Phytochemical and Antioxidant Activities of *Berberis integerrima* and *Berberis vulgaris* and Pharmacological Effects of the more Active Species on Alloxan-Induced Diabetic Rats

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Abstract

Background: Medicinal plants with biologically active components such as antioxidant and antidiabetic are useful sources of novel therapeutics. In Iran, traditionally believed that the aqueous extract of berberry fruit (*Berberis integerima* F.) improved health, especially in people with diabetes.

Objective: In this study, in vitro phytochemical and antioxidant activities of *B. integerrima* and *B. vulgaris* fruit aqueous extracts (BIFA and BVFA) were compared. Effects of more active species on fasting blood sugar (FBS), serum lipid, and malondialdehyde (MDA) were evaluated in alloxan-induced diabetic rats.

Methods: The phytochemical potential and antioxidant activity of these extracts were quantified in terms of total phenolic content and total reducing power, respectively. BIFA (500 mg/kg) and glibenclamide (2 mg/kg) were administered orally to alloxan-induced diabetic rats and FBS, body weight, lipid profile, and serum MDA were monitored at 0, 7, 14, and 21 days after induction of diabetes.

Results: BIFA showed the highest phenolic content (6.816 ± 0.132 mg/g of dry extract) and antioxidant activity (0.134 ± 0.022 EC_{50} mg/mL). LD_{50} of BIFA was found to be >2500 mg/kg. FBS, lipid profile, and serum MDA have been significantly reduced in BIFA-treated rats after 21 days versus diabetic control and glibenclamide-treated rats. Extract has significantly decreased FBS levels of rats from 138.1 ± 1.68 to 82.00 ± 4.02 mg/dL. Furthermore, body weight has significantly improved in treated groups.

Conclusion: The results indicate *B. integerrima* with high phytochemical and antioxidant activities, has the potential of suppressed hyperglycemia, hyperlipidemia and lipid peroxidation.

Keywords: Antioxidant activity, Fasting Blood Sugar, Glibenclamide, Hyperglycemia, Phytochemical

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from relative or absolute lack of insulin or insulin insensitivity. According to the World Health Organization (WHO), approximately 4% of the world's population are affected by DM and is estimated to increase by 5.4% in 2025 [1]. The origin of complications of DM, including neuropathy, nephropathy, retinopathy, and cardiovascular disease is related to yperglycemia and hyperlipidemia [2, 3]. Marked increases in production of free radicals (oxidative stress) such as superoxide, hydrogen peroxide and hydroxide, and deficiency in antioxidant defense mechanisms have also been demonstrated in DM [4]. Nowadays, different management strategies used for treatment of diabetes include improving lifestyle through diet modification, exercise, use of oral anti-diabetic drugs such as sulfonylureas, thiazolidinediones, α-glucosidase inhibitors and insulin treatment. These drugs are used as monotherapy or in combination to achieve better glycemic control [5]. Many synthetic anti-diabetic agents have shown various side effects. Hence, more attention has been focused to natural plant sources because of effectiveness, fewer side effects, acceptability, and affordability [4-6]. Therefore, plants as sources of biologically active substances including anti-oxidation, anti-hyperglycemia, and anti-hyperlipidemia, play an important role as new therapeutic agents for diabetes.

Berberis (Berberidaceae) contain about 650 species and 15 genera, which are often spiny plants or shrubs [7]. Barberry species (Zereshk in Persian) grows in different parts of the world such as Iran, China, middle Asia, and many countries in Europe, Africa and America [8]. Various parts of this plant, such as root, bark, leaf, and fruit have been used widely as traditional medicine for long time in Iran and other communities [9]. Among different species of barberry, two barberry species are famous in Iran; *Berberis vulgaris* (poloei) and *Berberis integerrima* (abi) [7].

B. vulgaris is a bush with light brown colored bark an obovate leaves. The plant has pendulous yellow flowers in spring succeeded by oblong red colored fruits (barberry). Its bright red fruits have no seeds [9]. *B. integerrima* is a thorny shrub with yellow wood with obovate leaves. Its fruits are small, red, and oval with two or three small oblong seeds [11]. Barberry fruit contains potentially bioactive constituents such as berberine, berbamine, berberuin, palmatine, oxyacanthine, malic acid, ascorbic acid, caffeic acid, ursolic acid, coumarin, beta carotene, and tannin [8-12]. The main active alkaloid with a benzyl tetra hydroxy quinoline chemical structure is berberin, which can be found mainly in the fruit (5.2-7.7%) [10-13]. Several pharmacological effects have been demonstrated for berberine such as antimicrobial, anti-inflammatory, antihistaminic, anticholinergic, antipyretic, anticancer, antidiaetic, anti-hyperlipidemic, and antioxidant [14]. It is known that the major function of berberine in regulating blood glucose and lipid includes inhibition of mitochondrial function and α-glucosidase, stimulation of glycolysis, and activation of

AMP-activated protein kinase (AMPK) pathway. Additionally, recent research has shown that in patients with type 2 diabetes, insulin sensitivity is increased in the presence of berberin. In patients with type 1 diabetes; berberine is able to increase insulin secretion via repairing destructed or exhausted islets [15]. Imbalance between reactive oxygen species level and the capacity of antioxidant defenses play an important role in the pathogenesis of DM. This research is aimed to compare the phytochemical and antioxidant activities of most widely used species of Berberis in Iran (*B. integerrima* and *B. vulgaris*) and also evaluated the effect of the extract with higher antioxidant potential on FBS, lipid profile and serum malondialdehyde in alloxan-induced diabetic rats.

Materials and Methods

Drugs and chemicals

Fasting blood glucose was measured by Glucose oxidaseperoxidase strip (Accue-check diagnostic kit, Roche, Germany). Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL)–cholesterol were estimated by using diagnostic kits (Pars Azmun, Tehran, Iran). Alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, USA) and all other chemicals used in this study were analytical grade.

Plant collection and identification

The root, stem and fruit of *Berberis integerrima* and *Berberis vulgaris* were collected in October-November 2013 from Shahmirzad suburb (Semnan, province, Iran). This process has been authenticated by the botany department of the Natural Resources and Animal Science, Research Center of Semnan, Iran. They were deposited in the herbarium (No. 836 and 194, respectively).

Preparation of aqueous extract

Dried fruits of these plants were prepared in the dark, grounded in a grinder and kept refrigerated (4°C) until use. Water extract was prepared by means of a soxhlet. Fruit samples were extracted with distilled water in a soxhlet apparatus until extraction water became colorless [16]. Both extracts were further filtered and evaporated to dryness in a vacuum dryer (Rotary evaporator, RE-52AA, China).

Determination of total phenolic contents

Determination of phenolic compounds was accomplished as suggested by Barros *et al.*, (2007). In order to estimate total phenolics, 1 mL of the aqueous extract (5 mg/mL) was combined with 1 mL Folin-Ciocalteu's phenol reagent (Merck, Germany). Then, 1 mL saturated sodium carbonate solution (Merck, Germany) was added to the mixture after 3 minutes and total volume of mixture was adjusted to 10 mL with distilled water. This reaction mixture was then kept in dark for 90 minutes and then absorbance was read at 725 nm. Standard curve was calculated using gallic acid.

Measurement of Reducing Power

The reductive potential of fruit aqueous extracts was determined according to the method described by Yen and Duh (1994). Different concentrations of the extracts were made (0.05-1.6 mg/mL) in 0.2 M phosphate buffer pH 6.6 containing 1% potassium

ferrocyanide (Merck, Germany). The mixture was incubated at 50 °C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10% w/v) was added to the mixture. It was then centrifuged at 3000 xg for 10 minutes. The upper layer was separated and mixed with 2.5 mL distilled water containing 0.5 mL of ferric chloride 1% (Merck, Germany). The absorbance of this mixture was measured at 700 nm. The intensity in absorbance showed the antioxidant activities of the extracts. Concentration of the extract, which can provide absorbance of 0.5 (EC_{50}) was determined from the graph of absorbance against concentration of extract.

Experimental animals

Healthy adult Wister albino rats were used in this study. Animals were housed in stainless steel cages and maintained under standard conditions (12 h light/dark cycle; 22 ± 3°C; 45-55% humidity). The animals were fed with standard commercial pellet diet and water was supplied *ad libidum.* All experimental procedures used in this study were approved by the Animal Ethical Committee of Semnan University, Semnan, Iran.

Determination of acute toxicity of the aqueous extracts

Acute toxicity of *Berberis integrima* fruit aqueous extract was determined in fasting Wistar rats according to acute toxic classic method as the OECD guideline No. 423 (Acut Toxic Class Method). Rats were maintained under standard conditions (12 h light/dark cycle; 22 ± 3°C; 45-55% humidity) and were fasted overnight prior to the experiment. It was observe that aqueous extracts of *Berberis integerrima* was not lethal to rats up to 2500 mg/kg dose. Therefore, the LD50 of these extracts are >2500 mg/kg. Hence 1/5th (500 mg/kg) of this dose were selected for further study [19].

Induction of experimental diabetes

Twenty-four male rats were selected for this experiment. The rats were kept fasted for 16 h. After 16 h of fasting, a blood glucose level in rats via tail vein was measured by ACCU-Check glucose meter. The Wistar rats were diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg i.p.) in sterile saline. After 6 days of alloxan administration, rats with blood glucose level of ≥ 126 mg/dl were considered diabetic [20].

Experimental design

The rats were randomly divided into four groups of six rats per group. Group 1 received normal saline (10 mL/kg) and served as a normal healthy control. Group 2 diabetic rats received normal saline (10 mL/kg) and served as a diabetic control. Group 3 diabetic rats received glibenclamide (2 mg/kg/day, p.o.), 6 days after alloxan administration as a reference drug, and Group 4 diabetic rats received BIFA 500 mg/kg/day, p.o.), 6 days after alloxan administration. These doses were selected from a study by Ashraf *et al.,* (2014). Administration of the vehicle, drug and plant extract to rats was done daily until 21 days. Blood glucose and body weights of the rats were measured at weekly intervals on days 0, 7, 14 and 21. All the animals, after 21 days, were anesthetized in a chloroform chamber. Blood samples were collected from the hearts for serum lipid profile and lipid peroxidation (MDA).

Statistical analysis

All the results were expressed as mean ± standard error mean (SEM). Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple tests and values of $P<0.05$ were considered statistically significant.

Results

Total Phenolic contents and Reducing Power of BIFA and BVFA

In this study BIFA showed higher Total Phenolic contents (three times) with significant differences from BVFA ($P < 0.05$). There was also significant difference in Reducing Power between two extracts (Reducing Power of BIFA was more than of BVFA) (Table 1). Therefore, the aqueous extract of *B. integerrima* was selected to evaluate its effect on blood glucose and lipid and malondialdehyde levels *in vivo* in alloxan-induced diabetic rats.

Effect of BIFA on the blood glucose levels of alloxan-induced diabetic rats

The hypoglycaemic effect of BIFA on the FBS of alloxan-induced rats is presented in Table 2. The FBS levels were significantly increased ($P < 0.001$) in alloxan-induced diabetic rats (Group 2) in comparison with normal healthy rats (Group 1). In glibenclamide (2 mg/kg) and BIFA (500 mg/kg) treated groups, the values of blood glucose significantly decreased ($P < 0.01$) as compared with untreated diabetic rats on the 21st day. There was also a significant reduction in blood glucose levels (on days 14 and 21) in diabetic rats treated with BIFA compared to diabetic rats treated with glibenclamide ($P <0.01$). Therefore, this effect of BIFA (500 mg/kg) is clearly better than glibenclamide (2 mg/kg).

Effect of BIFA on lipid profile of alloxan-induced diabetic rats

The level of serum TC, TG and LDL-cholesterol in untreated diabetic rats was significantly ($P < 0.001$) increased compared to the normal healthy rats, whereas the level of HDL-cholesterol was reduced in untreated diabetic rats (Table 3). In the glibenclamide and BIFA treated groups, the level of serum TC, TG, LDL-cholesterol reduced significantly ($P < 0.001$) as compared with untreated diabetic rats. The activity of BVFE at the dose of 500 mg/kg was comparable with the reference drug (2 mg/kg) on day 21.

Effect of BIFA on the body weight of alloxan-induced diabetic rats

As shown in Figure 1, there was a gradual increase in the mean body weight of normal healthy rats, but diabetic untreated rats showed significant reduction in body weight until day 21st. In the glibenclamide and BIFA-treated rats, there was a gradual increase in the mean body weight on days 7, 14, and 21.

Effect of BIFA on lipid peroxidation in alloxan-induced diabetic rats

Compared to normal healthy rats, the level of serum MDA was significantly ($P < 0.001$) increased in untreated diabetic rats (Figure 2). Glibenclamide (2 mg/kg) and BIFA (500 mg/kg) significantly ($P < 0.001$) reduced the levels of serum MDA of rats when compared with diabetic untreated rats.

Table 1- Reducing power and total phenolics of *B. integerrima* and *B. vulgaris*

Sample	Reducing Power EC_{50} value in mg/ml	Total Phenolics mg GA/ g DM
B. integerrima	0.134 ± 0.022*	6.816 ± 0.132**
B. vulgaris	0.042 ± 0.008	2.266 ± 0.263

Data expressed as means ± SD of triplicate analyses of two berberis species.
*P<0.01 and **P<0.001 reducing power and total phenol of *B. integerrima* were compared with *B. vulgaris*.

Table 2- Effect of glibenclamide and BIFA on the FBS of alloxan-induced diabetic rats treated for 21 d.

Group	Treatment	Mean fasting blood glucose levels ± SE				
		Pre-diabetic	Day 0	Day 7	Day 14	Day 21
1.	NS (10 ml/kg)	79.25 ± 2.86	79.50 ± 2.39	79.00 ± 2.73	78.00 ± 2.27	72.75 ± 4.02
2.	DC (150 mg/kg)	83.25 ± 4.17	134.50 ± 2.98#	134.50 ± 3.42#	136.00 ± 2.16#	139.75 ± 3.32#
3.	D+GC (2 mg/kg)	80.16 ± 1.49	140.33 ± 1.33	123.16 ± 3.72*	122.83 ± 2.83	113.83 ± 3.68***
4.	D+BIFA (500 mg/kg)	78.16 ± 3.00	138.16 ± 1.68	132.00 ± 1.77a	87.66 ± 7.02***c	82.00 ± 4.02***c

NS: Normal saline, DC: Diabetic control, D+GC: Diabetic + Glibenclamide, D+BIFA: Diabetic + BIFA.
#P<0.0001 Diabetic control rats were compared with normal control rats.
*P < 0.05, **P < 0.001 and ***P < 0.0001 Diabetic treated rats were compared with diabetic control rats.
aP < 0.05, bP < 0.01 and cP < 0.0001 Diabetic treated rats with BIFA were compared with Diabetic treated rats with glinenclamide on corresponding day.

Table 3- Effect of glibenclamide and BIFA on the lipid profile of alloxan-induced diabetic rats

Group	Treatment	Total cholesterol (mg/dL)	Teriglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
1.	NS (10 mL/kg)	63.75±5.54	50.00±6.89	47.90	48.00±2.87
2.	DC (150 mg/kg)	180.66±5.54#	155.00±15.56#	105.66±9.13#	44.00±7.37
3.	GC (2 mg/kg)	75.5±2.23*	81.83±7.19*	17.46±3.97*	41.66±2.99
4.	BIFA (500 mg/kg)	76.00±6.13*	88.50±12.83*	19.46±3.21*	38.83±2.05

NS: Normal saline, DC: Diabetic control, D+GC: Diabetic + Glibenclamide, D+BIFA: Diabetic + BIFA
#P<0.0001 Diabetic control rats were compared with normal control rats.
*P<0.0001 Diabetic treated rats were compared with diabetic control rats.

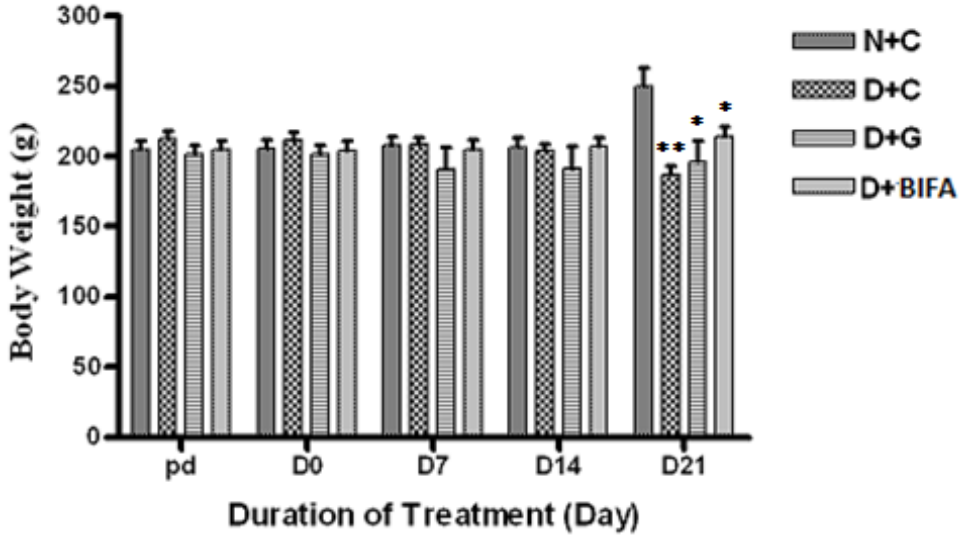

Figure 1- Effect of *B. integerrima* on mean body weights (g) of alloxan induced diabetic rats. N+C: normal control, D+C: diabetic control, D+G: diabetic glibenclamide, D+BIFA: diabetic Berberis integerrima fruit aqueous extract. *P < 0.05, **P < 0.001 Diabetic control rats and diabetic treated rats with glibenclamid and BIFA were compared with normal control rats.

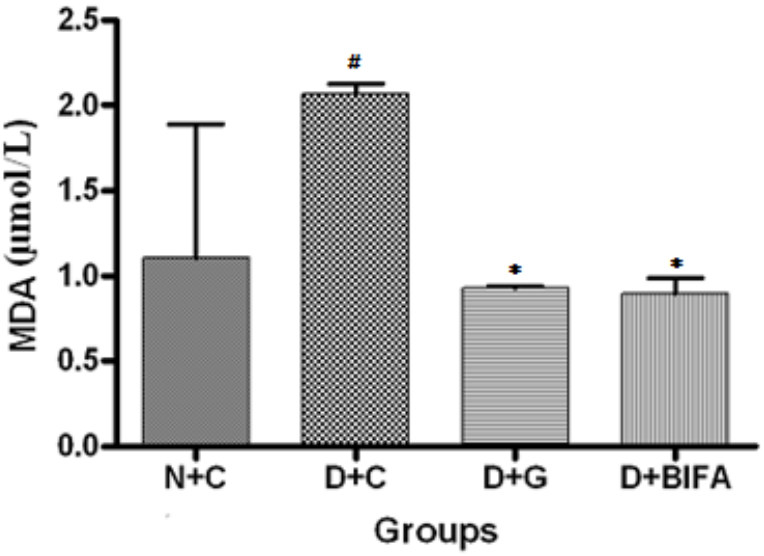

Figure 2- Effect of *B. integerrima* on serum MDA level of alloxan induced diabetic rats. N+C: normal control, D+C: diabetic control, D+G: diabetic glibenclamide, D+BIFA: diabetic Berberis integerrima fruit aqueous extract. #P<0.0001 Diabetic control rats were compared with normal control rats. *P<0.0001 Diabetic treated rats were compared with diabetic control rats.

Discussion

The most antioxidant tests have indicated the high antioxidant activity of Berberis species [21]. Most of the in vitro antioxidant research on *Berberis* concentrated on roots, twigs and leaves of plant [21-22]. Serteser *et al*., (2009) investigated the antioxidant activity of methanol extract of the leaves and fruit of *B. integerrima* and *B. vulgaris*. The effect of mixed extract of leaves and stems of *B. vulgaris* on antioxidant activity and cytotoxicity of human liver cancer cell lines has been studied by Hanachi *et al*., (2009). The correlation between antioxidant activity and total phenol content of *B. vulgaris* fruit extract has been shown in several studies [21-25, 26]. However, some studies did not find any relationship between total phenol content and antioxidant activity of *B. vulgaris* fruit extract [27-28]. Motalleb *et al*., (2005) demonestrated the aqueous extract of *B. vulgaris* has a higher antioxidant activity than its ethanol extract. To our knowledge, comparison of the antioxidant activity between fruit aqueous extracts of the two species of Berberis has not been studied.

In this study, in vitro antioxidant activity of most widely used species of Berberis in Iran (*B. integerrima* and *B. vulgaris*) were evaluated based on total phenol content and reducing power and the species with higher antioxidant potential was selected to evaluate its effect on blood glucose and lipid and serum malondialdehyde levels in vivo in alloxan-induced diabetic rats.

The value of total phenol content of the berberis aqueous extract determined by the Folin-Ciocalteu method was 6.816 ± 0.132 mg GAE/g DM for *B. integerrima* and 2.266 ± 0.263 mg GAE/g DM for *B. vulgaris*. Therefore, TP of *B. integerrima* was significantly higher than in *B. vulgaris* (Table 1). Results obtained for these Berberis species are consistent with those reported b Rezaeian

et al., (2015). The electron transfer ability from a compound is evaluated by reducing power assay. This method is commonly used as a significant indicator for assessing the antioxidant activity, since it has high sensitivity, and is rapid and inexpensive [21]. The value of reducing power of the Berberis aqueous extract was 0.134 ± 0.022 EC_{50} value in mg/mL for *B. integerrima* and 0.042 ± 0.008 EC_{50} value in mg/ml for *B. vulgaris*. Therefore, *B. integerima* showed a significant increase in reducing power than *B. vulgaris* (Table 1). RP values were in good agreement with literature report for Berberis samples [22].

The second goal of this study was to evaluate the anti-hyperglycemic, anti-hyperlipidemic and lowering of serum malondialdehyde activity of *B. integerrima* in alloxan-induce diabetic rats. In chemically induced type 1 diabetes two main compounds has been used: alloxan or streptozotocin (STZ). Mechanisms induced diabetes by alloxan are mainly related to rapid uptake of the compound by the beta cells of the islets of Langerhans of the pancreas and the formation of free radicals. Since the defense mechanisms of beta cells are weak, these cells are damaged and endogenous insulin secretion is reduced. This increased blood glucose, cholesterol and triglycerides and decreased body weight, consequently. Fasting animals are more susceptible to alloxan, because of the similarity of glucose with alloxan, can compete with it [3]. The results of this study showed that the aqueous fruit extract of *B. integerrima* at the dose of 500 mg/kg body weight significantly reduced the FBS levels ($P< 0.001$), to the extent that the blood glucose levels of diabetic rats treated with the extract (Group IV) restored to normal levels after 21 days (Table 2). Hypoglycemic effects of *B. integerrima* may be attributed to its main alkaloid (berberine). Previous studies have indicated that berberine is able to increase insulin sensitivity and increase insulin secretion by improving destructed or exhausted islets [29, 30]. Berberine acts as a α-glucosidase inhibitor in gut. Inhibition of the enzyme leads to decreased absorption of dietary carbohydrates. Berberine can also reduce FBS by direct inhibition of gluconeogenic genes, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) in liver [15]. Diabetic rates treated by sulfonylureas such as glibenclamide, as the reference drug for treatment of diabetes, at the dose of 2 mg/kg body weight showed a significantly decrease in FBS level ($P<0.001$) during 3 weeks of treatment in comparison with diabetic control rats. Although these results were inconsistent with the results of Ashraf *et al.,* (2014). They reported the aqueous fruit extract of *B. integerrima* was ineffective on blood glucose level in diabetic rats, but it entirely consistent with the results of Ashraf *et al*., (2014) who demonstrated the hypoglycemia effects of root aqueous extract of *B. Integerrima* on diabetic rats. Surprisingly, the effects of the aqueous fruit extract of *B. integerrima* on lowering blood glucose in diabetic rats in this study was significantly ($P<0.01$) higher than glibenclamide (Table 2).

Body weight loss is one of the features of uncontrolled diabetes mellitus and for

monitoring of severity or response to treatment, weight measurement is an important tool in the study of diabetes. The result of body weight loss in untreated diabetic rats in our study is consistent with other studies [4-6-11-19-31]. In the glibenclamide and BIFA-treated rats significantly improved the body weight of diabetic rats (Figure 1).

Marked increases in serum lipids and blood glucose levels in poorly controlled diabetes mellitus are considered as predisposing factors for coronary heart disease in diabetes [4]. Results of this study clearly indicated, marked increases ($P < 0.001$) in total cholesterol, triglycerides, LDL and decreases in HDL in untreated diabetic rats compared with normal rats (Table 3). In this study, in addition to the hypoglycemic role, serum triglyceride, total cholesterol, and LDL were significantly decreased ($P < 0.001$) with the glibenclamide and BIFA treatment. This finding suggests that BIFA can be beneficial to diabetic individuals with atherosclerosis [32]. These results were also compatible with results of earlier studies [4-6-11-19-31].

In Type 1 diabetes, it has been shown that β-cell damage resulting from lipid peroxidation, impaired insulin production. Determination of the serum MDA is an indicator of lipid peroxidation [4]. Glibenclamide (2 mg/kg) and BIFA (500 mg/kg) caused a significant reduction ($P<0.001$) in the level of MDA in treated diabetic rats in comparison with untreated diabetic rats (Figure 2). Zhou *et al*., (2008) Demonstrated that berberin significantly decreased the serum MDA level and increased catalase, superoxide dismutase, and glutathione peroxidase in rats.

Conclusion

In conclusion, *B. integerrima* fruit aqueous extract exhibited significant antihyperglycemic and antihyperlipidemic activities in alloxan-induced diabetes in this study. The results revealed that antioxidant potential of *B. integerrima* is high and it is able to inhibit lipid peroxidation. The most significant finding of this research is that the effect of aqueous extract of the plant on diabetes parameters shows more efficacy than standard anti-diabetic drug (glibenclamide). Antidiabetic activity and other effects of this plant may be result of its phytochemical constituents. Findings of this study indicate the potential of *B. integerrima* on suppressing of hyperglycemia, hyperlipidemia, and lipid peroxidation. It suggests a promising use of *B. integerrima* for the treatment of diabetes. However, studies are on-going to extract the main compounds responsible for the anti-diabetic activity and elucidate its molecular mechanism of action.

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