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RESEARCH ARTICLE

Effect of moringa oleifera with and without metformin on an experimental model of metabolic syndrome in rats

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Abstract

Metabolic syndrome is a multiplex risk factor characterized by the presence of obesity, hyperglycemia, insulin resistance and increased risk of cardiovascular disease. Moringa Oliefera is a herbal medicine which showed beneficial effects on blood glucose and lipid profile in experimental and human studies. So, the objective of this study was to evaluate the effects of Moringa Oliefera leaves extract in an experimental model of metabolic syndrome. High-carbohydrate, high-fat (HCHF) diet was used for induction of metabolic syndrome in rats which showed its effects through elevation of blood glucose and enhanced insulin resistance as evidenced by the homeostatic model assessment (HOMA- IR). Also, there were elevated levels of TNFα and leptin together with reduced adiponectin levels as well as development of hepatic steatosis as evidenced by liver histopathology and elevated hepatic enzymes. Rats treated with Moringa oliefera and metformin alone or in combination showed a significant improvement of lipid profile, insulin resistance, reduction of elevated TNFα and leptin and elevation of depleted adiponectin together with improvement of hepatic histopathological changes and liver enzymes. These beneficial effects were more pronounced when Moringa was combined with metformin which led to the conclusion that Moringa Oliefera leaves extract could be used as a supplement in addition to metformin for treatment of metabolic syndrome given the paucity of effective therapeutic modalities for this growing epidemic.

INTRODUCTION

Metabolic syndrome (MetS) is characterized by the presence of different physiological, biochemical, clinical, and metabolic factors that directly increases the risk of type 2 diabetes mellitus, and cardiovascular disease, and can lead to increased mortality. Several factors constitute the syndrome like chronic stress, genetic susceptibility, Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, elevated blood pressure, hyper-coagulable state (O'Neill & O'Driscoll, 2015). Metabolic syndrome is associated with chronic inflammation and is characterized by increased level of tumor necrosis factor α, interleukin-1 (IL-1), IL-6, leptin, and decreased level of adiponectin **(Kaur, 2014).**

The management of MetS is troublesome on the grounds that there is no direct strategy to counteract or enhance the entire disorder. Insulin resistance is the main pathogenic issue in MetS. So, Metformin is used for controlling diabetes and obesity, two characteristics of MetS **(Gangale, 2011, (Rojas and Gomes, 2013).**
Moringa oleifera is cultivated worldwide. It is used for many nutritional and medicinal properties **(Farooq et al., 2012).** Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids **(Manguro and Lemmen, 2007; Amaglo etal.,2010; Gowrishankar et al.,2010)**. Moringa oleifera has shown protective effects against streptozotocin(STZ) induced diabetes as it showed antioxidant and antidiabetic effects **(Jaiswal et al., 2009; Gupta et al., 2012).** It also showed antidiabetic effects in human **(Kumari, 2010; Ghiridhari et al., 2011).** Administration of moringa to rats along with high fat diet decrease total cholesterol in liver and serum (Ghasi et al.,2000). Moringa oleifera also showed antihyperlipedemic effects in rabbit with induced dyslipidemia ***(*Chumark et al., 2008*)*** and in rats fed a high-fat diet (HFD) **(Jain et al., 2010).** So ,the aim of this study was to evaluate the possible effects of moringa oliefera on an experimental model of metabolic syndrome regarding its components as well as insulin resistance.

Materials and methods:

Plant extract:
Ethanol extract of moringa oliefera leaves was prepared in the department of pharmacognosy, Faculty of Pharmacy, Mansoura University.

Experimental animals
Male *Sprague-Dawley rats (8–9 wk old, +/-250 g, n = 70)* were used throughout the present study. This study was carried out according to the guidelines and authorization of the Ethical Committee of Mansoura University, Faculty of Medicine. All efforts were made to minimize suffering of animals.

Induction of metabolic syndrome:
Rat model of metabolic syndrome was carried out through feeding the animals with, high-carbohydrate, high-fat (HCHF) diet contained 50% carbohydrate and 24% fat (mainly beef tallow) along with 25% fructose in drinking water (total 68% carbohydrate using mean food and water intakes) for16 weeks.
Rats with changes in metabolic variables as well as liver structure and function were considered to have metabolic syndrome and were included in the study. Control rats were fed a corn starch-rich (CS) diet contained 68% carbohydrate (mainly cornstarch) and 0.7% fat for 16 weeks ***(*Panchal et al, 2012).***

Grouping of Animals:
Rats were divided into the following groups (10 rats each):

- **Group 1:** Control CS fed rats for 8 weeks.
- **Group 2:** Control CS fed rats for 16 weeks.
- **Group 3:** HCHF fed rats for 8 weeks ***(*Panchal et al, 2012).***
- **Group 4:** HCHF fed rats for 16 weeks ***(*Panchal et al, 2012).***
- **Group 5:** HCHF fed rats treated with metformin, 200 mg/kg/day (Hussein et al 2011) starting from week 8 to week 16.
- **Group 6:**HCHF fed rats treated with moringa oleifera**,** 200 mg/kg /day **(Jain et al., 2010)** starting from week 8 to week 16.
- **Group 7:** HCHF fed rats treated with moringa oleifera, 200 mg/kg/day plus metformin 200 mg/kg/day starting from week 8 to week 16.
- During the study food intake, water intake and energy intake were measured daily. Final body weight and abdominal circumference were measured before scarification.

- Rats were sacrificed at the end of the study at 16 weeks and blood samples were taken for measurement of the following:

A. Metabolic assessment by measurements of:

1. Fasting plasma glucose level according to Trinder, (1969)
2. Fasting plasma insulin level according to Olsson & Carlsson, (2005)
3. Calculation of homeostasis Model Assessment (HOMA-IR) according to Mathews et al (1985) as follows;

$$\frac{fasting\ insulin\ (\mu U/l) \times fasting\ glucose\ (mmol/l\ )}{22.5}$$

4. Lipid profile (Plasma triglycerides according to fossati, (1982), Plasma total cholesterol, Plasma low density lipoprotein cholesterol, and Plasma high density lipoprotein cholesterol) according to Tietz, (1976).

B. Hepatic Enzymes:

1. Plasma Aspartate Transaminase (AST)and Plasma Alanine Transaminase (ALT) according to Tietz, (1976).

C. Tumor necrosis factor alpha (TNFα), Adiponectin, and leptin by Enzyme-Linked Immunosorbent Assay (ELISA) parmeters following manifacturer's instructions (Ray Biotech, Inc. USA)

Histopathological examination:

Rat livers were dissected after scarification, fixed in 10% formalin, embedded in paraffin. Liver sections were cut, stained and examined by light microscope for steatosis, inflammatory cell infiltration and fibrosis assessment ***(*Panchal et al, 2012*)***.

Statistical Analysis

All data are presented as Mean ±SD. One-way analysis ANOVA followed by post hoc (Tukey) multiple comparison test were used for comparison between groups to assess parameteric data. while Kruskal-Wallis test used to assess non parameteric data. A value of P <0.05 was considered statistically significant.

Results:

Table (1): Comparison of body weight and abdominal circumference of rats in different experimental groups

	8 weeks	**16 weeks**				
	HCHF	CS	HCHF	HCHF + MET	HCHF+ MOR	HCHF+ (MET + MOR)
Body weight(g)	210±8.9	248.3±8.1	331.7±22.2*	278.3±14.7#	270.0±14.1#	243.3±25.8#@
Abdominal circumference (cm)	16.2±0.7	15.8±0.7	22.3±1*	20.0±1#	18.7±1.2#	17.5±1#@

HCHF = high carbohydrate high fat **CS=** corn starch **MET=** metformin **MOR=** moringa oleifera. * difference from CS at 16 weeks, # difference from HCHF at 16 weeks, @ difference from metformin treated group.

Table (2): Comparison of metabolic and inflammatory markers in different experimental groups of rats

	8 weeks	**16 weeks**				
	HCHF	CS	HCHF	HCHF + MET	HCHF+ MOR	HCHF+ (MET+MOR)
FBG mmol/l	7.1±0.1	5.4±0.4	9.6±0.7*	7.1±0.5#	7.3±0.3 #	7.2±0.5#
Fasting insulin μu/l	12.8±0.7	8.9±0.6	15.4±0.8*	10.7±0.4#	11.0±0.6#	9.9±0.5#
HOMA-IR	4.0±0.5	2.1±0.3	6.5±0.7*	3.4±0.2#	3.5±0.2#	3.2±0.3#
Adiponectinpg/ml	29±1.5	66±1.9	20±1.4*	37±2#	41±2#	44±1.8#@
Leptinpg/ml	362.3±15	79.5±3.2	371.7±9*	230.2±15.7#	246.7±8.8#	194.8±9.9#@
TNF-αpg/ml	5.9±0.4	2.7 ±0.1	7.8±0.3*	6.1±0.3#	5.6±0.3#	4.8±0.5# @
TGmg/dl	133.7±11.7	106.7±13.3	175.2±11.4*	148.5±11.4#	142.0±7.1#	127.3±8.5#@
TCmg/dl	166.7±14	112.7±5	168.3±10.3*	240.7±11.1#	156.0±10.1#	143.2±9.7#@
HDLmg/dl	36.8±3.3	57.2±5.7	25.3±2.4*	37.6±1.6#	44.2±5.6#	49.5±3.8#@
LDLmg/dl	102.5±33.6	42.7±2.3	182.8±10.3*	148.0±3.3#	99.8±5.6#	83.8±12.3#@
ASTU/ml	23.7±1.6	9.3±0.8	39.0±1.5*	23.2±0.7#	21.0±1.1#	18.8±0.7# @
ALTU/ml	16.0±0.9	7.0±0.9	22.7±1.4*	17.0±0.9#	14.7±0.8#	10.2±1.2#@

HCHF = high carbohydrate high fat **CS=** corn starch **MET=** metformin **MOR=** moringa oleifera * difference from CS at 16 weeks, # difference from HCHF at 16 weeks, @ difference from metformin treated group.

A) Biochemical parameters

Induction of metabolic syndrome by HCHF diet for 16 weeks was evident by significant increase in body weight and abdominal circumference compared with CS fed rats (table 1). Treatment of rats with either metformin or moringa oleifera for 8 weeks resulted in significant reduction of body weight and abdominal circumference in relation to non- treated HCHF fed rats. While combined metformin and moringa oleifera treated group showed significant decrease in body weight and abdominal circumference compared with metformin treated group alone (table 1).

Administration of HCHF diet to rats for 16 weeks resulted in significant elevation of fasting blood glucose and insulin level and HOMA index compared with CS fed group. Metformin, moringa oleifera or combined therapy with both of them resulted in significant reduction in these parameters compared with non-treated HCHF fed group (table 2, fig 1).

HCHF fed rats showed increased levels of TG, TC, LDL, with decreased levels of HDL. Treatment of HCHF fed rats with either metformin or moringa oleifera or their combination resulted in significant reduction of TG, TC, LDL and significant increase of HDL compared with non-treated HCHF fed rats. Combination therapy with both metformin and moringa oleifera resulted in significant difference compared with metformin alone (table 2).

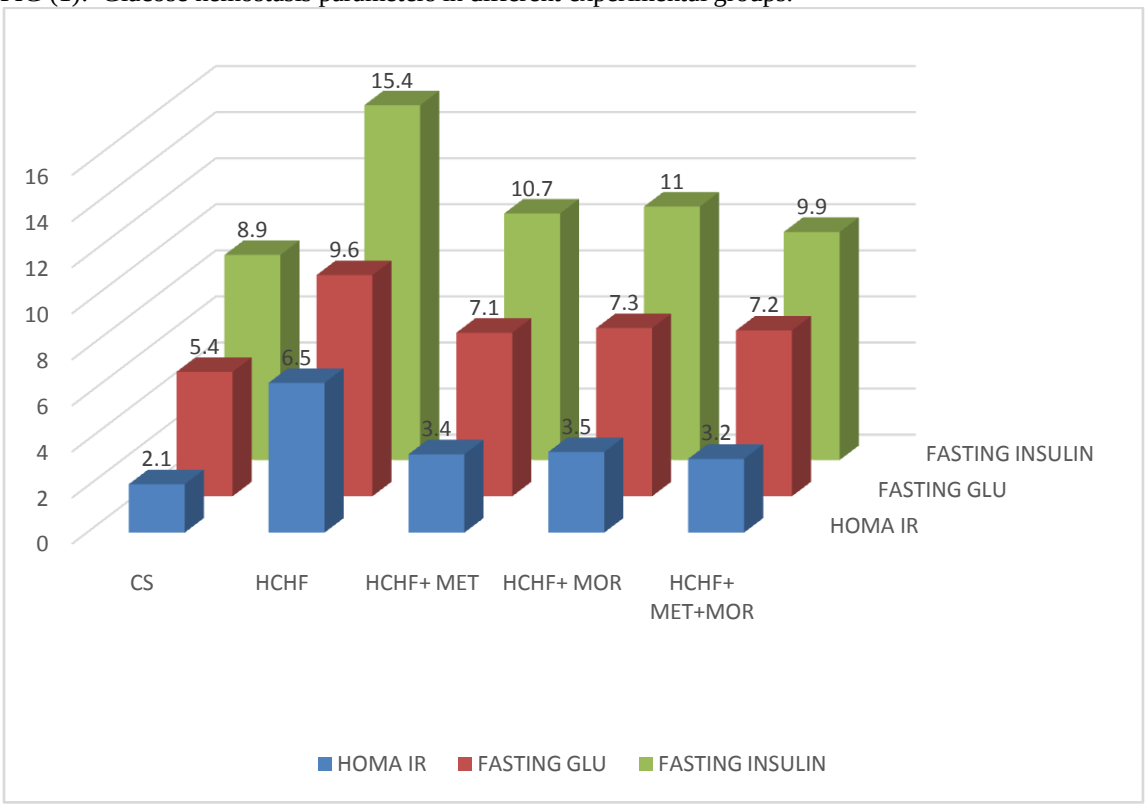
Serum ALT and AST levels were significantly higher in HCHF fed rats compared with CS fed rats. Metformin, moringa oleifera or their combination resulted in significant reduction in these enzymes. Combined therapy showed significant reduction compared with metformin treated group (table 2).

There were significant increase of TNFα and leptin levels and reduction in adiponectin levels in HCHF fed rats compared to CS group. Treatment with metformin or moringa oleifera alone or in combination resulted in significant changes of these parameters. Combined therapy showed significant changes in relation to metformin treated group (table 2, fig 3).

B) Histopathological examination:

Rats received HCHF diet showed severe degree of macro- and micro-vesicular steatosis. Treatment with metformin and moringa oliefera produced improvement of steatosis in both group more pronounced in rats treated with moringa oliefera. Combination of metformin and moringa oliefera produced marked improvement comparable to normal control group (fig:4).

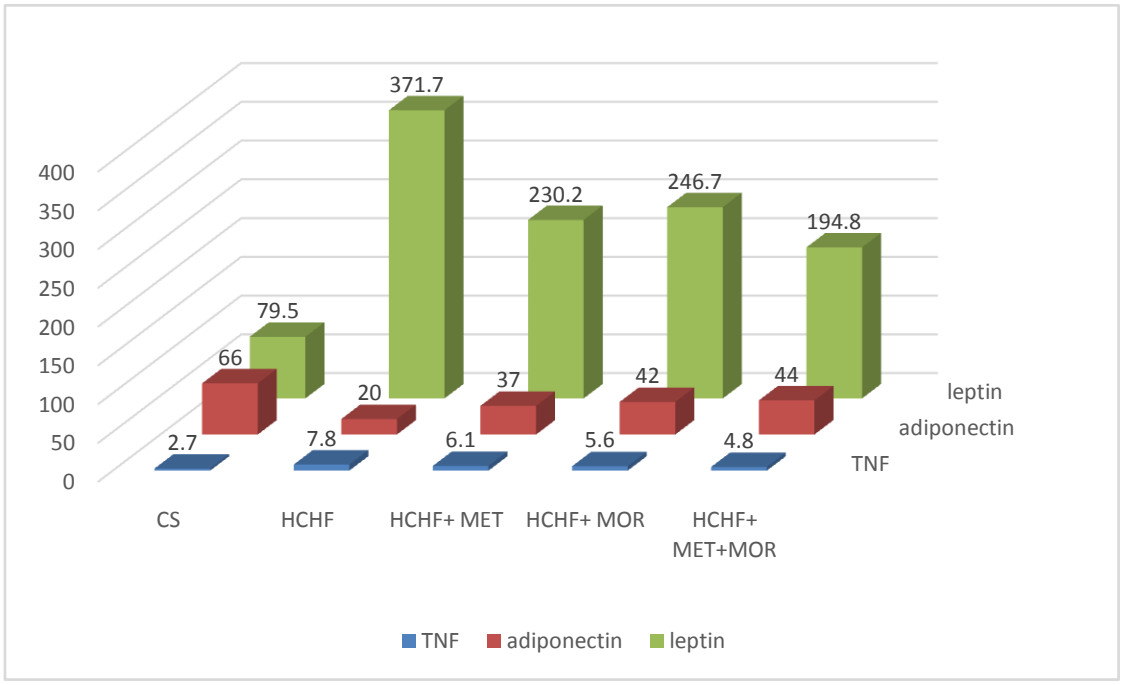
FIG (1): Glucose hemostasis parameters in different experimental groups.



HCHF = high carbohydrate high fat **CS**= corn starch **GLU**= glucose

MET= metformin **MOR=** moringa oleifera

Fig (2): Inflammatory mediators in different experimental groups



HCHF = high carbohydrate high fat **CS=** corn starch
MET= metformin **MOR=** moringa oleifera

Fig (3) Correlation between adiponectin and HOMA

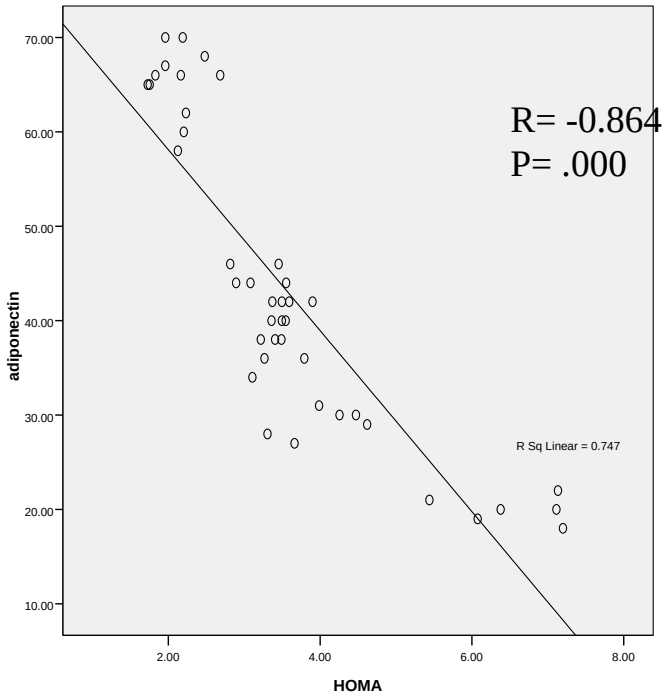
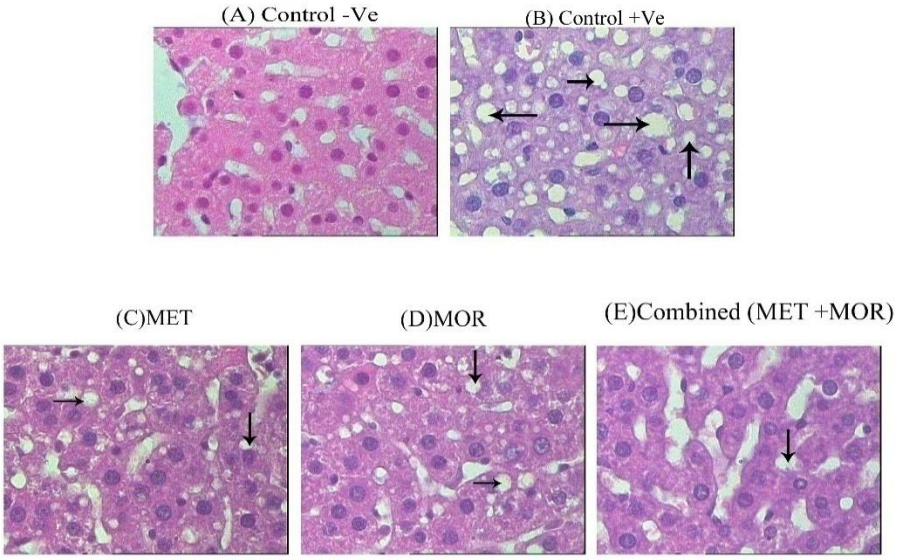


Fig. (4): Histopatholoical changes in the liver of different experimental groups.



(A) Normal control group. (B) Mice received HCHF diet showed sever degree of macro- and micro-vesicular steatosis. Treatment with MET and MOR produced improvement of steatosis in both group more pronounced in mice treated with MOR (C and D). Combination of MET and MOR produced marked improvement comparable to normal control group (E) Magnification is 400X

Discussion :

Induction of an experimental model of metabolic syndrome in rats was done using HCHF diet. HCHF fed rats is a well-established experimental model that mimic many of the features of human metabolic syndrome (Buettner et al., 2006)

Metformin remains the standard treatment for type 2 DM. it is used to control hyperglycemia, insulin resistance, lipid profile disturbance, fat distribution abnormalities, oxidative stress and it is an effective therapy for reduction of weight and lowering blood glucose level and improving insulin sensitivity. It also reduced leptin level and LDL and TG in diabetic obese persons **(Rojas and Gomes, 2013).** Metformin improved blood glucose levels, HA1c, TC, waist circumference in metabolic syndrome patients **(Mourão-Júnior et al., 2006)**. In the present study, metformin administration showed improvement of all parameters (table 2).

In this study, moringa oleifera improved insulin sensitivity and glycemic control in HCHF fed rats. This is in agreement with **Jaiswal et al. (2009); Toma et al. (2012); Luangpiomet al., 2013**, **Bais et al. (2014),** who showed the beneficial effects regarding hyperglycemia in diabetic rats, mice, and high fat fed rats respectively. Moringa oleifera significantly decreased blood glucose in type 2 DM model associated with decreased gastric emptying **(Ndong et al., 2007).**

The effect of moringa oleifera on glucose profile and insulin resistance was associated with an increase of adiponectin and a decrese of leptin levels. Leptin antagonises the effect of insulin and increase inflammation while adiponectin decreases the inflammation and improves insulin resistance (**Kaur, 2014**). These findings may explain the effect of moringa oleifera on insulin resistance as there was a negative correlation between adiponectin and HOMA (fig., 3).

Moringa oleifera showed a significant decrease in pro-inflammatory mediators (TNFα), which is in accordance with **Rajanandh et al., (2012).**This may play a role in improvement of insulin senstivity and decrease inflammation assocaited with metabolic syndrome **(Trayhurn & Wood, 2004; Saleem et al., 2009).**

Moringa oleifera treatement was associated with a significant decrease in TC, LDL, TG, and increase in HDL. This is in consistent with **Mehta et al. (2003);Jain et al. (2010); Bais et al. (2014),** Toma et al. (2015)**,** who showed that

moringa oleifera decreased cholesterol, triglycerides, VLDL, LDL, and increased HDL in hypercholestermic rabbits, rats fed with high fat diet and STZ induced diabetic ratsrespectively.

Moringa oleifera also showed anti-dyslipedemic effects in human patients with hyperlipedemia (**Nambiar et al., 2010).** It also improved lipid profile in patient with type 2 diabetes besides its beneficial effects on blood glucose ***(*Kumari, 2010*).***

An association between non-alcoholic fatty liver (NAFLD) and metabolic syndrome has been suggested and elevated markers of hepatic injury were found **(Hamaguchi et al,2005 ; Hanley et al,2005).** This is also confirmed in this study as shown in the histo-pathological findings as well as elevated liver enzymes (table 2, fig. 4). Treatment with Moringa oleifera alone or in combination with metformin showed significant improvement in these paremters.

In conclusion, it was shown in this study that the addition of moringa oleifera to metformin produced a significant improvement in parameters of metabolic syndrome. However, further studies are required to investigate the active ingredients of this herbal substance which is responsible for its beneficial effects and the possible mechanisms underlying this effect. Also, it could be suggested that moringa oliefera can be used as a herbal supplement besides metformin in subjects with metabolic syndrome, taking into consideration the shortage of therapies that directly address the growing epidemic of metabolic syndrome.

Conflict of interest: None declared

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