



The Effect of Standardized *Eryngium billardieri* Hydroalcoholic Extract on Glycated Hemoglobin and Hepatic Enzymes in Patients with Type 2 Diabetes: A Randomized Double-Blind Placebo-Controlled Trial

Fahimeh Khoushabi ¹, Motahareh Bazzi Allari ², Saideh Jahantigh ³, Alireza Bazziallahri ^{4,*}

¹ Department of Nutrition, School of Health, Zabol University of Medical Sciences, Zabol, Iran

² Student Research Committee, Zahedan University of Medical Sciences, Zahedan, Iran

³ Biotechnology and Plant Breeding, Faculty of Agriculture Unervercity of Zabol, Zabol, Iran

⁴ Nutrition, Health and Treatment Network, Zabol University of Medical Sciences, Zabol, Iran

*Corresponding Author: Department of Nutrition, Health and Treatment Network, Zabol University of Medical Sciences, Zabol, Iran. Email: alirezabazzii992@gmail.com

Received: 19 May, 2026; Revised: 1 June, 2026; Accepted: 17 June, 2026

Abstract

Background: As the global prevalence of type 2 diabetes mellitus (T2DM) increases and the limitations of conventional pharmacological treatments persist, scientific interest in plant-based adjuvants with multitarget therapeutic effects has grown.

Objectives: This study evaluated the efficacy of a standardized hydroalcoholic extract of *Eryngium billardieri* as an adjunctive intervention to improve glycemic control and hepatic enzyme parameters in patients with T2DM receiving stable metformin therapy.

Methods: This randomized, double-blind, placebo-controlled clinical trial enrolled 74 eligible participants aged 45 - 75 years with baseline HbA1c levels $\geq 7.5\%$ and no history of hepatic disorders. Participants were randomly assigned to 2 parallel groups. The intervention group received 25 drops of *E. billardieri* extract, providing 1.5 mg of polyphenols per 100 mL of aqueous solution, twice daily after lunch and dinner for 14 weeks. The control group received an identical placebo regimen. All participants continued their baseline metformin dose (500 mg twice daily). Primary outcomes, including serum HbA1c, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels, were assessed at baseline and at study completion. Data were analyzed using independent-samples t tests for between-group comparisons and paired-samples t tests for within-group changes, using SPSS version 26. No serious adverse events were reported during the trial.

Results: In the intervention group, HbA1c levels decreased significantly from $9.93 \pm 2.48\%$ to $8.61 \pm 2.35\%$ (mean difference, -1.32; 95% CI, -1.87 to -0.77; $P = 0.000$). In contrast, the control group showed no significant change in HbA1c levels, with values increasing marginally from $9.76 \pm 1.50\%$ to $10.30 \pm 2.05\%$ ($P = 0.066$).

Conclusions: Adjunctive administration of *E. billardieri* extract for 14 weeks significantly improved long-term glycemic regulation and liver enzyme profiles in patients with T2DM receiving metformin, with no safety concerns observed. These findings suggest that it may be a cost-effective complementary approach to diabetes management. Nevertheless, further large-scale, multicenter studies are required to confirm these outcomes and elucidate the underlying hepatoprotective mechanisms.

Keywords: Eryngium Billardieri, Diabetes Mellitus, Glycated Hemoglobin, Hepatic Enzymes

1. Background

Over recent decades, type 2 diabetes mellitus (T2DM) has emerged as a major global health challenge, affecting

more than 400 million individuals worldwide (1, 2). The World Health Organization estimates that the global population with diabetes, approximately 200 million in 2000, was projected to reach 380 million by 2025 (1, 2). In

Copyright © 2026, Journal of Advanced Immunopharmacology. This open-access article is available under the Creative Commons Attribution 4.0 (CC BY 4.0) International License (<https://creativecommons.org/licenses/by/4.0/>), which allows for the copying and redistribution of the material only for noncommercial purposes, provided that the original work is properly cited.

How to Cite: Khoushabi F, Bazzi Allari M, Jahantigh S, Bazziallahri A. The Effect of Standardized *Eryngium billardieri* Hydroalcoholic Extract on Glycated Hemoglobin and Hepatic Enzymes in Patients with Type 2 Diabetes: A Randomized Double-Blind Placebo-Controlled Trial. J Adv Immunopharmacol. 2026;6(2):e174245. doi: <https://doi.org/10.69107/jai-174245>

Iran, national forecasts indicate that the number of people with diabetes will exceed 6.4 million within the next decade (3).

Pathophysiologically, T2DM results from a complex interplay of metabolic defects, including reduced peripheral insulin sensitivity, progressive pancreatic β -cell dysfunction, and unregulated hepatic gluconeogenesis, culminating in sustained hyperglycemia (3). This chronic metabolic disturbance is strongly associated with microvascular and macrovascular complications, including retinopathy, nephropathy, neuropathy, and accelerated atherosclerosis (4, 5).

Glycated hemoglobin (HbA1c) remains the gold-standard marker of long-term glycemic control, with each 1% absolute reduction correlating with a 37% lower risk of microvascular events and a 21% reduction in diabetes-related mortality (4). The liver plays a central role in maintaining systemic glucose and energy homeostasis, and hepatic abnormalities are common in patients with diabetes. Elevated serum aminotransferase levels, including ALT and AST, are frequent biochemical findings in nonalcoholic fatty liver disease (NAFLD), which is more prevalent in patients with T2DM (6, 7). Routine liver function assessment is therefore recommended in diabetes care (6).

Despite available pharmacological options, including biguanides, sulfonylureas, and insulin, a substantial proportion of patients do not achieve optimal glycemic targets. Furthermore, tolerability issues, financial constraints, and disease progression often lead to discontinuation of conventional therapy, increasing interest in integrative approaches. Among complementary modalities, medicinal plants have gained considerable attention; more than 1200 botanical species have documented blood glucose-lowering activity (8, 9).

Eryngium billardieri, locally known as borage or eryngo, is a medicinal herb widely distributed across Iran and the Mediterranean basin. Traditional medicine has long used this plant for inflammatory disorders, oxidative stress-related conditions, and hyperglycemia (10). Phytochemical profiling has revealed a diverse array of bioactive constituents, including polyphenols, flavonoids, saponins, and coumarins, which are believed to mediate its pharmacological effects (8, 10, 11). Preclinical studies have provided encouraging evidence of its hypoglycemic and hepatoprotective potential (12). However, translation of these findings to human clinical settings remains limited, particularly regarding its effects on HbA1c and hepatic enzyme profiles.

2. Objectives

This randomized controlled trial was designed to evaluate the efficacy of a standardized hydroalcoholic extract of *E. billardieri* on glycemic parameters and aminotransferase levels in patients with T2DM. We hypothesized that active treatment would produce a clinically meaningful reduction ($\geq 1\%$) in HbA1c and significantly decrease serum aminotransferase activities compared with placebo.

3. Methods

3.1. Study Design and Participants

Eighty-two individuals with T2DM were assessed for eligibility. Eight were excluded: 5 did not meet the inclusion criteria, and 3 declined participation. Consequently, 74 eligible participants were randomized to the intervention group ($n = 37$) or the control group ($n = 37$). All participants completed the 14-week protocol, with no attrition; the per-protocol analysis included the entire cohort.

Inclusion criteria were as follows:

- Age 45 - 75 years
 - Confirmed T2DM diagnosis for ≥ 6 months
 - Baseline HbA1c $\geq 7.5\%$
 - Fasting plasma glucose ≥ 126 mg/dL
 - Stable metformin regimen (500 mg twice daily) for ≥ 3 months
 - Written informed consent
- Exclusion criteria were as follows:
- History of hepatic disease (hepatitis, cirrhosis, or NAFLD)

- Prior major surgery or malignancy
- Pregnancy or lactation
- Use of insulin or non-metformin antidiabetic agents
- Alcohol consumption
- Participation in structured exercise programs
- Acute illness within the preceding month

3.2. Sample Size Calculation

The sample size was calculated based on the primary outcome, AST, using a two-sided significance level of $\alpha = 0.05$ ($Z_{1-\alpha/2} = 1.96$) and 80% power ($Z_{1-\beta} = 0.84$). The standard deviation (SD = 14.46) was derived from a pilot study of 10 patients with T2DM at our center. A clinically meaningful between-group difference of 12 U/L in AST reduction was assumed based on previous studies by Khani et al. (2). The minimum sample size was calculated as follows:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2}{\Delta^2} = \frac{\left((1.96 + 0.84)^2 \times 2 \times (14.46)^2\right)}{12^2} \approx 23 \text{ per group}$$

Accounting for 20% attrition, $23 / (1 - 0.20) = 28.75$, which was rounded to 29 participants per group. We ultimately enrolled 37 participants per group, exceeding the minimum requirement.

3.3. Randomization and Blinding

Eligible participants were randomized 1:1 using a computer-generated sequence (Random Allocation Software 2.0). Allocation was concealed using sequentially numbered, opaque, sealed envelopes prepared by an independent statistician. A research assistant blinded to group assignment enrolled participants and opened the envelopes. The hospital pharmacist dispensed the corresponding study bottles. All laboratory technicians and outcome assessors were blinded to group allocation. Data analysis was performed with group codes masked until the final analysis.

3.4. Preparation and Standardization of the Herbal Extract

The hydroalcoholic extract of *E. billardieri* was manufactured by Iran Darouk Pharmaceutical Company, Iran.

- Plant part: Aerial parts (stems, leaves, and flowers)
- Authentication: Voucher specimen authenticated by Dr. M. R. Joharchi, Ferdowsi University of Mashhad Herbarium (voucher FUMH-4587)
- Extraction solvent: 70% ethanol (v/v)
- Extraction procedure: Maceration at room temperature for 72 hours with intermittent shaking, followed by filtration, concentration under reduced pressure at 40°C, and lyophilization
- Plant-to-extract ratio: 8:1 (w/w)
- Dry extract content: 45 mg of dry extract per 1 mL of liquid formulation (approximately 25 drops)
- Polyphenol content: 1.5 mg of total polyphenols per mL (Folin-Ciocalteu method, epigallocatechin gallate equivalents)
- Daily dose: 2 mL (50 drops = 90 mg dry extract, containing 3.0 mg polyphenols)

A single production batch was used throughout the study, and the extract was stored at 4°C until administration.

3.5. Placebo Preparation and Maintenance of Blinding

The placebo consisted of distilled water with the following components:

- Caramel coloring (0.1% w/v) to match the amber-brown color
- Quinine hydrochloride (0.01% w/v) to mimic the bitter taste
- 70% ethanol to match viscosity and odor

Both the active extract and placebo were packaged in identical 30-mL amber glass dropper bottles with matching labels. The placebo was visually, olfactorily, and gustatorily indistinguishable from the active extract, as confirmed by a pilot test involving 10 healthy volunteers.

3.6. Intervention Protocol

Participants in the intervention group received 25 drops (approximately 1 mL) of *E. billardieri* extract in 125 mL of water, taken 30 minutes after lunch and dinner for 14 weeks. Participants in the control group received an identical placebo regimen.

All participants continued metformin 500 mg twice daily. Adherence was assessed through weekly telephone calls and counts of returned bottles at visits during weeks 4, 8, 12, and 14. Adherence $\geq 90\%$ was achieved in both groups; no participant had adherence below 80%.

3.7. Safety and Adverse Event Monitoring

Participants were instructed to report any adverse events immediately via a 24-hour hotline. Structured adverse event questionnaires were administered at each visit (screening, baseline, and weeks 4, 8, 12, and 14). Adverse events were defined according to ICH-GCP guidelines. Criteria for treatment discontinuation included: 1) severe hypoglycemia (glucose < 50 mg/dL with symptoms), 2) ALT/AST > 3 times the upper limit of normal, 3) pregnancy, 4) withdrawal of consent, and 5) serious adverse events. Rescue therapy with additional oral agents was offered for fasting glucose > 300 mg/dL on 2 consecutive visits or symptomatic hyperglycemia; no participant required rescue therapy.

3.8. Outcome Measures

- Primary outcome: Change in HbA1c from baseline to week 14
- Secondary outcomes: Changes in ALT, AST, and ALP
- Exploratory outcomes: Correlations between glycemic and hepatic changes

For multiplicity control of secondary outcomes,

Bonferroni correction was applied ($\alpha = 0.05/3 = 0.0167$).

3.9. Laboratory Measurements

Fasting blood samples (5 mL) were collected after 10 - 12 hours of fasting. Participants were instructed to skip their morning metformin dose but to take the evening dose as usual. Participants rested for 5 minutes before sample collection.

- HbA_{1c}: A 2-mL whole-blood sample was collected in EDTA tubes and analyzed by ion-exchange high-performance liquid chromatography (DiaLab kit, BT3000 autoanalyzer, Italy).

- Liver enzymes (ALT, AST, and ALP): A 3-mL blood sample was collected in plain tubes and centrifuged at 2500 rpm for 15 minutes at room temperature. The supernatant was analyzed using enzymatic assays (BT3000 autoanalyzer, Italy).

3.10. Statistical Analysis

All analyses were performed using SPSS version 26. Normality was assessed using the Kolmogorov-Smirnov test. Nonparametric alternatives (Wilcoxon and Mann-Whitney tests) were used for nonnormally distributed data.

For the primary analysis, analysis of covariance (ANCOVA) was performed with postintervention values as the dependent variable, group as the fixed factor, and baseline values as covariates. Adjusted mean differences, 95% CIs, and Cohen d effect sizes were calculated. For the secondary analysis, a mixed-effects model was used with group, time (preintervention vs postintervention), and the group $\times$ time interaction. Within-group analyses used paired t tests or Wilcoxon signed-rank tests. Between-group baseline comparisons used independent t tests or Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables. Pearson r or Spearman rho was used for correlation analyses.

A P value < 0.05 was considered statistically significant for the primary outcome; for secondary outcomes, the Bonferroni-adjusted significance threshold was $P < 0.0167$. Data are presented as mean $\pm$ SD unless otherwise specified.

3.11. Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Zabol University of Medical Sciences (IR.ZBMU.REC.1405.056). Written informed consent was obtained from all participants.

4. Results

4.1. Participant Flow and Baseline Characteristics

Of 82 individuals screened, 74 met the inclusion criteria and were randomized to the intervention group ($n = 37$) or the control group ($n = 37$). All participants completed the 14-week trial (0% attrition) and were included in the per-protocol analysis (Figure 1).

Baseline demographic and clinical characteristics were comparable between groups (Tables 1 and 2). Most participants were female (68.3% in the intervention group and 61.0% in the control group). Most participants were married, had secondary education, and were homemakers or unemployed. The mean diabetes duration was 4.00 ± 0.67 years in the intervention group and 4.44 ± 0.73 years in the control group ($P = 0.18$). All participants reported being physically inactive.

4.2. Effect of the Intervention on HbA_{1c}

In the intervention group, HbA_{1c} decreased from $9.93 \pm 2.48\%$ at baseline to $8.61 \pm 2.35\%$ after 14 weeks of treatment with *E. billardieri* extract (mean difference, -1.32% ; 95% CI, -1.87 to -0.77 ; $P = 0.000$) (Figure 2). In contrast, HbA_{1c} slightly increased in the control group, from $9.76 \pm 1.50\%$ at baseline to $10.30 \pm 2.05\%$ after the intervention ($P = 0.066$).

Furthermore, HbA_{1c} levels differed significantly between the intervention and control groups after the intervention. Specifically, the intervention group had a lower HbA_{1c} level than the control group ($8.61 \pm 2.35\%$ vs $10.30 \pm 2.05\%$; mean difference, -1.69% ; 95% CI, -2.44 to -0.94 ; $P = 0.000$) (Figure 2).

4.3. Effect of the Intervention on Liver Enzymes

All 3 liver enzymes decreased significantly in the intervention group compared with the control group. In the intervention group, the mean change in ALT was -2.20 U/L (95% CI, -3.91 to -0.49 ; $P = 0.041$) (Figure 3), whereas in the control group, it was 0.61 U/L ($P = 0.153$). The mean change in AST was -2.77 U/L (95% CI, -4.79 to -0.75 ; $P = 0.038$) in the intervention group and 0.46 U/L ($P = 0.784$) in the control group (Figure 4). Finally, the mean change in ALP was -5.55 U/L (95% CI, -8.43 to -2.67 ; $P = 0.002$) in the intervention group and -1.11 U/L ($P = 0.292$) in the control group (Figure 5).

Baseline demographic and clinical characteristics were comparable between groups (Table 1 and 2). The results of the independent-samples t test for between-group comparisons of postintervention HbA_{1c} and liver enzymes are provided in Table 3. The comparison was not adjusted for covariates because no baseline differences were found between the groups. Table 4 presents the adjusted between-group differences using ANCOVA. Pearson correlation

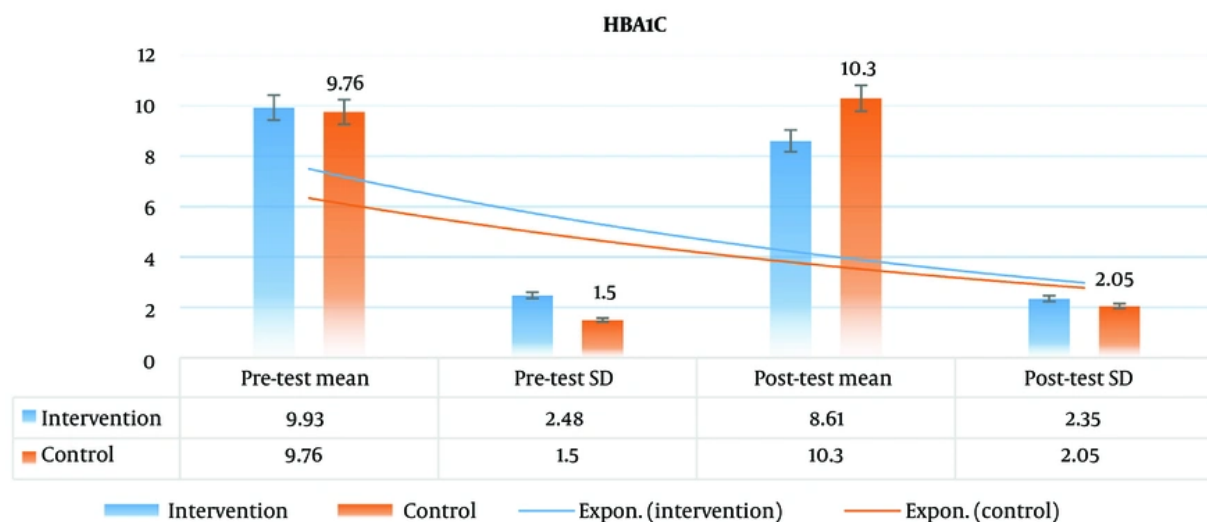


Figure 1. Participant flow through the randomized double-blind placebo-controlled trial

Table 1. Demographic Characteristics of Study Participants^a

Variables	Control Group (n = 37)	Intervention Group (n = 37)	P Value
Gender			0.47
Female	22 (59.5)	25 (67.6)	
Male	15 (40.5)	12 (32.4)	
Education			0.62
Below diploma	13 (35.1)	14 (37.8)	
Diploma	19 (51.4)	19 (51.4)	
Above diploma	5 (13.5)	4 (10.8)	
Occupation			0.48
Homemaker/Unemployed	28 (75.7)	23 (62.2)	
Retired	9 (24.3)	14 (37.8)	
Diabetes duration (y)			0.20
3	5 (13.5)	7 (18.9)	
4	13 (35.1)	23 (62.2)	
5	19 (51.4)	7 (18.9)	

^a Values are expressed as No. (%).

analysis in the intervention group showed a positive association between patient age and baseline ALT ($r = 0.64$, $P = 0.046$) and AST ($r = 0.737$, $P = 0.015$), whereas no other significant correlations were observed (Table 5).

The comparisons were not adjusted for covariates because no baseline differences were found between the groups. After the intervention, the intervention group had

significantly lower levels of all liver enzymes than the control group.

4.4. Correlation Analyses

Pearson correlation analysis in the intervention group showed a positive association between patient age and baseline ALT ($r = 0.64$, $P = 0.046$) and AST ($r = 0.737$, $P = 0.015$). No associations were found between HbA1c and

Table 2. Baseline Anthropometric and Clinical Characteristics^a

Variables	Control Group (n = 37)	Intervention Group (n = 37)	P Value
Age (y)	52.11 ± 5.78	57.80 ± 9.99	0.12
Height (cm)	163.78 ± 11.05	163.50 ± 2.29	0.88
Weight (kg)	75.28 ± 11.07	82.04 ± 13.77	0.35
Diabetes duration (y)	4.44 ± 0.73	4.00 ± 0.67	0.18
Baseline HbA1c (%)	9.76 ± 1.50	9.93 ± 2.48	0.72
Baseline ALT (U/L)	21.56 ± 6.36	20.30 ± 5.31	0.36
Baseline AST (U/L)	21.90 ± 8.75	20.78 ± 4.91	0.50
Baseline ALP (U/L)	229.10 ± 45.21	230.00 ± 89.46	0.96

^a Values are expressed as mean ± SD.

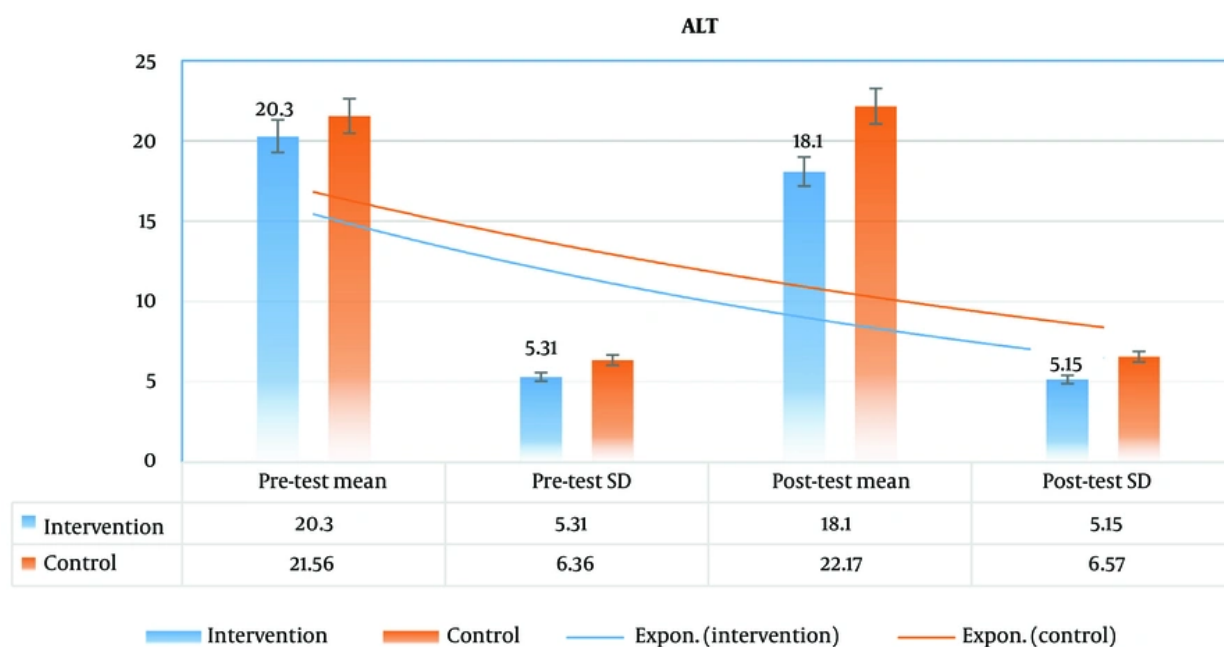


Figure 2. Comparison of mean glycated hemoglobin (HbA1c) levels between the *Eryngium billardieri* extract and placebo groups at baseline and after 14 weeks of intervention. Data are presented as mean ± SD. $P < 0.001$ for the within-group change in the intervention group (paired t test) and the between-group difference at week 14 (ANCOVA adjusted for baseline). ns = not significant ($P = 0.066$) in the placebo group.

liver parameters or patient sex, education, occupation, diabetes duration, or weight (Table 5).

5. Discussion

This randomized, double-blind, placebo-controlled trial provides evidence that adjunctive administration of a standardized hydroalcoholic extract of *E. billardieri* for 14 weeks significantly improves glycemic control, as

measured by HbA1c, and reduces serum aminotransferase levels in metformin-treated patients with T2DM. The mean 1.32% reduction in HbA1c observed in the intervention group is both statistically significant and clinically meaningful, as epidemiological data indicate that each 1% decrement in HbA1c reduces the risk of microvascular complications by 37% and diabetes-related mortality by 21% (4). This magnitude of effect is comparable to that of some

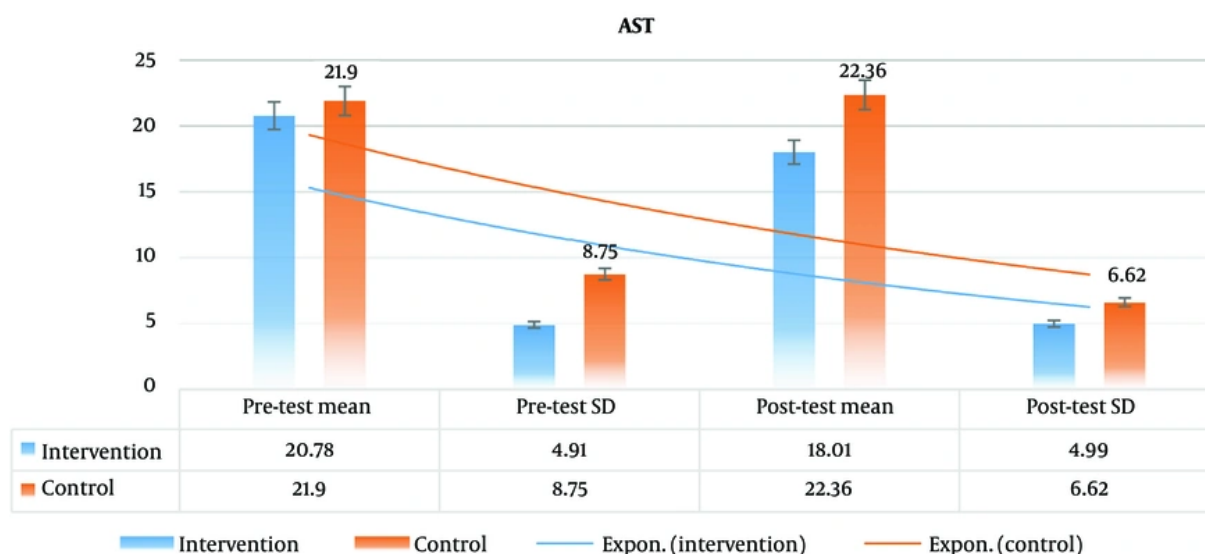


Figure 3. Effect of standardized *Eryngium billardieri* hydroalcoholic extract on serum alanine aminotransferase (ALT) levels in patients with type 2 diabetes: A 14-week double-blind placebo-controlled trial. Values are mean $\pm$ SD. The intervention group showed a significant reduction in ALT from baseline (mean change, -2.20 U/L; $P = 0.041$), whereas no significant change was observed in the placebo group ($P = 0.153$). Between-group comparison at week 14: $P = 0.041$ (ANCOVA).

oral antihyperglycemic agents, although direct comparative studies are needed.

5.1. Putative Mechanisms of Action

Although the present study was not designed to elucidate mechanisms, several biologically plausible explanations may account for the observed effects. *E. billardieri* is rich in polyphenolic compounds, particularly flavonoids and phenolic acids, with well-recognized antioxidant and anti-inflammatory properties (13). Phytochemical studies have identified quercetin and kaempferol as major flavonoid constituents, which have been shown to enhance pancreatic β -cell function, stimulate peripheral glucose uptake, and inhibit intestinal glucose absorption through modulation of sodium-dependent glucose transporters (14). The radical-scavenging capacity of these phenolic compounds may protect against oxidative stress-induced β -cell apoptosis, a critical factor in the progressive decline of insulin secretion in T2DM. Animal studies demonstrate that *E. billardieri* extract ameliorates pancreatic islet damage and restores β -cell mass in diabetic rodent models (15, 16). However, these mechanistic inferences are speculative and require confirmation through dedicated human studies involving measurements of insulin sensitivity, β -cell

function, and circulating markers of oxidative stress and inflammation.

5.2. Comparison with Previous Studies

Our findings are consistent with and extend previous preclinical studies on the genus *Eryngium*. Shirazi and colleagues reported that both aqueous and alcoholic extracts of *E. billardieri* attenuated pro-inflammatory cytokine production and oxidative stress markers in the hepatic tissue of diabetic rats while preserving pancreatic β -cell integrity. Similarly, Nowrozi and Valipour (17) demonstrated that a hydroalcoholic extract significantly lowered fasting blood glucose and improved lipid profiles in streptozotocin-induced diabetic rats.

Importantly, our study bridges a critical gap by translating these preclinical observations to a human clinical context. The 1.32% reduction in HbA_{1c} is comparable to that reported in trials of other plant-derived interventions. Moreover, the reductions in ALT, AST, and ALP levels align with the findings of Banankhojaste et al. (18), who demonstrated that *Rosa canina* extract mitigated hepatic oxidative injury in diabetic rats. However, direct comparisons should be interpreted cautiously because of differences in study design, intervention duration, and outcome measures.

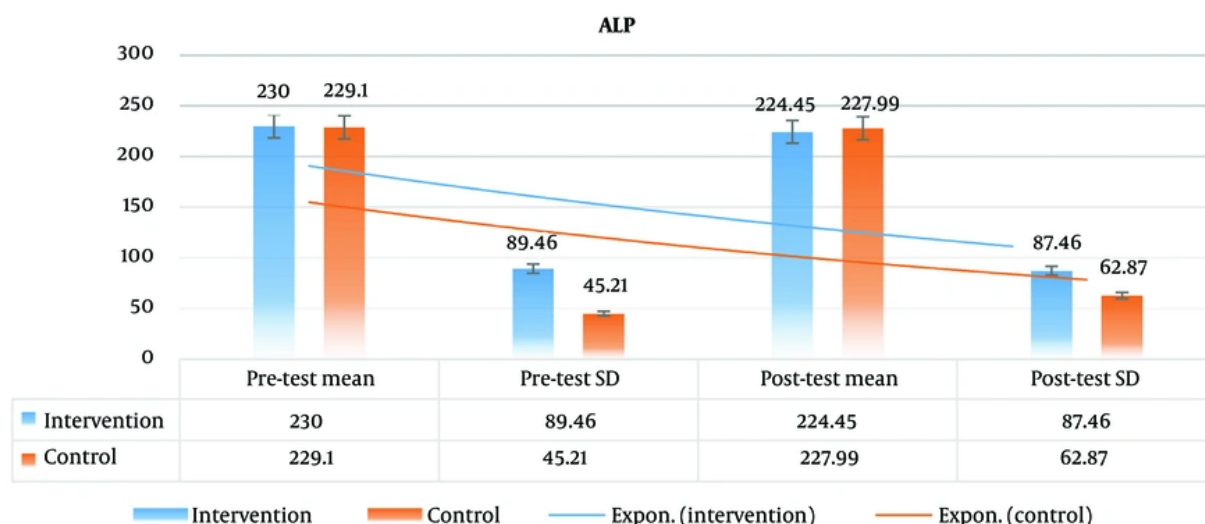


Figure 4. Changes in serum aspartate aminotransferase (AST) activity following adjunctive therapy with *Eryngium billardieri* extract versus placebo in metformin-treated patients with type 2 diabetes. Data are presented as mean $\pm$ SD. The extract group experienced a significant decrease in AST (mean difference, -2.77 U/L; 95% CI, -4.79 to -0.75; $P = 0.038$), whereas the placebo group did not show a meaningful change ($P = 0.784$). Adjusted between-group difference at the endpoint: $P = 0.038$.

Although previous human studies on *E. billardieri* have been reported by Hamami Chamgordani et al. (1), our study is among the firsts to use a standardized extract with quantified polyphenol content, employ a double-blind, placebo-controlled design, and evaluate simultaneous effects on both HbA_{1c} and liver enzymes with complete follow-up over 14 weeks.

5.3. Clinical Relevance and Implications

The substantial improvement in HbA_{1c} among patients with poorly controlled T2DM (baseline HbA_{1c} $\approx$ 9.9%) suggests that *E. billardieri* extract may serve as a valuable adjunct to metformin in patients who fail to achieve target glycemia with monotherapy. Given that metformin remains the cornerstone of initial pharmacotherapy, the additive effect observed offers a potentially safe and well-tolerated strategy to enhance glycemic outcomes without introducing additional synthetic agents. However, we caution against overinterpretation of these findings as a recommendation for widespread clinical adoption without confirmatory evidence.

The concurrent reduction in serum liver enzymes is noteworthy, given the high prevalence of NAFLD among populations with diabetes. However, it must be emphasized that:

- Baseline enzyme values were largely within normal ranges.
- The magnitude of changes was relatively small (ALT, -2.2 U/L; AST, -2.77 U/L; ALP, -5.55 U/L).
- NAFLD was not assessed using imaging (ultrasound or transient elastography) or histology.
- Therefore, the term "hepatoprotective" should be used cautiously, and our findings should be considered preliminary and hypothesis-generating.

The traditional use of *E. billardieri* as a culinary and medicinal herb, coupled with the absence of adverse events in our trial, supports a favorable safety profile at the administered dose. However, larger, long-term studies are necessary to confirm safety.

5.4. Strengths and Limitations

Strengths of this study include the following:

- Randomized, double-blind, placebo-controlled design minimizing bias
- Standardized extract with quantified polyphenol content
- Complete follow-up (0% attrition)
- Stable metformin regimen allowing isolation of the extract effect
- Comprehensive safety monitoring

Limitations include the following:

- The relatively small sample size (37 participants per group) limits generalizability.
- The 14-week intervention period is insufficient to assess long-term sustainability or safety.
- The study population consisted of patients with poorly controlled T2DM (mean HbA1c $\approx$ 9.9%) receiving metformin monotherapy; findings may not generalize to better-controlled patients or those receiving combination therapy.
- Mechanistic biomarkers, including insulin sensitivity indices, inflammatory cytokines, and oxidative stress markers, were not measured.
- Dietary intake and physical activity were not objectively monitored.
- Adherence was assessed by counting returned containers, which may overestimate adherence.
- Liver enzyme changes were small in absolute terms and within normal ranges; NAFLD was not confirmed by imaging.

5.5. Future Directions

Large, multicenter randomized trials with extended follow-up are needed to evaluate the durability of effects and confirm safety in diverse populations. Mechanistic studies incorporating surrogate biomarkers of insulin resistance, β -cell function, inflammation, and oxidative stress would elucidate pathways underlying the extract's actions. Head-to-head comparisons with established antidiabetic agents or other herbal formulations would inform its relative therapeutic position. Cost-effectiveness analyses would be valuable for assessing economic advantages.

5.6. Conclusions

In summary, this randomized controlled trial provides preliminary evidence that a 14-week course of *E. billardieri* hydroalcoholic extract, added to stable metformin therapy, produces clinically significant reductions in HbA1c and serum aminotransferase levels in patients with T2DM. The intervention was well tolerated, with no reported adverse events. However, given the limitations of this study, particularly the small sample size, short follow-up, and modest magnitude of liver enzyme changes, these findings should be interpreted with caution. Confirmation through larger, long-term investigations is imperative before this herbal extract can be recommended as adjunctive therapy in routine clinical practice. Until such evidence emerges, clinicians should exercise prudence in advising patients, emphasizing that this preparation does not replace, but

may complement, evidence-based pharmacological and lifestyle interventions.

5.7. Putative Mechanisms of Action

Although the present study was not designed to elucidate mechanistic pathways, several biologically plausible explanations may account for the observed glucose-lowering and hepatoprotective effects. *E. billardieri* is rich in polyphenolic compounds, particularly flavonoids and phenolic acids, which are well recognized for their antioxidant and anti-inflammatory properties (13). Phytochemical investigations have identified quercetin and kaempferol as major flavonoid constituents in this species; these molecules have been shown to enhance pancreatic β -cell function, stimulate peripheral glucose uptake, and inhibit intestinal glucose absorption through modulation of sodium-dependent glucose transporters (14). Furthermore, the radical-scavenging capacity of these phenolic compounds may protect against oxidative stress-induced β -cell apoptosis, a critical factor in the progressive decline of insulin secretion characteristic of T2DM. Histopathological evidence from animal studies corroborates this hypothesis, demonstrating that *E. billardieri* extract ameliorates pancreatic islet damage and restores β -cell mass in diabetic rodent models (15, 16). Nevertheless, these mechanistic inferences remain largely speculative and require confirmation through dedicated human studies involving measurements of insulin sensitivity, β -cell function, and circulating markers of oxidative stress and inflammation.

5.8. Comparison With Previous Investigations

Our findings are consistent with and extend the results of earlier preclinical studies on the genus *Eryngium*. Shirazi and colleagues reported that both aqueous and alcoholic extracts of *E. billardieri* attenuated pro-inflammatory cytokine production and oxidative stress markers in the hepatic tissue of diabetic rats while concurrently preserving pancreatic β -cell integrity. Similarly, Nowrozi and Valipor demonstrated that administration of the hydroalcoholic extract significantly lowered fasting blood glucose and improved the lipid profile in streptozotocin-induced diabetic rats. In another investigation, Muqtada'i and Khani observed a marked increase in the number and size of pancreatic islets following treatment with *E. billardieri* extract, providing morphological support for its hypoglycemic potential.

Importantly, the current study bridges a critical gap in the literature by translating these preclinical observations to a human clinical context. The 1.32% reduction in HbA1c observed here is comparable to that reported in trials of other plant-derived interventions. Moreover, the

hepatoprotective effect suggested by the significant reductions in ALT, AST, and ALP levels in our intervention group aligns with the findings of Banankhojaste et al., who demonstrated that *Rosa canina* extract mitigated hepatic oxidative injury in diabetic rats. Collectively, these parallels lend credence to the notion that polyphenol-rich botanicals may exert pleiotropic benefits in metabolic disorders.

5.9. Clinical Relevance and Implications

The results of this trial carry several practical implications for diabetes management. First, the substantial improvement in HbA_{1c} among patients with poorly controlled T₂DM (baseline HbA_{1c} approximately 9.9%) suggests that *E. billardieri* extract could serve as a valuable adjunct to metformin in individuals who fail to achieve target glycemia with monotherapy. Given that metformin remains the cornerstone of initial pharmacotherapy, the additive effect observed in this study offers a potentially safe and cost-effective strategy to enhance glycemic outcomes without introducing additional synthetic agents. Second, the concurrent reduction in serum liver enzymes is particularly noteworthy, given the high prevalence of NAFLD among populations with diabetes. Although our findings do not constitute definitive evidence of hepatoprotection, they raise the possibility that *E. billardieri* may exert favorable effects on hepatic health, warranting further investigation through liver imaging and histological assessment in future studies.

Third, the traditional use of *E. billardieri* as a culinary and medicinal herb, coupled with the absence of adverse events in our trial, supports its favorable safety profile at the administered dose. This is consistent with the generally recognized safety of many polyphenol-rich plant extracts when used in moderation. However, it is important to stress that these findings are preliminary and should not be interpreted as a recommendation for widespread clinical adoption without additional confirmatory evidence.

5.10. Strengths and Methodological Considerations

The principal strengths of this study include its randomized, double-blind, placebo-controlled design, which minimizes the risk of bias and confounding; the use of a standardized extract with verified polyphenol content, ensuring consistency across participants; and complete follow-up of all enrolled participants, with no attrition during the 14-week intervention period. Furthermore, the inclusion of patients receiving a stable metformin regimen

allowed isolation of the extract's effect independent of changes in baseline therapy.

5.11. Limitations and Future Directions

Notwithstanding these strengths, several limitations must be acknowledged when interpreting our results. The relatively small sample size (37 participants per group) limits the generalizability of our findings and reduces statistical power to detect smaller effect sizes. The 14-week intervention period, while sufficient to detect changes in HbA_{1c}, is inadequate to assess the long-term sustainability of the effect, tolerability, or safety beyond the study duration. Additionally, the study population comprised patients with poorly controlled diabetes (mean baseline HbA_{1c} approximately 9.9%) managed exclusively with metformin; whether similar effects would be observed in patients with better glycemic control or those receiving combination therapy remains unknown. We did not measure biomarkers directly related to the proposed mechanisms of action, such as insulin sensitivity indices, inflammatory cytokines (eg, TNF- α and IL-6), or oxidative stress markers (eg, malondialdehyde and superoxide dismutase). Such measurements would have strengthened the mechanistic interpretation of our findings. Furthermore, dietary intake and physical activity were not objectively monitored; although participants were advised to maintain their usual lifestyle habits, we cannot exclude the possibility of unmeasured confounding from lifestyle modifications. Adherence to the study medication was assessed by counting returned containers, which may overestimate actual compliance compared with more objective methods, such as electronic monitoring or biomarker validation.

Future research should prioritize larger, multicenter randomized trials with extended follow-up periods to evaluate the durability of the observed effects and confirm safety in diverse patient populations. Mechanistic studies incorporating surrogate biomarkers of insulin resistance, β -cell function, inflammation, and oxidative stress would help elucidate the pathways underlying the extract's actions. Additionally, head-to-head comparisons with established antidiabetic agents or other herbal formulations would inform its relative position in the therapeutic armamentarium. Finally, cost-effectiveness analyses would be valuable for assessing whether this botanical adjunct offers economic advantages over existing treatment-intensification strategies.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: Conceptualization, Methodology, Investigation, Writing – Original Draft, and Project Administration: A. B. A.; Supervision, Validation, Formal Analysis, and Writing – Review & Editing: F. K.; Data Curation, Software, and Visualization: S. J.; Resources, Investigation, and Writing – Review & Editing: M. B. A.; All authors read and approved the final manuscript.

Clinical Trial Registration: IR.ZBMU.REC.1405.056.

Conflict of Interests Statement: The authors have nothing to disclose.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study was performed according to the declaration of Helsinki and approved by the Ethics committee of Zabol University of Medical Sciences (IR.ZBMU.REC.1405.056).

Funding/Support: No funding was received for this study.

Informed Consent: Informed written consent was obtained from all participants.

References

- Hamami Chamgordani Z, Mazaheri M, Iraj B, Baghshahi H, Sabouhi F. Antidiabetic effects of Eryngium billardieri hydrosol in the treatment of type 2 diabetic patients: a double-blind randomized clinical trial. *Avicenna J Phytomed*. 2023;**13**(1):34-44. [PubMed ID: 36698734]. [PubMed Central ID: PMC9840773]. <https://doi.org/10.22038/AJP.2022.21175>.
- Khani S, Abdollahi M, Asadi Z, Nazeri M, Nasiri MA, Yusefi H, et al. Study on the hypoglycemic, cytoprotective, and lipid-lowering effects of a hydroalcoholic extract from Eryngium billardieri root in rats with nicotinamide/streptozotocin-induced type II diabetes. *Research in Pharmaceutical Sciences*. 2021;**16**(2):193-202. [PubMed ID: 34084206]. [PubMed Central ID: PMC8102925]. <https://doi.org/10.4103/1735-5362.310526>.
- Rajabali pour S, Ahangarpour A, Zakerkish M, Bineshfar F. A randomized controlled clinical trial assessing the impact of combined Eryngium billardieri and Boswellia serrata extracts on insulin-related markers, lipid profile, and liver enzymes in patients with type 2 diabetes. *Journal of Kerman University of Medical Sciences*. 2024;**31**(6):286-291. <https://doi.org/10.34172/jkmu.2024.44>.
- Yaghoobi M, Moridi Farimani M, Khan A, Asadollahi M, Omrani M, Luyten W, et al. Phytochemical analysis and evaluation of biological activities of a methanol extract from Eryngium billardieri, focusing on antimicrobial, antibiofilm, and anthelmintic properties. *Frontiers in Plant Science*. 2025;**16**: 1667335. [PubMed ID: 41281318]. [PubMed Central ID: PMC12631237]. <https://doi.org/10.3389/fpls.2025.1667335>.
- Yang J, Zheng X, Mahdi A, Zhou Z, Tratsiakovich Y, Jiao T, et al. In type 2 diabetes, red blood cells impair heart recovery after ischemia via arginase-mediated disruption of nitric oxide synthase and increased reactive oxygen species. *JACC: Basic to Translational Science*. 2018;**3**(4):450-456. [PubMed ID: 30175269]. [PubMed Central ID: PMC6115643]. <https://doi.org/10.1016/j.jacbs.2018.03.006>.
- Yang M, Hu Z, Yue R. A protocol for a systematic review and meta-analysis on the effectiveness of a decoction containing pueraria, scutellaria, and coptis in managing type 2 diabetes. *Medicine*. 2020;**99**(16): e19770. [PubMed ID: 32311982]. [PubMed Central ID: PMC7220274]. <https://doi.org/10.1097/MD.00000000000019770>.
- Jandari S, Hatami E, Ziaei R, Ghavami A, Yamchi AM. A systematic review and meta-analysis examining the influence of pomegranate (Punica granatum) supplementation on metabolic parameters in patients with type 2 diabetes. *Complementary Therapies in Medicine*. 2020;**52**: 102478. [PubMed ID: 32951728]. <https://doi.org/10.1016/j.ctim.2020.102478>.
- Mobasser M, Ostadrahimi A, Tajaddini A, Asghari S, Barati M, Akbarzadeh M, et al. A randomized, double-blind, placebo-controlled clinical trial assessing the effects of saffron supplementation on blood glucose levels and inflammatory markers in patients with type 2 diabetes. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2020;**14**(4):527-534. [PubMed ID: 32408117]. <https://doi.org/10.1016/j.dsx.2020.04.031>.
- Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine*. 1995;**2**(2):137-189. [PubMed ID: 23196156]. [https://doi.org/10.1016/S0944-7113\(11\)80059-0](https://doi.org/10.1016/S0944-7113(11)80059-0).
- Azimi P, Ghiasvand R, Feizi A, Hariri M, Abbasi B. Evaluation of cinnamon, cardamom, saffron, and ginger intake on glycemic control, lipid profile, oxidative stress, and inflammation markers in individuals with type 2 diabetes. *Review of Diabetic Studies*. 2014;**11**(3):258-266. [PubMed ID: 26177486]. [PubMed Central ID: PMC5397291]. <https://doi.org/10.1900/RDS.2014.11.258>.
- Nabavi SM, Nabavi SF, Alinezhad H, Zare M, Azimi R. Biological activities of flavonoid-rich fraction of Eryngium caucasicum Trautv. *Eur Rev Med Pharmacol Sci*. 2012;**16**(Suppl 3):81-87. [PubMed ID: 22957421].
- Sadiq A, Rashid U, Ahmad S, Zahoor M, AlAjmi MF, Ullah R, et al. Investigation into Eryngium caeruleum M. Bieb for managing hyperglycemia using in vitro α -glucosidase inhibition, antioxidant assays, in vivo antidiabetic tests, and molecular docking techniques. *Frontiers in Chemistry*. 2020;**8**: 558641. [PubMed ID: 33335883]. [PubMed Central ID: PMC7737655]. <https://doi.org/10.3389/fchem.2020.558641>.
- Wang P, Su Z, Yuan W, Deng G, Li S. Overview of phytochemical components and pharmacological actions of the genus Eryngium L. (Apiaceae). *Pharmaceutical Crops*. 2012;**3**(1):99-120. <https://doi.org/10.2174/2210290601203010099>.
- Korkina LG, Afanas'ev IB. Antioxidant and metal-chelating properties of flavonoids. *Advances in Pharmacology*. 1997;**38**:151-163. [PubMed ID: 8895808]. [https://doi.org/10.1016/S1054-3589\(08\)60983-7](https://doi.org/10.1016/S1054-3589(08)60983-7).
- Kalvandi R, Rajabi M, Kahramfar Z, Chaleh Cheleh T. Investigating the effects of artichoke (Cynara scolymus L.) on characteristics of fatty liver disease. *CMJA*. 2020;**10**(2):134-147. <https://doi.org/10.32598/cmja.10.2.891.1>.
- Alavian SM, Hajarizadeh B, Nematizadeh F, Larijani B. Prevalence and determinants of diabetes mellitus among Iranian patients with chronic liver disease. *BMC Endocr Disord*. 2004;**4**(1): 4. [PubMed ID: 15555059]. [PubMed Central ID: PMC538272]. <https://doi.org/10.1186/1472-6823-4-4>.
- Nowrozi M, Valipour S. The effect of Eryngium campestre hydroalcoholic extract on glucose and lipid indices of the blood in streptozotocin induced diabetic male rats. *J Animal Res*. 2021;**10**(5): 208-212.
- Banan Khojasteh SM, Basirat E. Effect of Alcoholic of Rosa canina on Hepatic Tissue and Hepatic Enzymes Activity in diabetic Rats. *J Ardabil University Medical Sciences*. 2017;**17**(1).