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Effect of *Hypericum perforatum* Aqueous Extracts on Serum Lipids, Aminotransferases, and Lipid Peroxidation in Hyperlipidemic Rats

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Abstract

Background: Patients with high levels of total cholesterol (TCH), low-density lipoprotein cholesterol (LDL-CH), and triglyceride (TG) are at increased risk of coronary heart disease. Studies have shown that flavonoids and antioxidant compounds have beneficial effects on hyperlipidemia.

Objectives: The aim of the present study was to evaluate the effects of extract of *Hypericum perforatum* (EHP) on the serum lipid profile (TCH, TG, and LDL-CH), aminotransferase, alkaline phosphatase, and lipid peroxidation in hyperlipidemic rats.

Materials and Methods: Thirty-two male rats weighting 200 ± 10 g were randomly divided into four experimental groups: 1) control, 2) control + EHP, 3) hyperlipidemia, and 4) hyperlipidemia + EHP. The rats in the hyperlipidemic groups were fed a high-fat diet for 60 days, and EHP (300 mg/kg) was injected intraperitoneally for 2 weeks in the rats in the second and fourth groups. At the end of the experimental period, blood samples from each group were analyzed.

Results: There was a significant reduction in LDL-CH in the control + EHP group and the hyperlipidemia + EHP group ($P < 0.05$). TCH was significantly reduced in the control + EHP group ($P < 0.05$). There were no significant changes in the levels of TG and HDL-CH. Malondialdehyde, aspartate aminotransferase, and alanine aminotransferase were significantly reduced in the hyperlipidemia + EHP group ($P < 0.05$), with no significant change in alkaline phosphatase.

Conclusions: EHP was able to both reduce LDL-CH and to significantly decrease markers of oxidative stress and lipid peroxidation induced by hyperlipidemia. Therefore, this herb, as a new pharmacological component, could be used to reduce certain blood lipids, lipid peroxidation, and aminotransferase markers.

Keywords: Hypericum, Transaminases, Alkaline Phosphatase, Hyperlipidemia, Lipid Peroxidation

1. Background

A growing body of evidence suggests that patients with high levels of total cholesterol (TCH), high levels of low-density lipoprotein cholesterol (LDL-CH), and low levels of high-density lipoprotein cholesterol (HDL-CH) are at a greater risk of atherosclerosis and coronary artery disease (1-3). Oxidative stress plays an important role in the etiology of these conditions. Hyperlipidemia has been found to induce oxidative stress, which causes cellular damage (4).

There has been a great deal of pharmaceutical research aimed at finding drug therapies to prevent the process of atherosclerosis formation. However, herbal and natural medicines are becoming increasingly popular due to fewer side effects and easier availability (5). *Hypericum perforatum* is one of the most widely used herbs in the world, and has been used traditionally in the treatment of mild-to-moderate depression, migraines, gastrointestinal disorders, and wound-healing.

Given that atherosclerosis is a complex inflammatory disease involving vascular endothelial cells, lipid-engorged macrophages, and smooth muscle cells, it appears that antioxidants can be used for the reduction of atherosclerosis formation (1, 6).

2. Objectives

The present study sought to investigate the lipid-lowering and antioxidative properties of EHP in terms of the lipid profiles and lipid-peroxidation products of rats fed a cholesterol-rich diet.

3. Materials and Methods

3.1. Animals

Adult male Wistar rats weighing 220 ± 10 g (Razi Institute, Iran) were housed five per cage under a 12-hour light/dark cycle at 22 - 24°C, with food and water ad libitum. The methods were approved by the Ethics Committee of

Shahed University of Medical Sciences, and the laboratory animals were afforded due care in accordance with the regulations of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The experiments were done by blinded researchers.

3.2. Drugs

H. perforatum was procured from the Isfahan Natural Resource Institute in August 2015 and authenticated by Dr. Ghaem-Maghami at the Isfahan University Herbarium, and voucher specimen number 13648 was assigned. The aerial parts of the plant were dried for 1 week at room temperature under shade, then powdered with an electric grinder. The extract was prepared with 10 g of powder and 100 mL of 70% ethanol as the solvent. The extraction was done using the maceration method for 72 hour at room temperature in the dark. Afterward, the solution was filtered three times and dried on a rotary evaporator at 40°C, yielding 1.85 g (18.5%) of extract. The final extract was kept at -20°C, and further dilution was prepared in cold normal saline. EHP at a dose of 300 mg/kg was injected intraperitoneally into the hyperlipidemic and control groups daily for two weeks. This dose was chosen on the basis of previous reports (7, 8).

3.3. Induction of hyperlipidemia

Hyperlipidemia was induced by feeding the rats a high-fat diet containing 25% corn oil for a period of two months (9). The induced hyperlipidemia manifested itself after 8 weeks as a significant rise in the mean TG and TCH levels ($P < 0.05$) in the rats fed a high-fat diet compared with those given a control diet.

3.4. Measurement of Lipid Profiles and Biochemical Factors

The lipid profile (TCH, LDL-CH, HDL-CH, and TG) was measured twice on separate days, first at baseline and then at the end of the tenth week. Blood samples were collected 12 hours after overnight fasting. Enzyme assay kits (Shimi Zist Co., Iran) were used. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured by centrifuging the blood samples at 2500 × g for 15 minutes, and then the levels of the biochemical factors were measured using appropriate kits.

3.5. Lipid Peroxidation Assay

The production of malondialdehyde (MDA) is used as a biomarker to measure the level of lipid peroxidation. Briefly, to evaluate the lipid peroxidation products (MDA), 1.0 mL of 20% trichloroacetic acid and 1.0 mL of 1% thiobarbituric acid reactive substances (TBARS) were added to 100 μL of a supernatant, mixed, then incubated at 100°C for 80 minutes. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 minutes and the absorbance of the supernatant was read at 532 nm (10).

3.6. Experimental Groups

The rats were randomly divided into four experimental groups of eight rats each: 1) control, 2) control + EHP, 3) hyperlipidemia, and 4) hyperlipidemia + EHP. Blood samples were obtained from all of the animals at baseline for the measurement of lipid profile, markers of lipid peroxidation, aminotransferases, and ALP. These factors were checked twice more, at eight and ten weeks later.

3.7. Statistical Analysis

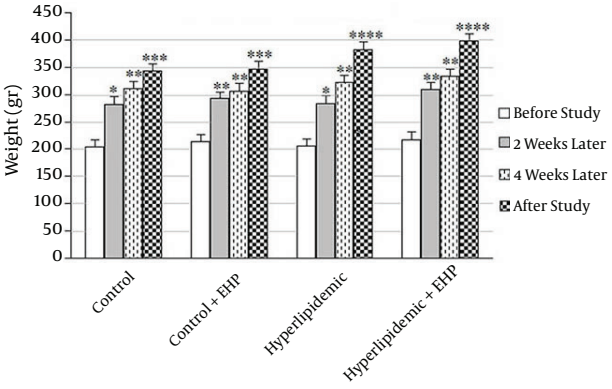
The data were expressed as mean ± standard deviation. The normality of the distribution of the variables was checked using the Kolmogorov-Smirnov test. For statistical analysis, the one-way analysis of variance (ANOVA) and post-hoc Tukey tests were used for intergroup differences. For this purpose, IBM SPSS Statistics 22 for Windows (IBM Inc., Armonk, NY, USA) was employed. A P value of < 0.05 was considered statistically significant.

4. Results

4.1. Animal weights

Figure 1 shows the weights of the animals in the different experimental groups. The findings demonstrated that weight increased significantly over time during the study. Between-group comparisons indicated that weights in the hyperlipidemia group and the hyperlipidemia + EHP group were higher than in the other groups, denoting the effect of the fat-rich diet in these two groups. However, the groups treated with the EHP had no statistically significant reduction in weight.

Figure 1. Comparison of Body Weights of Animals in the Experimental Groups



The data are expressed as mean ± standard error; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$ as compared with the baseline weight in each group.

4.2. Comparison of Lipid Profiles Before and After Treatment in All Groups

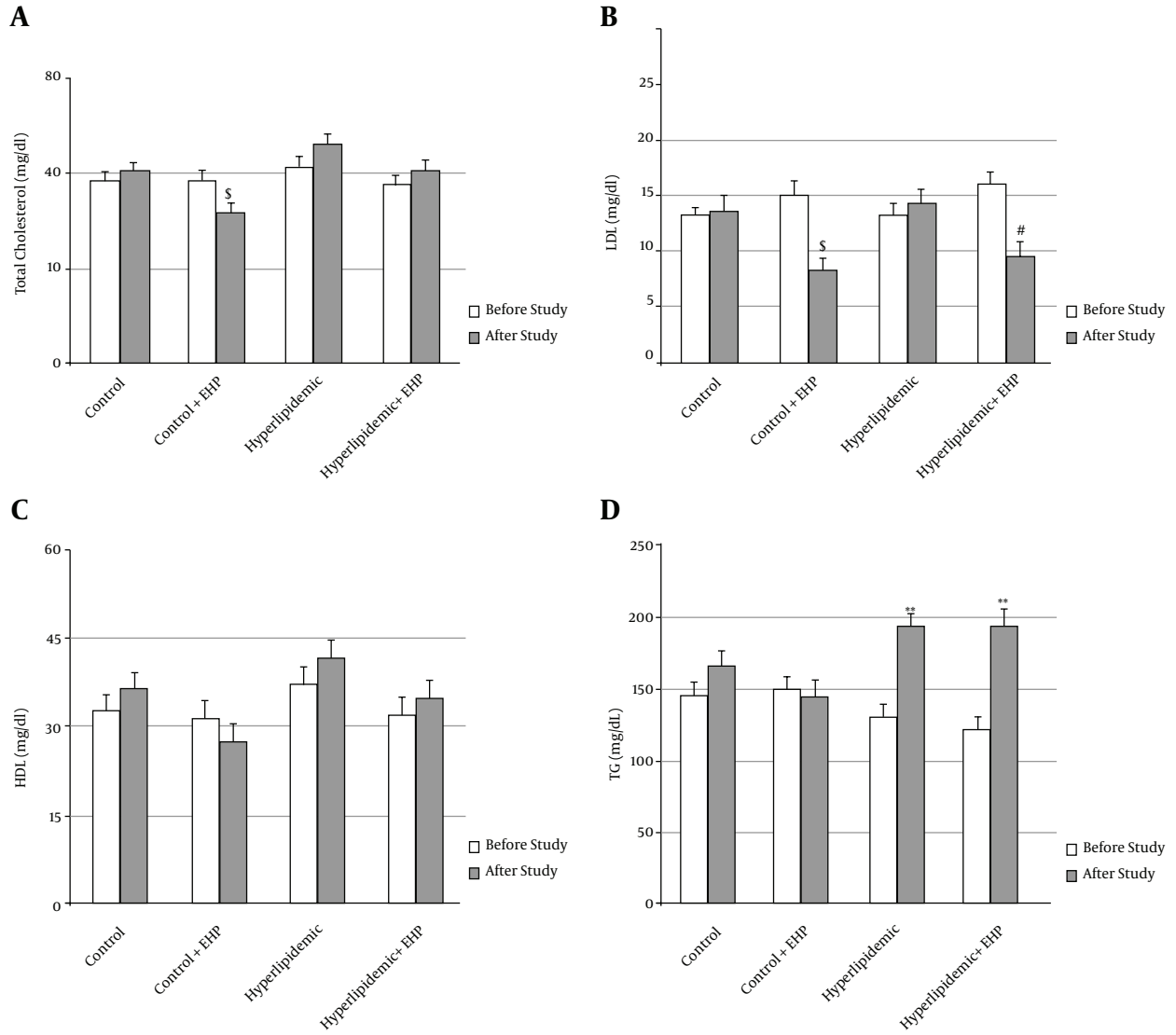
An ANOVA for TCH revealed no differences between the groups at baseline ($P > 0.05$). The TCH levels of the experimental groups at the end of the study are illustrated in Figure 2A. At the end of the study, the TCH level in the control + EHP group demonstrated a significant reduction ($P < 0.05$), while no significant reduction was seen in the hyperlipidemia + EHP group.

The data for LDL-CH are depicted in Figure 2B, which shows no statistically significant differences between the groups at baseline ($P > 0.05$). The ANOVA indicated a significant effect of EHP on LDL-CH in the control + EHP and hyperlipidemia + EHP groups ($P < 0.05$).

The HDL-CH levels in all of the experimental groups are illustrated in Figure 2C. The ANOVA indicated no significant differences between the groups regarding HDL-CH at baseline or at the end of the study ($P > 0.05$). This finding shows that EHP had no significant effect on HDL-CH levels.

The data for TG levels in all of the groups (Figure 2D) revealed that no significant differences existed at baseline ($P > 0.05$), whereas at the end of the study, the TG levels exhibited a significant increase in the hyperlipidemia + EHP group and the untreated hyperlipidemia group ($P < 0.01$). This finding indicates that the fat-rich diet was able to increase the animals' TG levels. On the other hand, in the hyperlipidemic rats treated with EHP, no significant reductions in TG levels were seen ($P > 0.05$). It appears that EHP had no significant effect on TG.

Figure 2. Effects of EHP on Serum Lipids



Data are expressed as mean ± standard error; A, Total cholesterol; B, LDL-CH; C, HDL-CH; D, TG; ** $P < 0.01$ (versus baseline values in the same group); $ $P < 0.05$ (versus control); # $P < 0.05$ (versus hyperlipidemia).

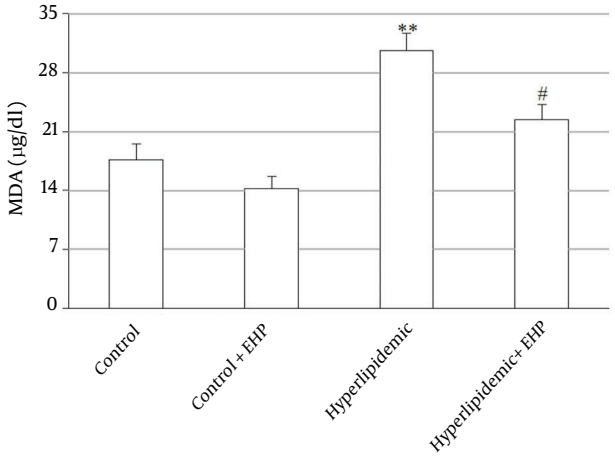
4.3. Effects of EHP on MDA, AST, ALT, and ALP Induced by Hyperlipidemia

The ANOVA test conducted on the MDA level showed a significant increase in the hyperlipidemia group ($P < 0.01$) and the hyperlipidemia + EHP group ($P < 0.05$) in comparison with the control groups. Meanwhile, MDA levels in the hyperlipidemia + EHP rats were statistically lower than in the untreated hyperlipidemic rats ($P < 0.05$). Therefore, aqueous EHP was able to reduce MDA levels in the hyperlipidemic animals (Figure 3).

Figure 4A shows the AST activity in all groups. ANOVA revealed that AST levels in the hyperlipidemic group were significantly increased in comparison with the control groups ($P < 0.05$). In the hyperlipidemia + EHP group, the activity of this enzyme was reduced when compared to the untreated hyperlipidemia group. This reduction, albeit not statistically significant, was considerable. These results suggest that aqueous EHP reduced the activity of this enzyme, which is a marker of oxidative stress injury caused by increased blood lipids.

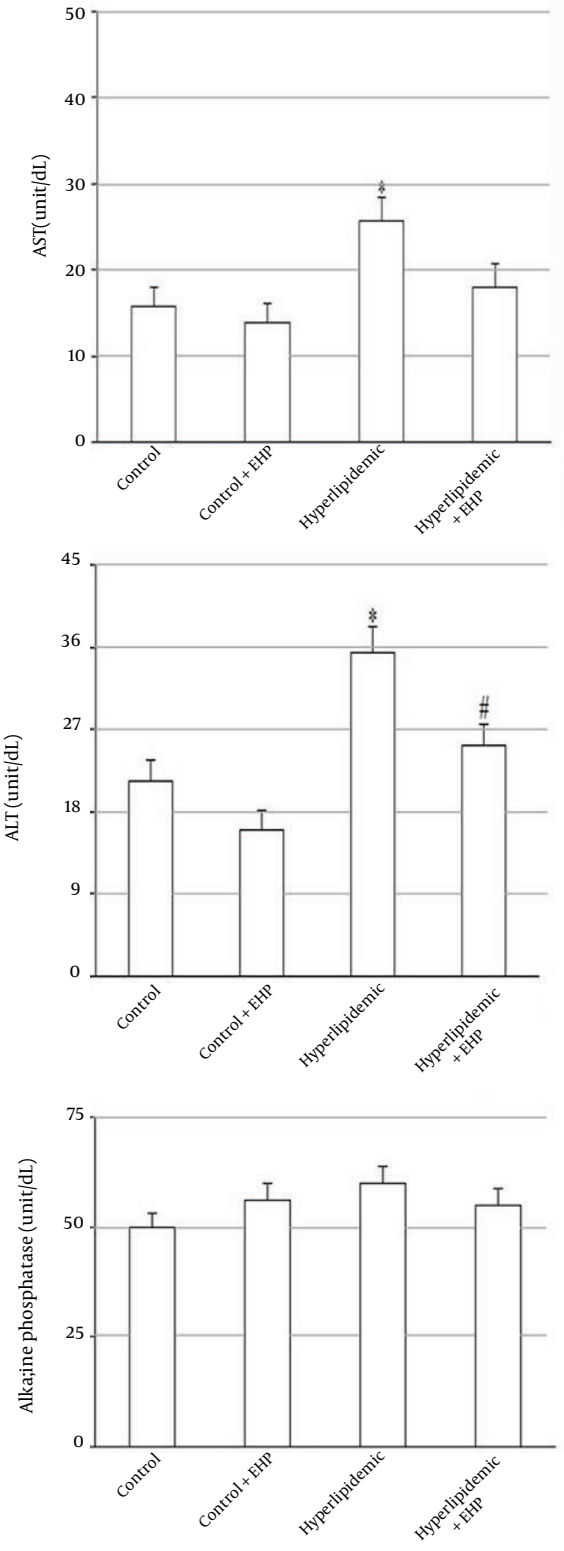
The ALT activity in each group is depicted in Figure 4B. ANOVA showed that ALT levels in the untreated hyperlipidemia group were significantly increased compared to the control groups ($P < 0.05$). Meanwhile, ALT was statistically lower in the hyperlipidemia + EHP group than in the untreated hyperlipidemic rats ($P < 0.05$). These findings suggest that aqueous EHP was able to effectively reduce ALT activity in the hyperlipidemic rats. Figure 4C shows that ALP activity exhibited no significant change in any of the four groups ($P > 0.05$).

Figure 3. Comparison of the MDA Concentrations Between the Groups



** $P < 0.01$ as compared with the control group; # $P < 0.05$ (versus hyperlipidemic)

Figure 4. Effect of EHP on Aminotransferases and ALP



Data are expressed as mean ± S.D.M.; A, AST; B, ALT; C, ALP; In A, * $P < 0.0001$ as compared with the control group; In B, *, # $P < 0.0001$ as compared with the control group.

5. Discussion

In this study, serum lipids, aminotransferases, and antioxidant status were evaluated to determine the effects of aqueous EHP on hyperlipidemic rats. In the treated hyperlipidemia group, LDL-CH was significantly decreased. A similar result was seen in the treated control group, demonstrating that EHP was able to reduce LDL-CH in the hyperlipidemic and even the normal rats. These results agree with those of previous studies.

Zou et al. reported that LDL-CH was significantly reduced in hypercholesterolemic rats treated with a flavonoid-rich extract of *H. perforatum* (FEHP). These researchers speculated that the deterrent effect of FEHP on hydroxymethylglutaryl coenzyme A (HMG-coA), or the increasing effect of the FEHP on the excretion of bile and cholesterol, was responsible for the reduction in LDL-CH (3).

Aqueous EHP effectively decreased TCH in the treated controls. This finding is concordant with those of several previous studies. A similar result was observed in the treated hyperlipidemia group; the difference, however, was not significant.

Habibi et al. studied the therapeutic effects of total EHP and calcic and magnesic sulfate mineral water on the lipid profiles of hyperlipidemic rats, and concluded that EHP significantly reduced TCH, TG, and LDL (11). Also in the study by Zou et al., FEHP reduced TCH in hyperlipidemic rats (3). In the present study, although the decrease in TCH in the treated hyperlipidemic group was not significant, it was in line with changes reported in previous studies.

Our results revealed no significant changes in HDL-CH levels in the rats treated with EHP. In contrast with Zou et al., Asgary et al. reported that EHP was able to increase HDL-CH levels (1, 3). In addition, it has been previously shown that the flavonoid components of *H. perforatum* can increase HDL-CH levels by augmenting the synthesis of apolipoprotein A-I (12). The difference in the results may be due to dissimilarities in the type of extract administered and the model of the study. Asgari et al. used a hydroalcoholic EHP administered to New Zealand rabbits, whereas Asgary et al. and Zou et al. utilized a flavonoid EHP (1, 3).

In the current study, the amount of TG in the untreated and treated hyperlipidemic groups increased significantly, indicating the mounting effects of a high-fat diet on TG. Nonetheless, there was no significant change in the TG levels of the EHP-treated hyperlipidemic group compared to the untreated hyperlipidemic group.

Khushbactova et al. reported that the proanthocyanidin content of *H. perforatum* was able to reduce TG levels (13). They speculated that this therapeutic effect occurred through the augmentation of vascular wall resistance, which prevents cholesterol penetration into the atherogenic lipoprotein composition. The discrepancy in the results may be due to differences in the active components of the different extracts of *H. perforatum*. Khushbactova et al. used polymeric proanthocyanidins derived from *H. perforatum* in their study, while we administered aqueous EHP (13).

Among the four groups in the current study, MDA concentrations were highest in the untreated hyperlipidemia group, but were reduced in the hyperlipidemic rats receiving EHP. Our results showed that *H. perforatum* was able to significantly reduce MDA due to its antioxidant properties, which is in agreement with the majority of previous studies.

In our study, ALT, AST, and ALP concentrations increased in the groups fed a high-fat diet. The elevation in the activity of these enzymes in hyperlipidemic rats represents an increase in oxidative factors due to the high blood-lipid concentration. At the end of the study, our findings showed that the ALT and AST activity in the treated hyperlipidemic group had reduced in comparison to the untreated hyperlipidemic group.

The findings of the present study are in concordance with those of several previous studies. For instance, Arhan et al. showed that increased blood lipids reduced antioxidant defenses and increased lipid peroxidation in the liver (14). In addition, oxidative stress eliminates the balance between peroxidants and antioxidants in biological systems, followed by a rise in lipid peroxidation and free-radical production.

Bayramoglu et al. investigated the hepatoprotective effects of *H. perforatum* on hepatic ischemia injury in rats, and showed that the injection of hydroalcoholic EHP significantly reduced AST, ALT, MDA, and LDH while increasing the levels of catalase and glutathione peroxidase (15), confirming our results that showed reduced aminotransferases. In the present study, we did not measure antioxidant defense systems such as glutathione, and we suggest that future studies take such systems into consideration.

5.1. Conclusions

In the present study, aqueous EHP reduced the serum lipid profile of rats in terms of LDL-CH. In addition, EHP effectively reduced the enzymes and markers of oxidative stress caused by hyperlipidemia.

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Footnotes

Authors' Contribution: Study concept and design: Mohammad Hassan Ghosian Moghaddam, Maryam Maleki; acquisition of data: Maryam Maleki; analysis and interpretation of data: Mehrdad Roghani, Maryam Maleki; drafting of the manuscript: Maryam Maleki; critical revision of the manuscript for important intellectual content: Maryam Maleki; statistical analysis: Mehrdad Roghani, Maryam Maleki.

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