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Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1601094

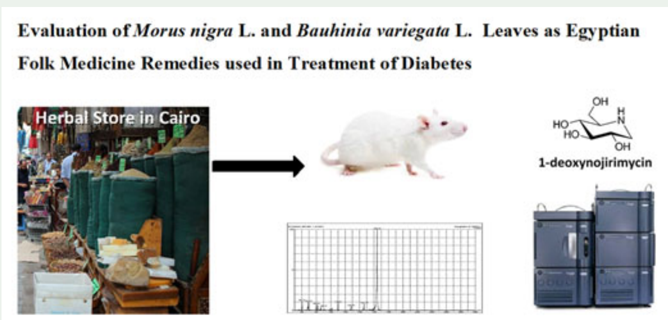
Evaluation of antidiabetic activity of *Morus nigra* L. and *Bauhinia variegata* L. leaves as Egyptian remedies used for the treatment of diabetes

Salma Hago[a], Engy A. Mahrous[a], Mohamed Moawad[b], Samia Abdel-Wahab[a] and Essam Abdel-Sattar[a]

[a]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt; [b]Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt

ABSTRACT

Morus nigra and Bauhinia variegata are used in the Egyptian folk medicine for their hypoglycemic effects. The standardized ethanolic extracts of both plants caused a significant decrease in fasting blood glucose level at two different doses (250 and 500 mg/kg) in streptozotocin-induced diabetic rats' model. Further, *in vitro* antioxidant activity and α-glucosidase inhibition assays were conducted as well as the measurement of insulin levels and the biomarkers for both liver and kidney functions in the treated animals. Beneficiary effects of BMLE and BVLE in the treatment of diabetes were found not to be limited to hypoglycemic effect but included preventing liver and kidney tissue damage that are associated with diabetes. A strong inhibition of the α-glucosidase enzyme by both extracts may be a contributing mechanism in the overall anti-diabetic effect that was observed. Further detailed study is needed in the future to explore the mechanism of action of both plants.



ARTICLE HISTORY
Received 7 January 2019
Accepted 23 March 2019

KEYWORDS
Morus nigra; *Bauhinia variegata*; diabetes; insulin; liver functions; kidney functions

CONTACT Essam Abdel-Sattar abdelsattar@yahoo.com; essam.abdelsattar@pharma.cu.edu.eg
Supplemental data for this article can be accessed athttps://doi.org/10.1080/14786419.2019.1601094.

1. Introduction

Diabetes mellitus remains one of the chronic diseases that are becoming more prevalent in middle- and low-income societies, especially in the Middle East region. Globally, 425 million adults aged over 18 years were living with diabetes compared to 108 million in 1980 (International Diabetes Federation 2017). According to International Diabetes Federation (IDF), approximately 38.7 million adults are living with diabetes in Middle East and North Africa (MENA) region in 2017, by 2045 this will rise to 76 million. In Egypt, over 8 million cases of diabetes of adults over 18 years (15.1%) were recorded in Egypt in 2017 (International Diabetes Federation 2017). Diabetic patients also suffer from diabetes complications such as retinopathy, kidney failure, and amputation of the extremities. In developing and underdeveloped countries, limited access to expensive pharmaceutical products and strong beliefs in customs and old traditions maintain a wide use of medicinal plants as an alternative therapy for many health ailments including diabetes. The leaves from *Morus nigra* and *Bauhinia variegata* which are low-cost plant materials that are of common availability and have been used in Chinese and Egyptian folk medicine for treatment of different health conditions including diabetes (Li et al. 2004; Araujo et al., 2015). Hosseinzadeh and Sadeghi (1999) and Barati and Momtaz (2012) reported the antihyperglycemic effects of the different extracts obtained from *M. nigra* leaves in diabetic rats and Xu et al. (2018) reported the α-glucosidase inhibitory activity of thirteen compounds isolated from the twigs of *M. nigra*, among which twelve were active. On the other hand, *B. variegata* bark extract was recently found to significantly lower blood glucose level when it was given by injection to alloxan-induced diabetic mice (Kumar et al. 2012). Meanwhile, Azevedo et al. (2006) attributed the hypoglycemic effect of *B. variegata* leaves extract to a chloroplast protein with a partial amino acid sequence identical to that of bovine insulin, while roseoside, a major secondary metabolite in the ethanolic leaves extract demonstrated insulinotropic activity toward pancreatic ß-cells in an *in vitro* assay (Wolfe et al. 2003). The current literature revealed that both *B. variegata* and *M. nigra* leaves are rich in phenolic compounds, especially flavonoids, which are known for their antioxidant potential and can be especially beneficial in many diseases where oxidative stress is a contributing factor as in diabetes.

This study was designed to evaluate the effects of oral administration of standardized black mulberry leaf extract (BMLE) and *B. variegata* leaf extract (BVLE) on hyperglycemia, insulin activity, kidney and liver function in an STZ-induced diabetic rat model.

2. Results and discussion

2.1. Chemical standardization of BMLE and BVLE

2.1.1. Profiling of phenolic and flavonoid contents of BMLE and BVLE

BMLE showed higher total phenolic and total flavonoids contents (65.44 mg GAE/g, 11.38 mg QE/g, respectively) than those of BVLE (60.55 mg GAE/g, 9.65 mg QE/g, respectively).

HPLC/DAD analysis of both extracts identified rutin, quercetrin, quercetin and hesperidin as the major flavonoids and chlorogenic and *p*-coumaric acids as phenolic acid (Table 1S). Quercetrin was identified as the major flavonoid in BMLE (2.75 mg/g), while rutin was the major one in BVLE (4.38 mg/g), which was quantified as the marker compound in BVLE.

2.1.2 Determination of radical-scavenging activity (DPPH assay) of BMLEa and BVLE

BMLE showed a moderate DPPH radical scavenging activity with an IC_{50} of 1.7, while the EC_{50} of BVLE was estimated at 1.02 compared to ascorbic acid (EC_{50} 0.28). The antioxidant activity may be related to high concentrations of flavonoids and other phenolic compounds.

2.1.3. Determination of 1-deoxynojirimycin (1-DNJ) content of BMLE

In previous studies, the anti-diabetic effect of *M. nigra* leaves has been attributed to the presence of α- glucosidase inhibitor (1-DNJ) which was isolated from leaves and barks of different *Morus* species (Li et al. 2005). Therefore, 1-DNJ was estimated in BMLE by LC-MS using soft ionization techniques such as electrospray ionization ESI. Under optimized experimental conditions, protonated 1-DNJ was detected at 164 *m/z* for $[M + H]^{+}$, and based on a standard calibration curve of 1-DNJ, its content in the BMLE was found to be 2.47 mg per gram of the dried extract.

2.2. Biological assessment of BMLE and BVLE

2.2.1. Determination of α-glucosidase inhibitory activity

The IC_{50} of BVLE in α-glucosidase inhibition assay was found to be 0.139 mg/ml compared to acarbose standard (0.789 mg/mL) revealing a strong α-glucosidase inhibitory effect. Meanwhile, BMLE showed only moderate α-glucosidase inhibition with an IC_{50} value of 1.076 mg/mL.

2.2.2. Determination of LD_{50} of BMLE and BVLE

Both plant extracts were safe by oral administration up to 13.5 g/kg b.w in male albino rats, when observed for 2, 4 and 48 hours. Both plants were considered safe and unclassified according to OECD (2001) guidelines. Therefore, the high value of LD_{50} was considered a satisfactory indicator of safety and could replace food and water intake in the current study.

2.2.3. Effects of BMLE and BVLE on STZ-diabetic rats

Although the folk use of the leaves of *M. nigra* and *B. variegata* in the treatment of diabetes have been practiced for centuries, no studies have been traced to evaluate the biological effect of administration of a standardized leaf extracts of the Egyptian plants

Administration of gliclazide (15 mg/kg BW), group III and either of BMLE or BVLE at two the different doses (250 and 500 mg/kg BW), groups IV through VII, resulted in a significant reduction of FBG (Figures 1 and 2) when compared to group II of diabetic

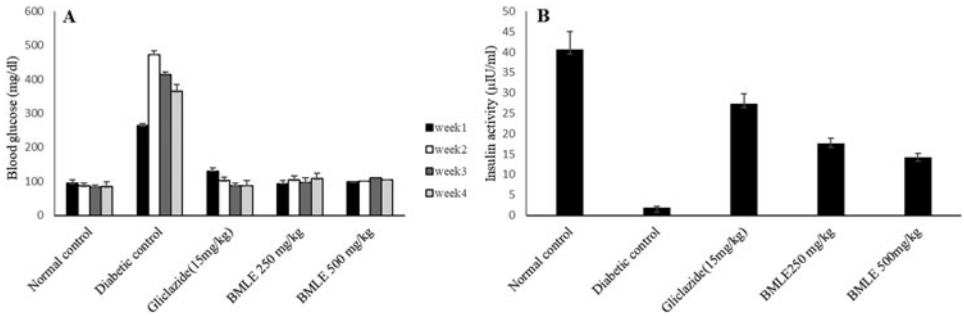


Figure 1. Effect of administration of BMLE on serum fasting blood glucose (FBG) concentrations and insulin activity. (A) FBG concentrations measured by the end of each week of the four weeks' study period in mg/dl. (**B**) serum insulin activity measured in µIU/ml at the end of the study period.

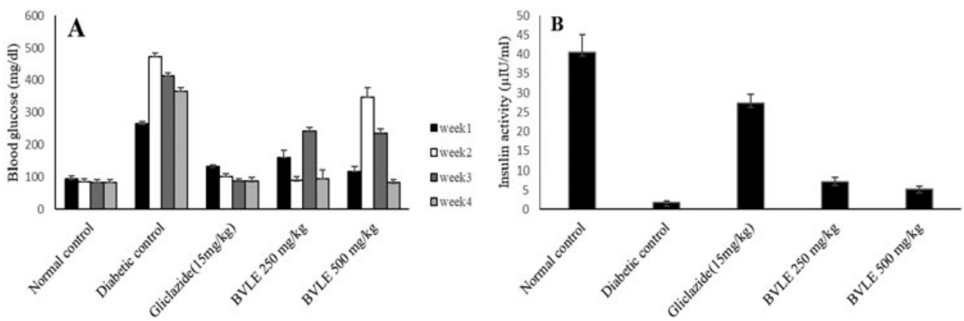


Figure 2. Effect of administration of BVLE on serum fasting blood glucose (FBG) concentrations and insulin activity. (A) FBG concentrations measured by the end of each week of the four weeks' study period in mg/dl. (B) serum insulin activity measured in µIU/ml at the end of the study period.

animals. In all cases, this significant reduction of FBG was maintained throughout the study period (4 weeks). After 4 weeks, serum insulin level was significantly higher in animals treated with gliclazide when compared with untreated animals (Figures 1 and 2). This is consistent with the mode of action of gliclazide which stimulates "first phase" release of insulin from pancreatic β-cells and increases the sensitivity of β-cells to glucose stimulus to control hyperglycemia (Giacco and Brownlee 2010). Treatment with BMLE at both doses resulted also in a significant elevation in serum insulin levels when compared to untreated animals (Figure 1). In previous studies, the anti-diabetic effect of *M. nigra* leaves has been attributed to the presence of α-glucosidase inhibitor (1-DNJ) which was isolated from leaves and barks of different *Morus* species (Li et al. 2005). However, our results suggested that the antihyperglycemic effect observed upon treatment with BMLE could not be attributed solely to its 1-DNJ content. In fact, BMLE investigated in this study revealed a low amount of the 1-DNJ alkaloid (2.47 mg/g dry extract) which varied widely depending on the variety of the plant and its time of collection (Giacco and Brownlee 2010). This was also supported by the relatively weak α-glucosidase inhibition effect observed for the extract (IC_{50}= 1.076 mg/

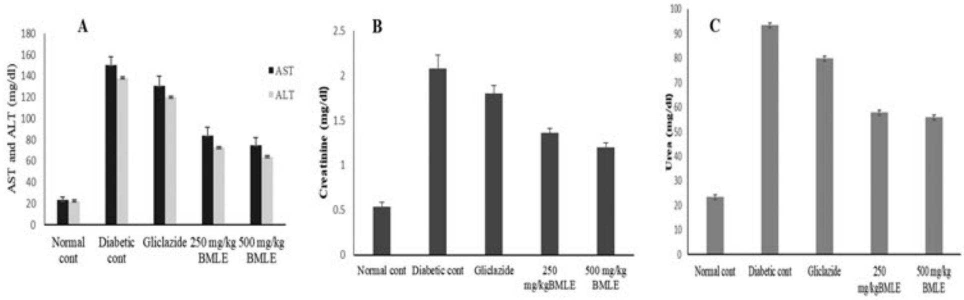


Figure 3. Effect of administration of BMLE on serum biomarkers for both liver and kidney functions. (A) serum concentrations of liver enzymes AST and ALT in mg/dl. (B) serum concentrations of creatinine in mg/dl and (C) serum concentrations of urea in mg/dl as measured by the end of the study period.

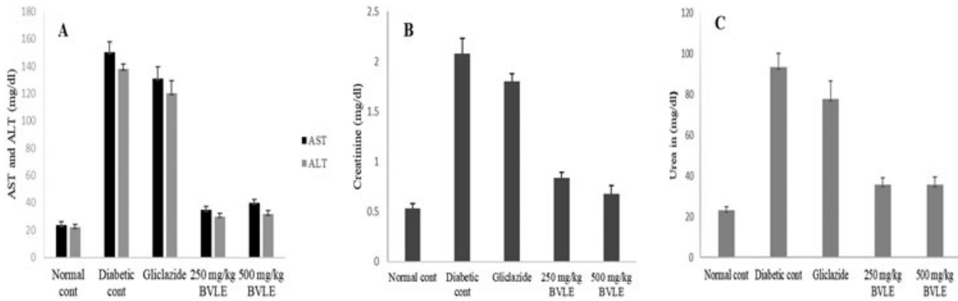


Figure . Effect of administration of BVLE on serum biomarkers for both liver and kidney functions. (A) serum concentrations of liver enzymes AST and ALT in mg/dl. (B) serum concentrations of creatinine in mg/dl and (C) serum concentrations of urea in mg/dl as measured by the end of the study period.

mL). Meanwhile, the elevated levels of serum insulin in animals treated with BMLE strongly suggest other possible complementary mechanism for the anti-hyperglycemic effect of BMLE which may include boosting endogenous insulin secretions. Other bioactive secondary metabolites identified in the extract such as rutin, quercetin and chlorogenic acid might play a role in stimulating secretion of insulin which will require further investigation. Additionally, oral administration of BMLE was found to be superior to the treatment with gliclazide in restoring serum level of AST, ALT, urea, and creatinine (Figure 3). Such effect is possibly due to the high content of polyphenols in the extract that contribute to its antioxidant effect in preserving the integrity of vital organs (liver and kidney) from possible tissue damage associated with hyperglycemia in the model used (Ha et al. 2010; Dennis and Witting 2017).

On the other hand, administration of BVLE extract lowered the FBG level at the two different doses (Figure 2), without causing any significant elevation in the serum insulin level. Accordingly, the hypoglycemic effect of BVLE may be due to other mechanisms which likely includes α-glucosidase inhibition as it can be concluded from the strong *in vitro* inhibition of BVLE in the *in vitro* assay and was similarly reported for other *Bauhinia* species (Elbanna et al. 2017). Similar to BMLE, serum levels of AST, ALT, creatinine, and urea were significantly reduced upon administration of BVLE (Figure 4), when compared to untreated diabetic control rats, indicating that the extract can ameliorate the damage to the liver and kidney that was caused by diabetes. This may be attributed to the high phenolic content of BVLE which is further translated into high antioxidant activity.

3. Conclusion

The results of the current *in vitro* and *in vivo* study verified the efficacy of both plants used in Egyptian folk medicine as remedies for the treatments for diabetes, which can not only reverse hyperglycemia but also provide necessary hepatoprotective and nephroprotective effects in diabetic patients to help combat the serious systemic complication associated with the disease. Our report also confirmed results from previous studies carried on both plants collected from other locations.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study received no funding.

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