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Research Paper

The Synergistic Glucose-lowering Effects of Metformin and Bavachinin on Type II Diabetic Rats

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ABSTRACT

Background: Diabetes is one of the most prevalent endocrine disorders in humans, and its first-line medication is metformin. Peroxisome proliferator-activated receptor gamma (PPAR–γ) agonists are the adjuncts to metformin. Bavachinin is a PPAR pan-agonist with fewer side effects than metformin" into PPAR–γ agonists. In this study, the synergistic effects of metformin and Bavachinin were investigated on type II diabetic rats.

Methods: After four weeks of a high fat and glucose diet, type II diabetes was induced in 28 male Wistar rats, using injection of streptozotocin and nicotinamide. The animals were distributed into five groups of seven each: 1) Normal control (N), 2) Diabetic control (D), 3) Diabetic rats receiving metformin (DM), 4) Bavachinin (DB), and 5) Metformin plus Bavachinin (DMB). Oral glucose tolerance test (OGTT), fasting blood glucose (FBG), fasting insulin (FINS), homeostasis model assessment of β-cell function (HOMA-β), homeostasis model assessment of insulin resistance (HOMA-IR), and insulin sensitivity index (ISI) were obtained.

Results: The OGTT results in DM, DB, and DMB groups were significantly improved compared to that of D group. The FBG levels were significantly lower in DMB than in DB, DM, and D groups. The FINS levels of DMB were significantly less than those of DB, DM, and D groups. The HOMA-IR and HOMA-β were comparable between DMB and N groups. The ISI improved significantly in DMB compared to those in DM, DB, and D groups.

Conclusion: Bavachinin may be used combined with metformin for the treatment of type II diabetes at lower doses of metformin, thus having fewer side effects.

Keywords: Bavachinin, Diabetes mellitus type 2, Insulin resistance, Metabolic syndrome, metformin

Introduction

Diabetes is one of the most common endocrine diseases in humans [1]. It is anticipated that by 2040, this disease would affect over 600 million people globally [2]. Diabetes is characterized by the elevated blood glucose level as a result of decreased insulin secretion or insulin insensitivity [3, 4]. Chronic high blood glucose results in microvascular and macrovascular complications [5]. There is no cure for diabetes, however, it is manageable with currently available medications, including biguanides and thiazolidinediones (TZDs) [6].

Metformin is a biguanide derived from Galega officinalis, and the first line oral therapy in patients with type II diabetes mellitus. It mostly reduces glucose production in the liver with some implications on peripheral glucose uptake. By inhibiting mitochondrial respiratory chain, metformin impedes adenosine triphosphate (ATP) production, which leads to the activation of hepatic adenosine monophosphate-activated protein kinase (AMPK). This enzyme activates the catabolic ATP generating pathways, and inhibits ATP consuming processes, such as hepatic gluconeogenesis. Also, in an AMPK-independent manner, by inhibiting fructose-1,6-bisphosphatase, metformin inhibits gluconeogenesis. By phosphorylating acetyl-CoA carboxylases 1 and 2 (ACC1 & ACC2), AMPK modulates fat metabolism and enhances hepatic insulin sensitivity. In the gut, metformin increases the consumption of anaerobic glucose and secretion of glucagon-like peptide-1 (GLP-1), which lowers gluconeogenesis.

Metformin is also associated with gastrointestinal (GI) adverse effects, leading to treatment discontinuation [7]. If metformin fails to adequately lower blood glucose, other hypoglycemic agents, such as TZDs, e.g. Rosiglitazone, could be used as an adjunct [6]. Because of the full agonism of nuclear peroxisome proliferator-activated receptor gamma (PPAR-γ), TZDs decrease gluconeogenesis in the liver, increase peripheral uptake of glucose, and improve insulin sensitivity in cells [8]. These agents elevate adiponectin levels, a cytokine secreted by adipose tissue that improves insulin sensitivity. Moreover, upon insulin release from the pancreas, TZDs enhance the expression of glucose transporter type 4 (GLUT-4), resulting in increased glucose uptake in adipocyte and skeletal muscle cells. Furthermore, TZDs increase insulin receptor substrates 1 and 2 (IRS-1 & IRS-2) [9]. These compounds are associated with multiple side effects, including weight gain and hepatotoxicity, which limit their continued use [10].

The PPARs, including α, β, δ and γ, are known as the key modulators of lipid and glucose metabolism. The a species of PPAR agonists regulate gene expression for fatty acid β-oxidation, therefore, they are useful in the management of dyslipidemia. The PPAR-β or -δ ligands improve lipid profile, insulin sensitivity, and minimize weight gain. The PPAR-γ agonists are known to impact adipogenesis, glucose homeostasis, and insulin sensitivity [11].

More than a hundred plant-derived compounds have been identified as PPAR-γ agonists with potential antidiabetic effects [12]. An *in vivo* study has shown that several natural PPAR-γ activating agents (e.g. amorphastilbol, amorfrutin-1, amorfrutin-B & honokiol) with fewer side effects than thiazolidinediones, and refined metabolic indices in diabetic animals [12]. *Psoralea corylifolia Leguminosae* from the Fabaceae family of plants, is a herb utilized in traditional Chinese medicine to treat metabolic disorders, such as diabetes and hyperlipidemia [13]. Bavachinin is a PPAR pan-agonist that is extracted from the seeds of this plant. In addition to being a partial agonist, PPAR-γ activates PPAR-α, -β and –δ, inhibits weight gain, and hepatotoxicity associated with TZDs [14]. Considering the different hypoglycemic mechanisms of metformin and bavachinin, this study aimed to assess the possible synergistic and glucose-lowering effects of combined meftormin and bavachinin on the treatment of Wistar rats with diabetes type II.

Materials and Methods

Animals: Male Wistar rats, weighing 200-250 g (n=35) were purchased from the vivarium of Qazvin University of Medical Sciences. The animals were kept in separate cages under 12 hours of light and dark cycles, at a temperature of 22-24˚C. The animals had free access to food and water. All of the experimental procedures were consistent with the guidelines of the Ethics Committee of the Qazvin University of Medical Sciences.

Materials: Metformin (PHR1084), bavachinin (SMB00100), and ketamine hydrochloride or xylazine hydrochloride (K113) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carboxymethyl cellulose 0.5% was used as a metformin vehicle for oral administration. Potassium citrate buffer (1%) was used as the bavachinin solvent for intraperitoneal injection. To induce type II diabetes in rats, streptozotocin (S0130) was obtained and used based on the established method [15] (Sigma-Aldrich Inc, USA).

Type II diabetes induction: To induce type II diabetes, the rats were given a high glucose and fat regimen for four weeks (3.5% cholesterol, 10% egg yolk powder, 20% saccharose and 66.5% regular diet). After this period, streptozotocin (50 mg/kg) and nicotinamide (120 mg/kg) were injected into the rats, intraperitoneally. Ninety-six hours after the injection, a random blood glucose measurement was performed in rats. The tail vein was used to collect the blood samples, and the blood glucose was measured, using an electronic glucometer. A blood glucose level of more than 300 mg/kg in a rat was considered as being diabetic [16, 17].

Experimental groups: Thirty-five mature Wistar rats were distributed randomly into five groups of seven rats each. The normal control group included healthy rats, but the remaining groups included diabetic rats. They were treated as outlined below for two weeks:

Normal control group (N): Healthy rats without receiving any drugs.

Diabetic control group (D): Diabetic rats without receiving any drugs.

Metformin group (DM): Diabetic rats receiving metformin (320 mg/kg/day) orally, as described in a previous study [18].

Bavachinin group (DB): Diabetic rats receiving bavachinin (5 mg/kg/day) intraperitoneally.

Metformin+bavachinin group (DMB): Diabetic rats receiving an intraperitoneal injection of bavachinin at 5 mg/kg plus oral metformin at 320 mg/kg/day as described earlier [17].

Oral glucose tolerance test (OGTT): On day one, i.e.immediately after the administration of the first drug dose, and on day 14, the blood glucose levels were measured in both the healthy and diabetic rats after eight hours of fasting. Subsequently, they received an oral dose of 1 g/kg glucose through a gavage needle. After glucose loading, the blood glucose levels were determined at zero, 30, 60, and 120 minutes afterward [19], using the kit for glucose oxidase assessment [20] (Pars Azmun Co., Tehran, Iran).

Evaluations of β-cell Function and Insulin Resistance: On day 14, blood samples were collected from the rats' tail vein, after an 8-hr of fasting. The fasting blood glucose (FBG) and fasting insulin (FINS) levels were measured, using a locally available kit (Pars Azmun Co., Tehran, Iran), and Mercodia rat insulin ELISA kit (Sweden), respectively. The FBG and FINS were used to calculate the homeostasis assessment of beta cell function (HOMA-β) and insulin resistance (HOMA-IR). These measures provided both the insulin secretion and resistance, based on the following Equations (Equations 1-3):

1. $HOMA\text{-}\beta=20\times FINS/(FBG-3.5)$

2. $HOMA\text{-}IR=FBG\ (mmol/l)\times FINS\ (mU/mL)/22.5$

3. $ISI=Ln\ [(FBG\times FINS)-1]$ [21].

Statistical analyses: The statistical analyses were carried out, using Graphpad Prism software, version 8. The results were expressed as Mean±SEM per rat group (n=7). To compare the data in various groups, Tukey's test was performed followed by one-way analysis of variance (ANOVA). The statistical significance between the pairs of data sets was set at $P<0.05$.

Results

OGTT on day 1: The results showed that on day one, the blood glucose levels of D, DM, DB, and DMB groups at 0, 30, 60, and 120 minutes post feeding were considerably greater than that in the normal control group ($P<0.001$, Figure 1). The findings indicated that type II diabetes had been induced in rats. No significant differences were observed after comparing the blood glucose levels in DM, DB, DMB, and D groups ($P>0.05$, Figure 1).

OGTT on day 14: Except for the DMB group ($P>0.05$), significant differences were found among the D, DM and DB groups versus the N rats at zero minute ($P<0.0001$, $P<0.05$ & $P<0.05$, respectively, [Figure 2]). The glucose levels in all of the intervention groups were dramatically lower than that of D group. The difference was more pronounced in DMB group than in DM and DB groups ($P<0.001$, $P<0.01$, & $P<0.05$, respectively, [Figure 2]). There were no significant differences among the treatment groups.

At 30 minutes after treatment, there were noticeable differences among the diabetic groups compared to the normal group, however, the difference was less significant between the DMB and N groups ($P<0.01$). The differences among the treatment groups were not significant, although, the difference between DMB and D groups was greater ($P<0.01$) than those of DM and DB groups ($P<0.05$).

At 60 minutes post treatment, there were no significant differences among DB, DMB, and N groups. The differences among the DM, DB, DMB and diabetic control groups were statistically significant at $P<0.05$, $P<0.01$ and $P<0.01$, respectively. No significant differences were noted among the treatment groups.

At 120 minutes post treatment, no significant differences were observed among the treatment groups versus the normal controls. Also, significant differences were found among all treatment groups versus the diabetic controls. However, this was more pronounced between the DMB and D groups ($P<0.01$). Lastly, no statistically significant differences were observed among the treatment groups.

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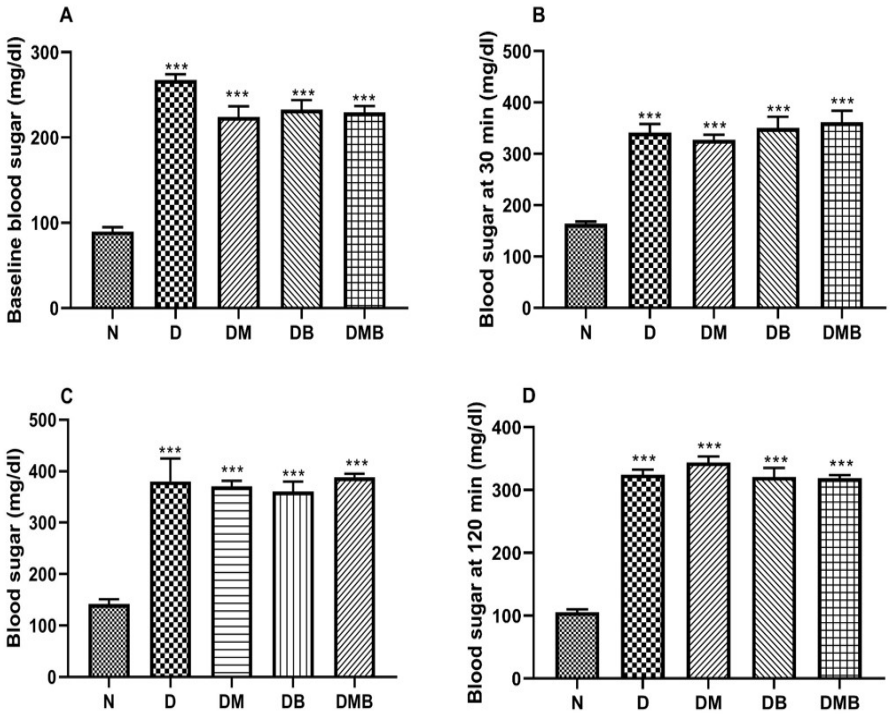

Figure 1. OGTT results in rats on day 1

Data are shown as Mean±SEM (n=7). Blood glucose levels are reported as mg/dL at 0 (A), 30 (B), 60 (C) and 120 (D) minutes after glucose administration.

Abbreviations: OGTT: Oral glucose tolerance test; N: Normal control group; D: Diabetic control group; DM: Diabetic group receiving metformin (320 mg/kg/day, PO); DB: Diabetic group receiving bavachinin (5 mg/kg/day, IP); DMB: Diabetic group receiving metformin (320 mg/kg/day, PO) and bavachinin (5 mg/kg/day, IP); Min: Minute. ***P<0.001, compared to N.

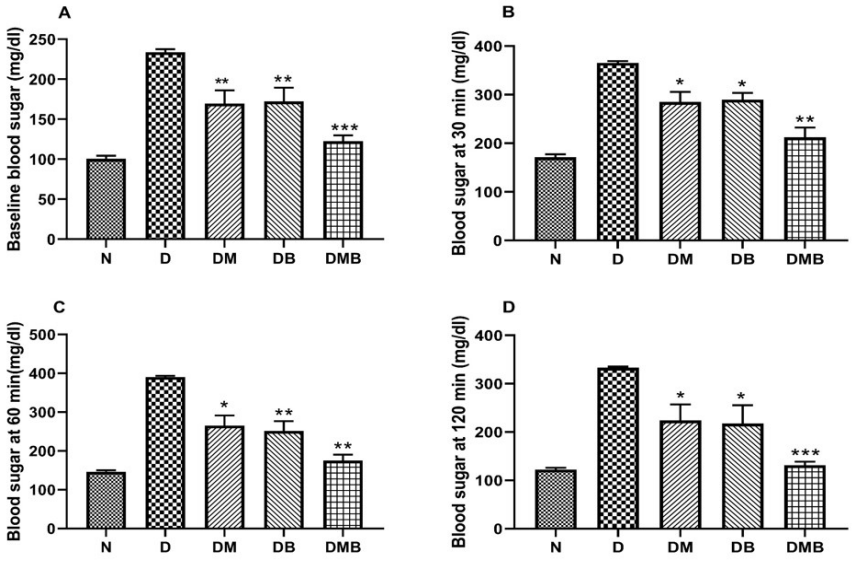

Figure 2. OGTT results in rats on day 14

Data are depicted as Mean±SEM (n=7). Blood glucose levels are reported as mg/dL at 0 (A), 30 (B), 60 (C) and 120 (D) minutes after glucose administration.

Abbreviations: OGTT: Oral glucose tolerance test; N: Normal control group; D: Diabetic control group; DM: Diabetic group receiving metformin (320 mg/kg/day, PO); DB: Diabetic group receiving bavachinin (5 mg/kg/day, IP); DMB: Diabetic group receiving metformin (320 mg/kg/day, PO) and bavachinin (5 mg/kg/day, IP); Min: Minute. *P<0.5, **P<0.01, ***P<0.001 compared to D.

Table 1. FBG, FINS, ISI, HOMA-β and HOMA-IR on day 14

Group	Mean±SEM				
	FBG	FINS	ISI	HOMA-IR	HOMA-β
N	5.306±0.45	17.72±0.31	-4.51±0.07	4.157±0.3	203.8±22.93
D	18.28±0.67****	30.64±0.87****	-6.31±0.03****	24.81±0.75****	42.16±2.78****
DM	12.25±0.79$^{****, ####}$	26.33±0.87$^{****, ##}$	-5.75±0.07$^{****, ####}$	14.32±0.93$^{****, ####}$	63.22±5.96^{ns,***}
DB	14.29±0.67$^{****, ##}$	25.62±0.42$^{****, ###}$	-5.89±0.05$^{****, ##}$	16.3±0.91$^{****, ####}$	48.58±3.07 ^{ns,****}
DMB	8.78±1.13$^{*, ####}$	21.22±1.03$^{*, ####}$	-5.16±0.01$^{****, ####}$	7.22±0.87$^{ns, ####}$	124.1±38.44 $^{ns, ns}$

Results are expressed as Mean±SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to the normal control group. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, and $^{\#\#\#\#}$P<0.0001 compared to the diabetic control group.
Ns: Not statistically significant.

The statistical significance illustrated by * is compared to the normal control group, and the statistical significance illustrated by # is compared to the diabetic control group (the diabetic rats without receiving any treatment).

FBG levels on day 14: The FBG levels in all diabetic rats were significantly greater than that in the N group, however, the difference was less significant between the DMB and N groups (P<0.05, Table 1).

FINS levels on day 14: The insulin levels in all diabetic rats were significantly higher than that in the N group (P<0.0001). However, the difference was less pronounced, comparing DMB and N groups (P<0.05). A comparison of the diabetic groups showed significant differences among the insulin levels in DM (P<0.01), DB (P<0.001), DMB (P<0.0001) and D groups. The difference was more pronounced between the DMB and D groups. Also, the insulin level in the DMB group was much lower than those found in DM (P<0.001), and DB groups (P<0.01), (Table 1).

HOMA-IR, HOMA-β, and ISI: The HOMA-IR, HOMA-β, and ISI were determined, using the FBG and FINS measures (Table 1). The HOMA-IR was considerably higher in the diabetic than in normal rats (P<0.0001). The exception was for DMB, where no statistically significant difference was evident. Metformin, bavachinin, and metformin combined with bavachinin significantly reduced the HOMA-IR measure compared to that seen in the diabetic control group (P<0.0001). There was no significant difference between DM, and DB groups, but a significant difference was observed between those and DMB groups (P<0.0001), (Table 1). The HOMA-β measure was significantly different among the D, DM, DB, and N groups. But, no obvious difference was detected between the DMB and N groups.

There was a clear difference in the ISI measures among all diabetic groups versus the N group (P<0.0001), and the DM, DB, DMB and D groups. No significant difference was found between the DM and DB groups, however, the insulin sensitivity improved significantly in the DMB group compared to the DM and DB groups (P<0.0001) (Table 1).

Discussion

Principal findings: This study aimed to assess the role of treatment with combined bavachinin and metformin and the blood glucose and insulin sensitivity in type II diabetic rats. Before treatment, as expected in diabetes, the OGTT results showed that the glucose levels in diabetic rats were dramatically higher than those in normal animals. The combined treatment with bavachinin and metformin was associated with lower blood glucose levels, and better glucose tolerance in diabetic rats than using either bavachinin or metformin alone. Our study is one of the few that evaluated the bavachinin effects on fasting blood glucose level, glucose tolerance, fasting insulin level, and insulin sensitivity in type II diabetic rats.

The potency of combination therapy: Following the combined treatment, the blood glucose levels of the diabetic rats were almost comparable to that of the normal control group. This finding reflects the synergistic, glucose-lowering effect of using bavachinin and metformin concurrently. No significant differences were observed in the HOMA-IR and HOMA-β measures between the DMB and N groups. Also the differences in the FBG and FBI levels were lower than those found in other groups.

Despite the significant ISI difference between the DMB and N groups, the combined therapy with metformin plus bavachinin significantly improved the insulin sensitivity index in the diabetic rats, compared to administering either agent alone. These findings further emphasize the possible synergistic effects of bavachinin and metformin in the management of type II diabetes. Comparing the FBG and OGTT results, it appeared that the concurrent use of bavachinin and metformin lowers the postprandial glucose levels more effectively than that of the normal fasting blood glucose.

A new PPAR agonist: Type II diabetes accounts for about 90% of all diabetic cases, which is manifested by hyperglycemia and insulin resistance [3, 4]. Metformin is the first-line and most commonly used oral hypoglycemic agent prescribed for patients with type II diabetes [22]. Various hypoglycemic agents may be added to metformin in the management of type II diabetes, including PPAR-γ agonists [23]. These agonists, e.g. thiazolidinediones (TZDs) have known effects on type II diabetes, including modulating gene expression, binding to PPAR-γ nuclear receptors, reducing insulin resistance, and lowering the blood glucose level in type II diabetes [24]. Bavachinin is a natural flavanone with PPAR pan-agonist properties [13, 25].

Synergism of bavachinin and TZDs: Feng, et al. in their study on leptin-deficient and diet-induced obese mice, showed that treatment with bavachinin significantly lowered the fasting and non-fasting blood glucose levels. It also ameliorated glucose tolerance, hyperinsulinemia, and insulin sensitivity without inducing weight gain or hepatotoxicity. Moreover, these authors demonstrated that bavachinin synergizes with rosiglitazone (a TZD drug), and alleviates the associated hepatotoxicity and weight gain. The *in vitro* phase of that study demonstrated that by up-regulating the gene expression of glucose transporter-4 (a PPAR-γ marker gene), bavachinin improves glucose transport and the metabolism in skeletal muscles and adipose tissue. Furthermore, their findings showed that bavachinin enhances the expression of other PPAR target genes involved in insulin sensitivity [14].

Mechanisms of action: In a more recent study, Feng, et al. used nuclear magnetic resonance, biochemical assays, and molecular dynamic analyses to reveal the specific mechanisms interaction for the synergistic effects of bavachinin and rosiglitazone on each other. They concluded that a hotspot between Met329 and Ser332 in helix 5 enables bavachinin to allosterically stabilize Rosiglitazone-bound PPAR-γ [8].

In another study at cellular level, Lee, et al. have shown that bavachin, a compound similar to bavachinin with known effects on PPAR-γ, increases the PPAR-γ gene expression dose-dependently. They concluded that the bavachin derived from *Psoralea corylifolia*, increased the glucose uptake via translocation of GLUT-4, and through activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) and AMPK pathways in the presence or absence of insulin [25].

Bavachinin, a promising hypoglycemic agent: The results of our study indicate that bavachinin is a promising agent for the treatment of diabetes mellitus type II. In agreement with earlier studies, our findings suggest that bavachinin lowers blood glucose, improves glucose tolerance, hyperinsulinemia, and insulin sensitivity in type II diabetic rats. The reduction in liver glucose synthesis is the main effect of biguanides, such as metformin, while PPAR-γ agonists, such as TZDs, improve both the glucose uptake in peripheral tissues and insulin sensitivity [26, 27]. Therefore, considering the activities of PPAR-γ agonist in bavachinin, the concomitant administration of metformin and bavachinin might increase the insulin sensitivity via effects on various organs. For instance, metformin affects the liver while bavachinin interacts with adipose tissue and skeletal muscles. Therefore, using it in the treatment of diabetic subjects is likely to allow a lower dose of metformin with better tolerance [28]. Finally, our results suggest that the combination therapy with bavachinin and metformin improves blood sugar level and insulin resistance in type II diabetic rats more effectively than using either agent alone.

Limitations of the study: This study had a few limitations: a) The optimal single or combined drug dosage was not determined. b) The animals were not weighed repeatedly throughout the study, which could have influenced the findings, since body mass index has its effect on insulin resistance. c) Because of a two-week follow-up period, the possible long-term side effects of the treatments were not fully investigated.

Conclusions

Based on the study findings in the Wistar rats model for type II diabetes, we conclude that bavachinin demonstrates synergism with metformin and could be a proper candidate for combination therapy to improve blood glucose level and its tolerance, insulin level and the sensitivity in patients with type II diabetes at lower doses of either agent alone, thus having less side-effects.

Ethical Considerations

Compliance with ethical guidelines

This study fully complied with the established ethical guidelines: The study protocol was approved by the Ethics Committee of the Qazvin University of Medical Sciences (Code: IR.QUMS.REC.1398.124).

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Authors' contributions

Conceptualization and methodology: Yazdan Naderi and Sara Khosraviani; Data analysis and experiments: Sara Khosraviani, Ali Emami, Yazdan Naderi and Samaneh Keshavarz Hedayati; Writing original draft: Sara Khosraviani, Yazdan Naderi, Ehsan Aali. Writing, review and editing: Sara Khosraviani, Ehsan Aali, Sanaz Keshavarz Shahbaz; Supervision and visualization: Yazdan Naderi.

Conflict of interest

The authors declared no conflict of interest.

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