) (Fig.2.a), inflammatory cells infiltration between pancreatic acini (I) (Fig.2.b), and necrosis of the islets of Langerhans (N) (Fig. 2.c). Pancreas sections of rat treated with 5 mg BM/kg BW showed vacuolation (V) and necrosis of the islets of Langerhans (N) (Fig. 3). Pancreas sections from rats treated with 10 mg BM/kg BW showed no histopathological changes (Fig.4.a), expect some sections showed congestion of blood capillaries (C) (Fig.4.b). Pancreas sections from rats treated with BM (15 mg /kg BW) showed no histopathological changes (Fig.5.a), while other sections showed hyperplasia of the islets of Langerhans (HL) (Fig.5.b). (H & E X 400)

DISCUSSION

Metabolic syndrome becomes a public health problem throughout the world. Close links exist between MetS and oxidative stress due to an imbalance between pro-oxidant and antioxidant [19]. Bitter melon (*Momordicacharantia*) may help to treat or reduce MetS disorders by assisting the body homeostasis mechanisms [20]. The hypothesis of this study is to investigate the possible role of BM against HF-induced MetS in male rats.

In the present study, feeding HF diet-induced significantly increased in BW compared with control group. The obtained results agree with Mahmoud and Elshazly [21]. This may be attributed to chronic fructose consumption could lead to enhance caloric overconsumption, induce leptin and insulin resistance which have a vital role in food intake regulation, energy homeostasis, expenditure, acceleration high fat-induced obesity and oxidative stress [22][23]. Administration of BM to MetS rats induced a significant difference in BWG % compared with MetS untreated group. This deceleration in BW may be explained by Alam et al.[24] who reported that peroxisome

proliferator-activated receptors (PPARs) and liver X-receptors (LXRs) are two regulatory proteins, which have the pivotal role in metabolic homeostasis regulation, BM reduced the lipid accumulation by down-regulated PPARγby its antioxidant activity.

The obtained results showed that systolic and diastolic BP in MetS untreated group were significantly increased after 8 and 14 weeks of feeding HF diet compared with the corresponding values in control rats. These results agree with the results obtained by Hwang et al. [25] who found that fructose has been shown to induce hypertension and increase BP in rats. Treatment MetS with BM induced a significant reduction in BP compared with MetS untreated group. In agreement with these results are the results obtained by Lee et al. [26] who reported that BMextract induced normalizing blood pressure in subjects with metabolic syndrome. This effect of BM may be attributed to the presence of bioactive compounds which act to induce ammonia detoxification by removing excess ammonia, urea and uric acid [27].

In the obtained results, MetS untreated rats showed significantly elevated in glucose and insulin concentrations compared with control group. These results agree with Amini and Janghorbani [28]. This may be explained by HF diet-induced excess insulin secretion, which leads to raising blood glucose and further deterioration of ß-cells functions and insulin sensitivity *via* glucose toxicity [29]. Oral administration of BM resulted in significant reduce in glucose and insulin concentrations in MetS treated groups at all doses used, especially medium dose as compared with untreated MetS rats. The present finding is in accordance with Chen et al. [13]. This may be explained by BM that has the ability to stimulate insulin sensitivity and reduce oxidative stress [5]. Moreover, BM contains bioactive antioxidant components with anti-diabetic properties such insulin-like protein; p-insulin, v-insulin, or polypeptide-p and other, which regulate insulin release and altered glucose metabolism [30].

Feeding HF diet-induced significantly increased in leptin hormone level as compared with control group. This effect may be attributed to the exogenous leptin impaired to transport across the blood-brain barrier and reach target sites in the brain [31]. There was a significant decrease in leptin hormone level in all treated groups as compared with MetS untreated group. Chen et al. [13] found that BM induced decrease in the level of leptin appetite-controlling hormone in rats fed HF and treated with BM compared to untreated group, this may be attributed to biologically active compounds in BM, which have a hypoglycemic effect, and this effect is associated with reduced adiposity [24], thus confirmed in the obtained results.

Rats in MetSgroup showed a significant disturbance in lipid profile parameters compared with control rats. Fructose overconsumption leads to dyslipidemia and ectopic lipid deposition, along with increase in hepatic lipids and insulin resistance [21]. This results may be explained by HF can be converted to TG through de novo lipogenesis thus elevate blood lipid levels [32]. Treatment with BM significantly improved lipid profiles compared with untreated MetS group, the medium dose showed the most effective hypolipidemiceffect. Treatment with BM significantly corrected the hyperlipidemia in MetS rats, this effect may be explained by BM that has a potent inhibitor of lipogenesis and lipid deposition [13].

Oxidative stress has been identified as a major mechanism of vascular complications in the MetS. The results showed that, there was significantly increased in MDA level in MetS group compared with control group. These results agree with the results obtained by Kostogrys and Pisulewski [33]. This effect may be explained *via* fat accumulations induced obesity-leads to un-regulation production of adipocytokines, thus induced increase in ROS production [23]. Administration of BM significantly reduced MDA in all MetS treated groups compared with untreated group. This effect could be attributed to BM possessed potent antioxidant and free radicals scavenging activities [24].

Feeding HF diet induced a significant increase in UA level compared with control rats. These results agree with the results obtained by Suhaila [20]. This may be explained by the hyperinsulinemia in MetS that can lead to impairment in urate excretion [34]. Administration of BM induced reduction in UA when compared between MetS untreated group and MetS treated groups. Administration of BM significantly decreased serum urea [5], this may be explained by bioactive phenolic compounds of BM which act as xanthine oxidase inhibitors [35].

Histopathological examination of liver sections of MetS untreated rats showed focal hepatic haemorrhage, necrosis of sporadic hepatocytes, inflammatory cells infiltration in the portal triad and fibroblasts proliferation in the portal triad. These results agree with Tsutomu et al. [36]. Treatment MetS rats with BM showed an improvement in liver tissues, the most effective treatment was shown in medium BM dose (10mg/kg BW). This may be attributed to the antioxidant activity of BM, thus maintaining the structural cellular integrity of the hepatocellular membrane. In the present study, histopathological examination of the pancreatic sections of MetS group showed dilatation of pancreatic duct, infiltration between pancreatic acini and necrosis of Langerhans cells. Examination of the pancreatic sections from MetS rats treated with the medium dose of BM showed nearly no histopathological changes. These findings may be due to the hypoglycemic role of BM by increasing the renewal and allowing the recovery of destroyed of β-cells in the pancreas [37].

CONCLUSION

Overconsumption of fructose increases the incidence of MetS. In the present study, different doses of BM were evaluated for their beneficial effects as an antioxidant in treatment MetS symptoms in male rats. The results revealed that the most effective treatment was the medium dose (10 mg/kg BW), which has therapeutic effects as antioxidants, hypoglycemic, hypolipidemic and hypotensive activities.

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