



Effects of *Brassica Rapa* on Fructose-Induced Metabolic Syndrome in Rats: A Comparative Study

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ABSTRACT

Increased fructose consumption is strongly associated with incidence of metabolic syndrome (MS). This study aimed to elucidate the role of turnip (*Brassica rapa*) on fructose-induced metabolic syndrome. MS was induced by administration of fructose as 10% solution in drinking water for 8 weeks. Three groups of rats (n=8) were administered fructose as 10% solution in drinking water for 8 weeks. One served as fructose fed control while the remaining two groups were treated with metformin (10 mg/kg/day) and turnip (400 mg/kg/day) for two weeks. At the end of the experiment, blood samples were withdrawn for estimation of markers related to MS. Analysis of the data was performed by one way Analysis of Variance and subsequent analysis was performed using Tukey-Kramer test. The P values smaller than 0.05 were selected to indicate statistical significance between groups. Induction of MS was associated with increased body weight gain and elevated levels of blood glucose, MDA, nitric oxide, total triglycerides and total cholesterol. It also reduced levels of blood GSH and liver glycogen. *Brassica Rapa* attenuated most of the changes associated with MS. It reduced weight gain and blood glucose, MDA, nitric oxide, total triglycerides and total cholesterol. It also elevated blood GSH and liver glycogen. The findings of this study prove the benefits of turnip in fructose-induced model of MS.

Keywords: *Brassica Rapa*, metabolic syndrome, insulin, glucose, oxidative stress, total triglycerides.

INTRODUCTION

Fructose is used extensively in carbonated beverages, baked goods, canned fruits and dairy products¹. The increasing consumption of fructose could play a potential role in the etiology of obesity and metabolic syndrome.²

MS is a cluster of pathologies compromising insulin resistance, hyperinsulinemia, hypertiglyceridemia, accelerated atherosclerosis and hypertension^{3,4}. In addition, it is associated with morbidities such as increased risk of developing cardiovascular disease, type 2 diabetes mellitus and renal disease.^{5,6} In contemporary times, MS is becoming an alarming concern for the developing world. The prevalence of metabolic syndrome is increasing worldwide and is a growing threat to global health. People with metabolic syndrome are twice as likely to die from heart attack or stroke compared with people without MS and has a fivefold greater risk of developing type 2 diabetes⁷. The use and acceptance of traditional medicine have increased in recent years⁸. A variety of natural products have been proposed as pharmacological treatment of type 2 diabetes and MS. These natural products include cinnamon, green tea⁹ and oats¹⁰.

Brassica rapa species constitute one major source of food and can be considered as important natural source of antioxidants¹¹. Moreover, in traditional medicine, *brassica rapa* is used to treat a variety of diseases such as hepatitis, jaundice, furuncle and sore throats¹². Chandra et al.,¹³ also showed that *brassica rapa* has anti-thyroid effects and indole fraction from *brassica rapa* roots is known to possess anti-inflammatory potential via

inhibition of pro inflammatory mediators such TNF α and interleukin-6¹⁴.

Turnip contains numerous biologically active compounds, such as flavonoids (isorhamnetin, kaempferol and quercetin glycosides), phenylpropanoid derivatives¹², indole alkaloids and sterol glucosides (glucosinolates)¹⁵ which could be attributed to its activity.

The aim of present study was to evaluate the effect of turnip on fructose induced metabolic syndrome in rats.

MATERIALS AND METHODS

Animals

Male Sprague Dawely rats of body weight from 220-230 g were used. They were housed in animal room in Pharmacology department, Faculty of Pharmacy under conventional laboratory conditions on a 12 hours light/dark cycle and constant temperature (22 \pm 1°C). Throughout the study food and water were supplied *ad libitum*. All experimental procedures were conducted in accordance with ethical procedures and policies approved by the Ethics Committee of Faculty of Pharmacy, Beni Suf University.

Collection and extraction of Turnip

500gm of fresh turnip bulbs (*Brassica rapa* subsp. Rapa Fam. Brassicaceae) were collected on March 2012. The leaves were detached and the tap root bulbs were washed with water. The clean bulbs were shredded and homogenized in a blender then soaked in ethanol 70% for 12 hours three times until exhaustion. The filtered ethanol extracts were combined and dried under vacuum

at 40°C. The dried extract was kept in freezer at -20°C until the biological screening.

Toxicity Study

The alcoholic extract of turnip at the dose of 2 g/kg /body weight was given to ten animals. The animals were continuously observed for 14 days for mortality and general behavior. No death was observed till the end of the study. The drug was considered safe up to the dose of 2 g/kg body weight. From the results, dose of 400mg/kg body weight was chosen for the biological studies.

Experimental design

After acclimatization period of one week rats were divided into 4 groups (n=8 rats in each group) as follows:

Group 1: This group received regular diet and water *ad libitum*. This group didn't receive any medication and served as control.

Group 2: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 8 weeks.

Group 3: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 8 weeks followed by metformin (10 mg/kg/day p.o) for 2 weeks.

Group 4: This group received regular diet and water *ad libitum* and fructose was administered as 10% solution in drinking water for 8 weeks followed by turnip (400 mg/kg/day p.o) for 2 weeks.

Body weights of animals were recorded at the baseline and after the treatment. By the end of the treatment period, animals were fasted for 12 hours and blood samples were collected by retro-orbital plexus for the estimation, of glucose, insulin, nitric oxide, MDA, GSH, glycogen level, total triglycerides and total cholesterol levels.

Methods

i- Induction of Metabolic syndrome:

Administration of 10% fructose solution as drinking water was used to achieve the pathological model.

ii- Fasting serum glucose was determined using glucose kit (Biolabo SA, France).

iii- Determination of serum insulin was performed using rat ELISA kit (Biovendor, Czech Republic).

iv- Total cholesterol and triglycerides were estimated using test reagent kits (Spinreact, Spain).

v- Serum malondialdehyde, blood glutathione and Serum nitric oxide were determined according to the methods described previously¹⁶⁻¹⁸.

vi- Liver glycogen was estimated according to the method described previously¹⁹.

Statistical analysis

Analysis of the data was performed by one way ANOVA and subsequent analysis was performed by Tukey test. The P values smaller than 0.05 were selected to indicate statistical significance between groups.

Table 1: Changes in body weight

	Normal – control (n= 8)	Fructose-fed (n= 8)		
		Control	Metformin (10 mg/kg)	Brassica rapa (400 mg/kg)
Body weight (g) (Initial)	232.23±5.99	225.83±6.13	227.93±4.98	224.13±5.17
Body weight (g) (Final)	326.78±9.73	379.07±5.77*	324.12±4.78*	319.72±3.55*

Data was expressed as mean ± s.e.m.; Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.; *: Significantly different from the normal control group at P <0.05. @: Significantly different from the diabetic control group at P < 0.05.

Table 2: Changes in oxidative stress biomarkers, total triglycerides and total cholesterol

	Normal – control (n= 8)	Fructose-fed (n= 8)		
		Control	Metformin (10 mg/kg)	Brassica rapa (400 mg/kg)
GSH (mg%)	58.17±3.2	35.36±3.7*	54.85±3.9 [@]	57.00±3.5 [@]
MDA (nmol/ml)	1.67±0.32	4.77±0.32*	1.81±0.37 [@]	1.71±0.24 [@]
Nitric oxide (µM)	54.24±3.67	87.16±3.81*	57.77±3.66 [@]	49.58±4.71 [@]
Total triglycerides (mmol/l)	1.37±0.15	3.09±0.25*	1.63±0.16 [@]	1.46±0.17 [@]
Total cholesterol (mmol/l)	2.17±0.10	3.22±0.19*	2.31±0.18 [@]	2.50±0.19 [@]

Data was expressed as mean ± s.e.m.; Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.; *: Significantly different from the normal control group at P <0.05.; @: Significantly different from the diabetic control group at P < 0.05.

RESULTS

Body weight

Fructose for 8 weeks significantly increased body weight as compared with control group. Body weights were decreased during the treatment of metformin or *brassica rapa* at the end of the experiment (table 1).

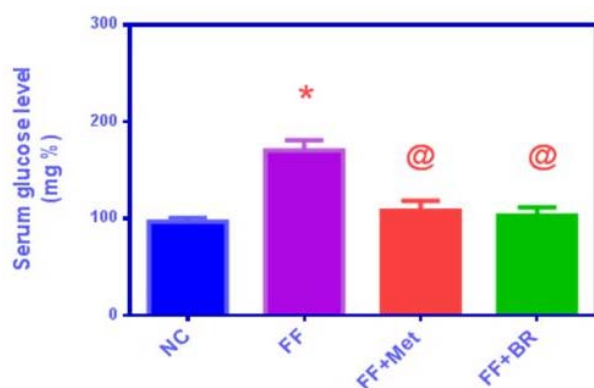
Oxidative stress biomarkers

The effect of metformin and *Brassica rapa* on oxidative stress biomarkers is summarized in table (2). Metformin and *brassica rapa* significantly increased blood GSH level as compared to fructose fed rats. In addition, metformin and *brassica rapa* showed a significant decrease in both serum MDA and nitric oxide.

Serum glucose and insulin levels

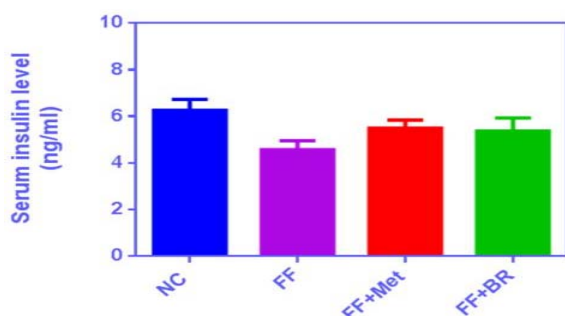
Serum glucose level was increased in fructose fed rats at the end of the experiment. Metformin, *brassica rapa* interrupted the rise in glucose level. Insulin level was increased in the serum of fructose fed rats. Treatment with metformin and *Brassica rapa* did not affect this increased level (figures 1 and 2).

Figure 1: Effect of *Brassica rapa* on serum glucose level.



n = 6 rats per group.; Data was expressed as mean \pm s.e.m.; Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.; *: Significantly different from the normal control group at P < 0.05.; @: Significantly different from the diabetic control group at P < 0.05. NC: normal control group.; FF: fructose-fed group.; FF+MET: fructose fed + Metformin.; FF+BR: fructose fed + Brassica rapa.

Figure 2: Effect of *Brassica Rapa* on serum insulin level



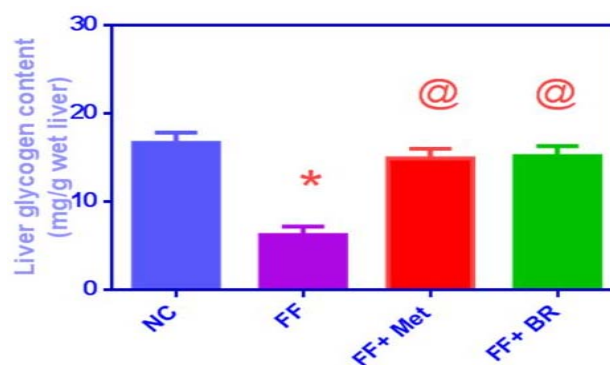
n = 6 rats per group.; Data was expressed as mean \pm s.e.m.; Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.; *: Significantly different from the normal control group at P < 0.05.; @: Significantly different from the diabetic control group at P < 0.05.; NC: normal

control group; FF: fructose-fed group; FF+MET: fructose fed + Metformin; FF+ BR: fructose fed + Brassica rapa.

Liver glycogen

Liver glycogen content was decreased in fructose fed rats. Treatment with metformin and *brassica rapa* was accompanied by normalization of this decrease (Figure 3).

Figure 3: Effect of *Brassica rapa* on liver glycogen content



n = 6 rats per group; Data was expressed as mean \pm s.e.m.; Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.; *: Significantly different from the normal control group at P < 0.05.; @: Significantly different from the diabetic control group at P < 0.05.; NC: normal control group; FF: fructose-fed group; FF+MET: fructose fed + Metformin; FF+BR: fructose fed + Brassica rapa.

Total triglycerides and total cholesterol

Total triglycerides and cholesterol levels in fructose fed rats were significant different from normal control rat. *Brassica rapa* and metformin nearly normalized total triglycerides and cholesterol levels (table 2).

DISCUSSION

Results of the present study revealed that maintenance of rats on fructose (10%) for 8 weeks was associated with increased weight gain, hyperglycemia, dyslipidemia, hyperinsulinemia and oxidative stress. These findings are quite consistent with that of Stanhope & Havel²⁰ who indicated that fructose rich diet induced the development of pathophysiological characteristics associated with metabolic syndrome. The high fructose flux leads to enhanced accumulation of hepatic triglycerides and resulting in impairment of glucose and lipid metabolism²¹.

The antidiabetic effect of *brassica rapa* could be attributed to the presence of quercetin which inhibits competitively the uptake dehydroascorbic acid and methyl glucose (a substrate that enters the cells through facilitative hexose transporter GLUT4)²². Quercetin is also known to protect pancreatic β cells from oxidative stress and damage resulting in increased insulin secretion²³. This is in accordance with Kannappan & Anuradha Who suggested that quercetin could improve insulin signaling and sensitivity in rats with insulin resistance²⁴. In addition, quercetin is known to decrease the activity of small intestine and inhibition of alpha glucosidase enzyme and thus could reduce the digestion of dietary carbohydrates²⁵.

In addition, the increased glycogen content observed after administration of *brassica rapa* in this study reinforces the evidence of antidiabetic and extrapancreatic effect of turnip in liver adipose tissue and could be attributed to enhanced insulin stimulated glycogen synthesis²⁶.

Results of the present study also revealed that supplying rats with a fructose for 8 weeks increased body weight. This finding is quite consistent with Xolalpa-Molina²⁷ who reported that studies in humans and animal models suggest that dietary components such as high fat and fructose can affect fatty infiltration and lipid peroxidation. The lipolytic effect of the ethanolic extract of *brassica rapa* could be attributed to the inhibition of adipocyte lipid accumulation and the stimulation of beta(3)-AR-dependent lipolysis²⁸.

Disturbed lipid metabolism is considered as another characteristic feature of MS. The current study showed that supplying rats with fructose (10%) elevated the levels of total triglycerides and total cholesterol in serum. These results are in harmony with those of other investigators.^{29,30} This is may be as a result of reduced lipoprotein lipase activity secondary to reduced plasma insulin level³¹. Dyslipidemia could also be attributed to increased level of cholesterol ester transfer protein which is important in regulating lipoprotein lipid composition³². Turnip improved dyslipidemia most probably due to the presence of the flavonoid quercetin which is known to reduce hepatic fat accumulation³³ and the presence of glucosinolates which are known to reduce triglycerides level³⁴.

Our study showed that treatment with long term fructose was associated with an increase in oxidative stress. Delbosc et al., found that fructose increased oxidative stress and is associated with MS in rodents³⁵. The enhanced lipid peroxidation in fructose fed rats could be associated with high circulating glucose levels which enhances free radical production from glucose autoxidation and protein glycation³⁶.

Brassica rapa improved GSH and consequently decreased cellular oxidative damage. This is in accordance with Kim et al.,³⁷ who reported that ethanol extract of *brassica rapa* has attenuated oxidative stress. The antioxidant effect of *brassica Rapa* is further supported by the decrease in MDA level. This reduction could be attributed to the presence of quercetin which is known to reduce TBARS level, scavenging free radicals and chelating transitional metal ions and thus retarding oxidative degradation.^{38,39} In addition, the antioxidant activity of *brassica rapa* is related to the presence of phenolic compound which can play an important role in neutralizing ROS via quenching singlet and triplet oxygen or decomposing peroxides⁴⁰.

CONCLUSION

In conclusion, the present data supports the beneficial effects of turnip (*Brassica rapa*) in management of

metabolic syndrome. Future work needs to be done in the direction of use of *Brassica rapa* for clinical studies with the aim to elucidate the molecular and cellular mechanisms involved with the usage of turnip for the prevention of metabolic syndrome.

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