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Original Article

Comparison of *Momordica charantia* effect on *Mcl-1* gene expression in the livers of streptozotocin diabetic and healthy rats

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Abstract

Background and aims: The positive effects of medicinal herbs on diabetes have been proved in previous studies. The aim of the present study was to evaluate the effect of active *Momordica Charantia* on the treatment of liver diseases resulting from diabetes and the expression level of the *Mcl-1* gene, which is a proapoptotic gene and becomes antiapoptotic in the event of damage.

Methods: In this study, 42 adult male Wistar rats were randomly divided into 7 groups including healthy, diabetic, metformin, 150 mg/kg *M. charantia* controls, and three groups that received the active *M. charantia* with doses of 50, 100, and 150 mg/kg. All groups became diabetic with streptozotocin injected intraperitoneally except for the control and *M. charantia*. Afterward, they received the active *M. charantia* by gavage for four weeks (three times a week). Finally, the Kruskal-Wallis method was used for comparison among the groups. The statistical tests were analyzed using SPSS software, version 22.

Results: The level of *Mcl-1* expression in the diabetic control group (C) was significantly higher than that in the healthy control (A) and the *M. charantia*-receiving control group (B, $P<0.05$). The group receiving 150 mg/kg dose of *M. charantia* drug (G) had a better effect compared to the group that received 100 mg/kg (F), and this difference was significant ($P<0.05$). This increase indicated that the medication was dose-dependent.

Conclusion: In general, a reduction in the level of *Mcl-1* gene expression relied on the *M. charantia* dose. After the development of diabetes, this level significantly increased in the diabetic groups, but decreased after receiving *M. charantia*, leading to a decrease in the side effects and symptoms associated with diabetes.

Keywords: Diabetes, Streptozotocin, Liver, *M. charantia*, *Mcl-1* gene

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Introduction

Diabetes mellitus (DM) is a chronic disorder in the metabolism of carbohydrates, fat, and protein, and is characterized by increased blood sugar in the diabetic samples. In addition, cardiovascular failure, renal failure, and decreased neurological activity are among the long-term complications of this disease. DM is caused by the cell absorption of the glucose from decreased insulin secretion or the resistance of the cells to insulin (1,2). According to the World Health Organization (WHO), the number of people affected by the disease will increase by 60% in developing countries by 2030 if effective measures are not taken to prevent diabetes. However, with over 8% of diabetes, Iran has the highest rate of prevalence. So far, previous studies have focused on several genes in the pathogenesis of DM (3,4). The impaired liver function is considered as one of the side effects of diabetes. It seems that the planned death (apoptosis) in the liver cells increases and the tissue is damaged in this case. Apoptosis is a natural process that removes old, damaged, and impairing cells. Any disorder in the process of apoptosis results in the development of the disease, which can be due to a reduction in the cell death that leads to the development and growth of cancer cells or autoimmune disorders (5,6). Various factors affect the differentiation of the liver cells, including caspase 9, *Bcl-xl*, *P53*, *Bcl-2*, *Bim*, *Bad*, *Mcl-1*, *Bax*, and *Bak*. The cooperation of *Bcl-2*, *Bcl-xl*, and *Mcl-1* proteins brings forth the anti-apoptotic activity (7,8). *Mcl-1* belongs to the group of *Bcl-2*, which has two isoforms. Isoform 1 increases cell survival by inhibiting apoptosis,

and Isoform 2, which is the product of the shortened cut gene and causes the death of the cell (9). This gene is located on chromosome 1 in humans and on chromosomes 3 and 5 in mice (10). To prevent apoptosis, Fas and *Bcl-2* should be prevented and IAPS concentration should be increased. Further, the Akt-kinase protein causes cell survival, which occurs through this process. The phosphorylation of the Akt gene prevents *Bax* from acting and the Akt protein activates the IKKA molecule, which activates the NF-KB molecule, and ultimately, leads to the expression of genes that are anti-apoptotic (11,12). Furthermore, physical activities modulate cell amplification and death through cytokines, hormones, growth factors, and metabolic pathways (13). Before the discovery of insulin, as well as common antidiabetic drugs, diabetic cases were treated with medicinal herbs and traditional therapies. Up to now, more than 1200 herbal medicines have been known to be effective in reducing blood glucose levels or its complications (14). According to (15), more than 2100 herbal medicines have so far been identified for reducing blood glucose levels or its complications. (16) indicated some of these plants such as bitter cucumber (*Momordica charantia* L.), fenugreek (*Trigonella foenumgraecum* L.), Black Guillaume (*Vaccinium arctostaphylos* L.), and Abojahl watermelon (*Citrullus colocynthis*). The bitter cucumber (*M. charantia*), also called Karela, is one of the tropical and subtropical plants of the Cucurbitaceae family. It naturally grows in Asia, Africa, and India and its fruits are edible with a very bitter taste, which is why it is called bitter cucumber (17). Moreover, this plant can be used in the green or yellowish stage and its green leaves and stems are used in the preparation of foods, soups, and salads. High consumption of this plant causes some side effects thus this type of cucumber should be taken under the supervision of a traditional medical practitioner (18). Cucurbitacin, as the active ingredient of this plant, consists of a skeletal structure and a base ring. The reason for this naming is due to the presence of a specific cucurbitacin in the *M. charantia*, which has never been detected in any other plant. Triterpenoid cucurbitacin found in *Momordica* fruit is remarkable for anti-diabetic and anti-cancer activities (19-21). Due to the high rates of diabetes and the resultant liver complications in Iran and the world, as well as due to the lower side effects of medicinal plants compared to industrial and chemical drugs, this study investigated the effect of the bitter cucumber on the expression of *Mcl-1* gene, which is one of the key genes for apoptosis in the liver.

Materials and Methods

Study type and grouping

The present study was experimental and data collection was done by a laboratory-observational method. In this study, 42 adult male Wistar rats with an average weight of 240 to 300 g were purchased from the animals' nests of Shahrekord University of Medical Sciences. The animals were transferred to the animals' nest of Shahrekord Azad University under standard conditions of 23 to 25°C and a cycle of 12/12 hours of light/darkness with adequate and standard water and food. After adapting to the environment, the rats were randomly divided into 7 groups of 6.

Group A (control group) consisted of six healthy rats which received only regular water and a standard diet per day. Group B (control group), which had the regular intake of *M. charantia*, received 150 mg/kg of *M. charantia* in gavage once every three other days for 30 days.

Group C (negative control/diabetic control) included diabetic rats with streptozotocin which received only every day.

Group D (standard group) included streptozotocin-diabetic rats which received this drug every day, as well as metformin in gavage every three other days for 30 days, each time 50 mg/kg (10 gavages).

Group E included streptozotocin-diabetic rats which received this drug every day, along with *M. charantia* in gavage every three other days for 30 days, each time 50 mg/kg (10 gavages).

Group F (the second treatment group) included streptozotocin-diabetic rats which received this drug every day as well as *M. charantia* in gavage every three other days for thirty days, each time 100 mg/kg (10 gavages).

Group G or the third treatment group included streptozotocin-diabetic rats that received this drug every day, as well as *M. charantia* in gavage every three other days for thirty days, each time 150 mg/kg (10 gavages). The *M. charantia* was purchased and used as an extract from Merk Company, Germany (24).

It should be noted that the blood glucose in the rats was measured by a glucometer before becoming diabetic and the weights of the rats were estimated and recorded using digital scales. After a month, the rats were anesthetized by chloroform in completely sanitary conditions and in accordance with the ethical values, followed by removing their liver tissues during the subsequent operation. The 0.5 in 0.5 tissue of the liver was then placed in RNA for further experiments, stored at -20°C in a freezer for 24 hours, and was eventually transferred to a -70°C freezer.

In the next step, RNA was extracted using the cDNA Synthesis Kit (YTA) and its quantitative and qualitative analyses were finally carried out using the nanodrop spectrophotometer and agarose gel 1%. Then, cDNA was synthesized after assuring the purity of the extracted RNA using the cDNA Synthesis Kit (14,15).

To evaluate the studied gene, the primers used in this study were synthesized based on gene sequence and in line with Primer 3D software by Kohangen Company. The sequence of the *Mcl-1* gene is shown in Table 1.

Gene expression using polymerase chain reaction

The expression of *Mcl-1* gene was carried out in a volume of

Table 1. Primers used in this research

	Sequence (5´→3´)	Tm
Forward *Mcl-1*	GTG CCT TTG TGG CTA AAC ACT	61
Reverse *Mcl-1*	AGT CCC GTT TTG TCC TTA CGA	61
Forward GAPDH	ATG GTG AAG GTC GGT GTG AAC	62
Reverse GAPDH	TTG AAC TTG CCG TGG GTA GAG	62

15 µL in such a way that 7.5 µL Master Mix was poured into a vial and then a 1 µL primer containing 0.5 µL of the leading primer and 0.5 µL of the follower primer were added to the vial. In addition, 1.5 µL of the cDNA was added to the vial, and finally, 4 µL of the injection water was added to obtain the 15 µL volume. The samples were then placed in polymerase chain reaction (PCR), which consisted of the following programmed steps: Initial denaturation step for 5 minutes at 94°C, denaturation for 30 seconds at 94°C, and annealing for both genes for 30 seconds. However, the temperature was 61°C and 62°C for *Mcl-1* and GAPDH genes, respectively. The extension was adjusted for 30 seconds at 72°C and final extension for 5 minutes at 72°C. The number of cycles was 33, and finally, PCR products were examined on 1% agarose gel.

Real-time polymerase chain reaction technique

To perform the RT-PCR technique based on the PCR Kit Takara protocol, 6.5 µL of SYBR Green was added to 1 µL of cDNA, and then 0.5 µL of reverse and forward primers was added, and finally, 4.5 µL of the injected water was added to it such that the volume of 13 µL could be obtained. Eventually, the samples were placed in the RT-PCR machine and the device was adjusted according to Table 2, followed by evaluating the results (23).

Data analysis method

The collected data were analyzed using SPSS software, version 22 and the analysis of variance was used to obtain the significance relationship. The statistical level of 5% was considered as the significant level. The average data were collected and the charts were aggregated using Excel software. Charting was drawn using the Kruskal-Wallis and ANOVA software and the confidence level was 95%.

Gene expression analysis using the PCR technique

To ensure the accuracy of the cDNA synthesis for the samples, RT-PCR was performed for the internal control gene of GAPDH and the intended gene of *Mcl-1*. Based on the results, 160 bp and 130 bp bands were amplified for the internal control gene and the intended gene of *Mcl-1*. The results of the RT-PCR product electrophoresis are shown in Figures 1 and 2.

GAPDH and *Mcl-1* amplification curves at real-time polymerase chain reaction

Figures 3 and 4 illustrate the amplification curve for *Mcl-1* and homed genes.

Melting curves obtained for GAPDH and *Mcl-1*

The GAPDH and *Mcl-1* melting curves in healthy samples, as well as those that received different doses of *M. charantia,* are depicted in Figures 5 and 6. Single and aligned curvatures indicate the specific amplification of the *Mcl-1* gene.

After the RT-PCR and drawing the threshold line, the

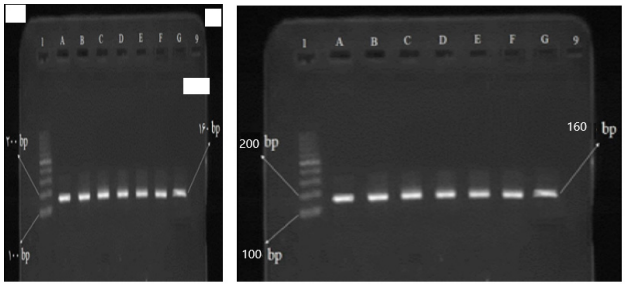

Figure 1. GAPDH bands with the100 bp marker.

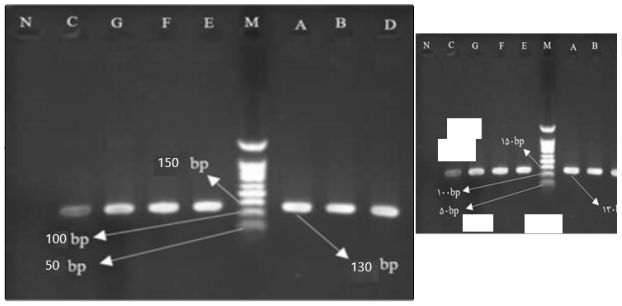

Figure 2. *Mcl-1* bands with the 50 bp marker.

Table 2. Temperature stages of *Mcl-1* gene real-time

Stages	Time	Temperature	Cycle
Initial Denaturation	5 minutes	95	1
Denaturation	20 seconds	95	
Annealing for *Mcl-1* gene	30 seconds	61	40
Annealing for GAPDH gene	30 seconds	62	
Extension	30 seconds	72	1

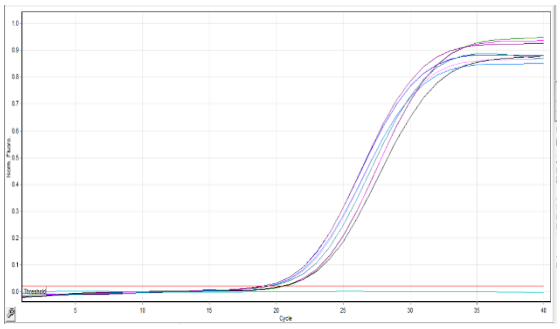

Figure 3. GAPDH Amplification Curve

Table 3. Comparison of the Level of *Mcl-1* Gene Expression in All Samples

Groups	SD ± Mean
Healthy control	0.002089 ± 0.018[a]
Healthy control receiving the dose of 150 mg/kg of *M. charantia*	0.00208 ± 0.0005[a]
Diabetic control	1.0007 ± 0.0539[b]
Receiving 50 mg/kg of metformin	0.9043 ± 0.0002[b]
Receiving 50 mg/kg of *M. charantia*	0.77820.1178 ± [b]
Receiving 100 mg/kg of *M. charantia*	0.541 ± 0.0556[c]
Receiving 150 mg/kg of *M. charantia*	0.1672 ± 0.0640[e]

Unsimilar letters indicate the significance of the groups together ($P< 0.05$).

results of which are provided as follows.

Examining the level of *Mcl-1* gene expression in diabetic cases and samples receiving different doses of *M. charantia*

In this study, the diabetic group was taken as the reference for the other groups and their *P*-values were obtained based on this reference. By definition, there is a significant difference between the groups if $P<0.05$, but there is no significant difference between them if $P>0.05$. The expression level of this gene in the diabetic control group (C) was significantly higher than those in the healthy (A) and the *M. charantia*-receiving (B) control groups ($P<0.05$). However, the *Mcl-1* gene expression gradually decreased depending on the amount of the drugs received in each group, where a non-significant reduction ($P<0.05$) in gene expression was observed in the groups receiving 50 mg/kg of metformin (D) compared to the diabetic control group (C). On the other hand, a significant reduction ($P<0.05$) was found in the level of gene expression in the diabetic group receiving 50 mg/kg of *M. charantia* (E) compared to the diabetic control group (C) and the groups receiving the dose of 50 mg/kg of metformin (D). However, the level of *Mcl-1* gene expression in the group receiving 100 mg/kg of *M. charantia* (F) was significantly lower than those in C, D, and E groups ($P<0.05$).

The amount of *Mcl-1* gene expression in the group receiving 150 mg/kg pf *M. charantia* (G) was significantly lower ($P<0.05$) than those in the other diabetic groups (diabetic control group and drug-receiving groups). In addition, the group receiving the dose of 150 mg/kg of the *M. charantia* drug (G) had a better effect compared to the group which received the dose of 100 mg/kg (F), and this difference was statistically significant ($P<0.05$) (Figure 6 and Table 3). This reduction in expression demonstrates the dose-dependency of *M. charantia*.

Discussion

In a review article, Fallah Huseini et al investigated the effect of medicinal plants on diabetes and found that these plants reduce blood sugar and are considered the best way to treat diabetes due to their low side effects (16).

In another investigation over the effect of the extract of Karela plant leaf on the amount of glucose, lipid, and malondialdehyde serum streptozotocin-diabetic rats, Hajinejad et al found that the blood sugar-reducing effect of the plant leaf varies depending on the glucose concentration. These effects may be due to flavonoids and antioxidant compounds. According to the obtained results, flavonoids and anti-oxidant compounds in M. charantia also reduce the level of the *Mcl-1* gene expression (22).
Similarly, Fernandes et al showed that 10 weeks of regular swimming in male rats increased anti-apoptotic proteins of Bcl-2 and Bcl-xl and reduced apoptotic Bad protein. This occurs with Bad phosphorylation and leads to a reduction

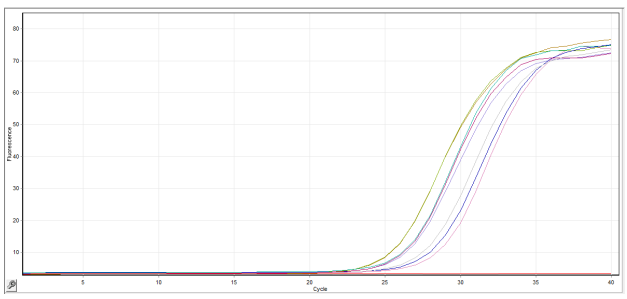

Figure 4. Amplification Curve for *Mcl-1* Gene

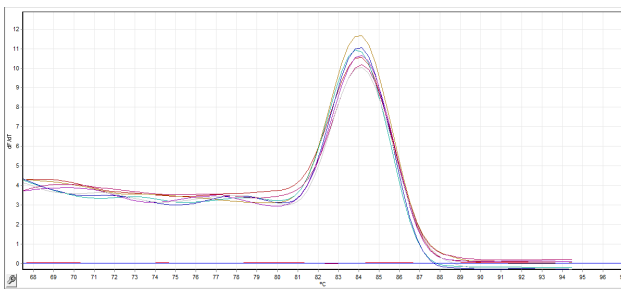

Figure 5 . Melting curve for GAPDH.

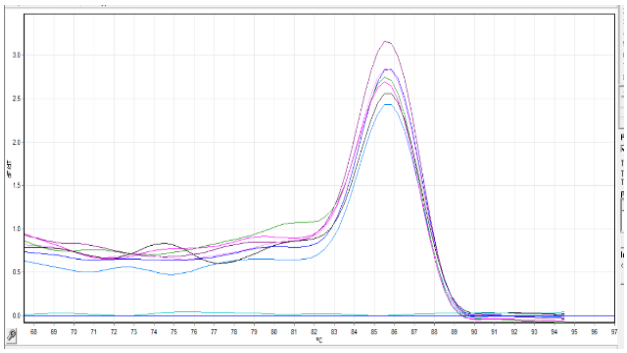

Figure 6. Melting curve for *Mcl-1* findings from the statistical analysis.

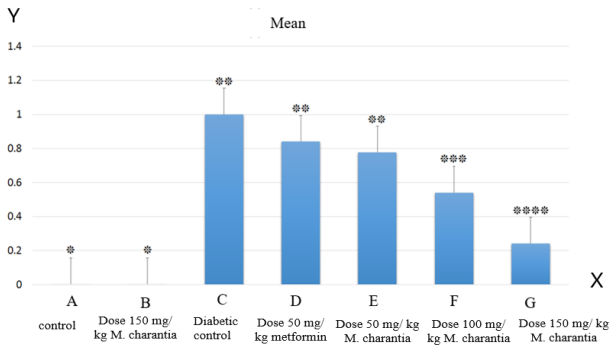

Figure 7 . Level of *Mcl-1* gene expression in all groups.

in the ratio of Bad compared to Bcl-2 (23). The results of the above-mentioned study are consistent with our results, where a reduction was also observed in *Mcl-1* gene expression. Given that *Mcl-1* is a key gene in apoptosis, this concept can be understood in light of the present study.

By studying the chromosomal map of the anti-apoptotic genes in myeloid cancer, Craig et al concluded that *Mcl-1* is an anti-apoptotic gene of the *Bcl-2* family which is found on chromosome 1 in humans and on chromosomes 3 and 5 in mice (11).

Evaluating the effect of streptozotocin injection on the rats, Aksu et al found that there was a direct correlation between blood glucose (diabetes) and liver damage, which is in line with the results of the present study, indicating that streptozotocin can cause liver damage (24).

Further, Kermany et al conducted a study on the effect of the aqueous and methanolic extract of the Karela fruit on blood glucose and liver enzymes in male rats and found that the aqueous extract of this fruit has a greater effect on diabetes compared to the methanolic extract and that the methanolic extract brings forth a significant reduction in the activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase enzymes (25). Thus, it can be argued that Karela affects liver factors.

In a study entitled "Effect of bitter gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabetic rats", Shetty et al found that feeding the dry matter powder of the *M. charantia* extract with a diet improved the levels of glucose and the condition in diabetic mice (21).

By examining the preventive effects of *M. charantia* on hyperglycemia and insulin resistance in mice fed with fatty diets and *M. charantia* without taking Rosiglitazone, Shih et al concluded that *M. charantia* is effective in improving hyperglycemia due to a fatty diet, as well as hyperlipidemia and visceral obesity (26).

In a review study regarding the effect of nettle plant on diabetes, Hasani-Ranjbar et al found that this plant significantly reduces blood glucose and various mechanisms of pancreatitis and non-pancreatitis. They further reported its side effects in the liver and kidneys (27). Based on the findings of the above-mentioned studies and the present study, streptozotocin causes damage to the liver and thus the pancreas, as well as the development of diabetes and its complications. However, *M. charantia* can be effective in improving this damage, and the obtained results are corroborated with those of the studies conducted by Kermany et al (25), Shih et al (26), and Hasani-Ranjbar et al (27) and Aksu et al (24).

Conclusion

Overall, a reduction in *Mcl-1* gene expression by *M. charantia* was dose-dependent due to the destructive effect of streptozotocin on the liver, and the most reduction was observed in the dose of 150 mg/kg. After the development of diabetes, the reduction increased sharply in diabetic groups. In addition, the level of *Mcl-1* gene expression was reduced again after using the *M. charantia* and weaker symptoms and complications were found to be associated with diabetes.

Conflict of Interests

None.

Ethical Considerations

This paper was derived from a research project approved by the Research Deputy of Islamic Azad University of Shahrekord with the ethics code of IR.IAU.SHK.REC.1398.016.

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