The assessment of the antihyperglycemic effect of a standardized ethanolic extract of *Morus nigra* L. fruits growing in Egypt in streptozotocin – induced diabetic rats

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Introduction

Diabetes Mellitus (DM) is considered the most common endocrine disorder, affecting 200 million world-wide. By the year 2025 it is expected that 333 million people of the world will have diabetes as their main ailment (king et al., 2013).

According to the International Diabetes rates Federation (2007); Egypt records one of the highest diabetes rates in the Middle East and North Africa region with 11% of its population suffering from the disease.

Since DM is considered as free radical – mediated disease, there has been renewed interest in the use of flavonoid or phenolics in the treatment of some diabetes complications (Dembinska –Kiec et al., 2008).

Black mulberry (*Morus nigra* L.) family Moraceae , is a plant with reported hypoglycemic effects in both human and animal (Babu et al., 2006). Its edible fruits, dark purple almost black when ripe, is enriched in phenolics especially anthocyanins, a group of powerful antioxidant plant secondary metabolites. It was reported that cyanidin and its derivatives are the major constituents in black mulberry fruits. These compounds had been reported to exhibit anti-diabetic activity in animal model (Nasri et al., 2011), and were found to be beneficial for the prevention of obesity and diabetes (Tsuda et al., 2003).

Therefore, the aim of this study was to assess the possible beneficial antihyperglycemic effect of a standardized black mulberry fruits ethanolic extract (BMFE) in streptozotocin (STZ)-induced diabetic rats and evaluate its effect on liver and kidney functions.

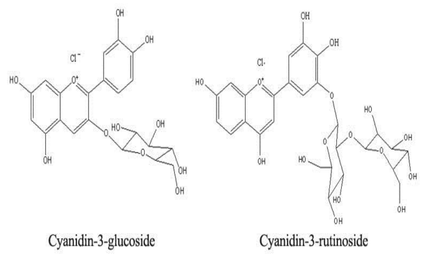

Fig.1: Major identified cyanidin compounds in BMFE

Fig.2: Fruits of *Morus nigra* L

Materials and Methods

Plant material

The fruits of *Morus nigra* were collected in May 2013 from Orman park.

Extraction of *Morus nigra* fruits

Frozen fruits were defrosted at room temperature for 30 min and extracted three times with 80% ethanol (1:4) by homogenizer at 4000 rpm for 10 min at room temperature .The mixture was filtered and evaporated to dryness using rotary evaporator at 45°C under reduced pressure to give purple –brownish semi solid residue.

Determination of total flavonoids and total phenolics

Total flavonoids were determined using $AlCl_3$ method ,while, total phenolics were determined using Folin- Ciocalteu method according to (Egyptian Pharmacopoeia 4 th edition, 2002)

HPLC/UV analysis of *Morus nigra* fruit extract

20 µl of 0.1g/ml of the extract and standards were injected into an Agilent 1200 HPLC system. Separation was achieved using RP C18 chrosphere (250 x 4.6 mm, 5 µm) and a 30 minutes gradient of 5% acetic acid (solvent A) and 5% acetic acid in ACN (solvent B). Anthocyanins were detected at λ=530nm.

Acute toxicity measurement :

An approximate LD_{50} was estimated for the ethanolic extract by exposing the experimental animals (four animals / group) to various doses of test extract. The animals were observed for first 2 hours, 24 hours and 48 hours for mortality.

Assessment of antihyperglycemic activity:

Antihyperglycemic activity of the black mulberry fruit extract (BMFE) was assessed using STZ-induced diabetes in male Albino rats model. After establishment of hyperglycemia, STZ-treated rats were divided into 4 groups (6 animals each) and were given oral doses of either 250 mg BMFE, 500mg BMFE, 15mg gliclazide per kg b.wt or vehicle only (untreated control). Results were further compared to normal control group (6 animals). Blood glucose levels were checked at one week intervals for a total period of 4 weeks. By the end of the experiment, animals were sacrificed and serum was collected.

Assessment of blood glucose level:

Blood samples were collected from the tail vein of the over night (12-15 h) fasting rat for determination of fasting blood glucose level (FBG) using GlucoDR (Koria)

Determination of liver and kidney function:

AST, ALT, creatinine and urea were measured after the 4-week treatment of STZ-induced diabetic rats using standard methods at commercial medical laboratory.

Statistical analysis:

Data are presented as mean ±SD. Unless other wise indicated statistical analysis were performed by one-way ANOVA.

Results and Discussion

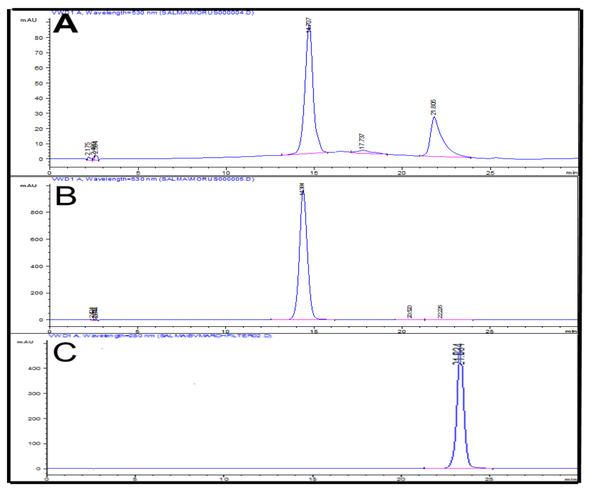

Table1: Concentration of total phenolics, total flavonoids, cyanidin-3-O-glucoside and cyanidin-3-O- rutinoside

Item	Concentration
Total phenolic mg gallic acid equivalent/g	13.53
Total flavonoid mg quercetin equivalent/g	3.02
Cyanidin- 3- O- glucoside mg/g	0.276
Cyanidin -3- O– rutinoside mg/g	0.269

Fig.3: HPLC chromatogram of:
- A. Ethanol extract of Black mulberry
- B. Cyanidin-3-O- glucoside standard
- C. Cyanidin-3-O- rutinoside standard

Comparative HPLC/UV profiling revealed the presence of two anthocyanin compounds: cyanidin -3-O- glucoside and cyanidin -3-O- rutinoside in BMFE as shown in fig.3 A and table1. The identification of anthocyanins was based on comparison of their retention times with those of external standards. Similar results have been revealed by other authors (Dugo et al.,2001)

The value of total phenolics and total flavonoids are shown in table1, comparable values were recorded by (Ercisli and Orhan, 2006)

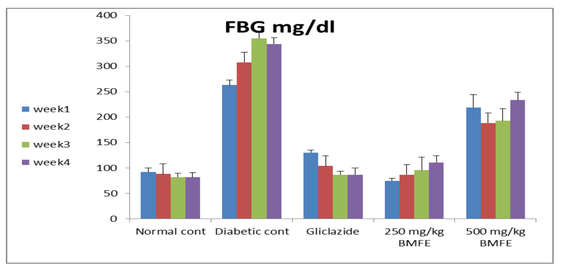

Fig.4: FBG concentration in normal control and diabetic rats during4- week of treatment

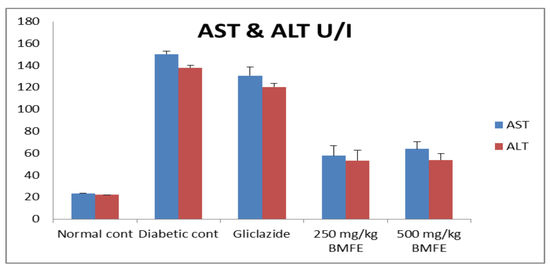

Fig.5: levels of biochemical markers of liver function (AST and ALT) in normal, diabetic and BMFE treated rats after 4-weeks.

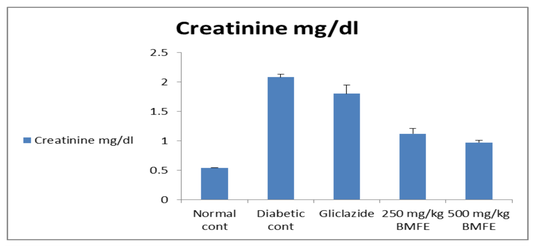

Fig.6: Measurement of serum creatinine level in normal, diabetic and BMFE treated rats after 4-weeks.

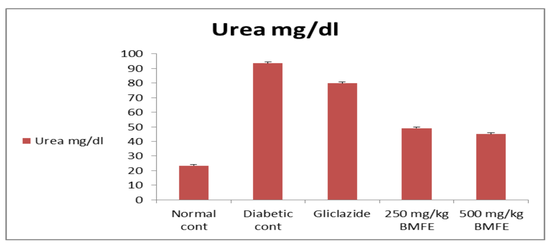

Fig.7: Measurement of serum urea level in normal, diabetic and BMFE treated rats after 4-weeks.

Oral administration of BMFE in doses up to 13 g/kg b.wt. failed to kill rats within 2, 24 &48 hours. We concluded that BMFE extract was safe according to (Buck et al.,1976).

Fig.4 showed that the administration of BMFE at 250 mg/kg b.wt. significantly reduced the FBG level and kept it without any significant variation until end of the experiment (4 weeks) when compared to rates that were not given any treatment.

Biochemical measurement of liver function (AST and ALT) and kidney function (creatinine and urea) indicated significant improvement in both function by administration of BMFE at both doses in STZ- induced diabetic rats when compared to diabetic untreated control as shown in fig. 5-7.

Improvement of liver and kidney functions is probably due to the protective anti oxidant properties of cyanidin rich black mulberry extract. Cyanidin -3-O- glucoside was shown to exert a hypoglycemic effect by decreasing fasting blood glucose level, increasing insulin sensitivity, lowering malondialdehyde (MDA) content and reducing activity of superoxide dismutase. (Guo et al., 2012).

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Conclusion

To the best of our knowledge, this is the first study to report *in vivo* treatment of diabetic rats with ethanolic extract of *M. nigra* fruits. Standardizing of the BMFE using HPLC/UV revealed the presence of two major anthocyanin compounds cyanidin-3-O- glucoside and cyanidin-3-O- rutinoside with concentrations of 0.276 and 0.269 mg/g, respectively. Total phenolic content was estimated at 13.53 mg gallic acid equivalent/g of frozen fruits and total flavonoid content was determined to be equivalent to 3.02 mg quercetin /g frozen matter.

Our finding indicated that rats receiving BMFE showed significant reduction on FBG level at a dose of 250mg/kg. Furthermore, administration of BMFE at two different doses significantly improved liver and kidney function in diabetic rats. The results suggest that BMFE can be used as a safe nutraceutical agent to ameliorate hyperglycemia associated with type II diabetes and it might also be useful in preventing secondary diabetic complications