Effects berberine–silymarin on liver enzymes: A systematic review and meta-analysis of randomized controlled trials

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Abstract

ABSTRACT Objectives: Despite controversies, no study has systematically summarized findings from earlier studies on the effect of berberine (BBR)–silymarin on liver enzymes. Therefore, the current systematic review and meta-analysis aimed to investigate the effect of berberis aristate and silybum marianum on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in adults. Methods: Relevant studies, published up to June 2020, were searched through PubMed/Medline, Scopus, ISI Web of Science, EMBASE and Google Scholar. The mean differences and standard deviations were pooled using a random-effects model. The studies' quality was evaluated using Cochrane Risk of Bias Tool. Out of 80 citations, 5 trials that enrolled 549 participants were included. Results: Berberis aristate and silybum marianum resulted in no statistically significant change in ALT (weighted mean differences (WMD): -0.39 mg/dl; 95% CI: -1.67 to 0.89, P=0.55), and AST (WMD: -0.44 mg/dl; 95% CI: -2.02 to 1.14, P=0.58). We did not find any significant reduction in liver enzymes following BBR–silymarin consumption in adults. Conclusion: Further clinical trials with high quality according to challenges mentioned seem to be helpful to use BBR–silymarin as a supplement for improving liver function. Keywords: Berberis aristate, Silybum marianum, ALT, AST, Meta-analysis

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Running title: Berberine–silymarin and liver enzymes

DISCLOSURE

The authors declare that they do not have any conflict of interest. We declare that none of the authors listed on the manuscript are employed by a government agency that has a primary function other than research and/or education. Also we declare that none of the authors are submitting this manuscript as an official representative or on behalf of the government.

ABSTRACT

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Methods : Relevant studies, published up to June 2020, were searched through PubMed/Medline, Scopus, ISI Web of Science, EMBASE. The mean differences and standard deviations were pooled using a Fixed effect model. The studies' quality was evaluated using Cochrane Risk of Bias Tool. Out of 80 citations, 5 trials that enrolled 549 participants were included.

Results : Berberis aristate and silybum marianum resulted in no statistically significant change in ALT (weighted mean differences (WMD): -0.39 mg/dl; 95% CI: -1.67 to 0.89, P=0.55), and AST (WMD: -0.44 mg/dl; 95% CI: -2.02 to 1.14, P=0.58). We did not find any significant reduction in liver enzymes following BBR–silymarin consumption in adults.

Conclusion : Further clinical trials with high quality seem to be helpful to use BBR–silymarin as a supplement for improving liver function.

Keywords: Berberis aristate, Silybum marianum, ALT, AST, Meta-analysis

Review Criteria: Randomised clinical trials were identified using prespecified search terms in PubMed/Medline, Scopus, ISI Web of Science, EMBASE electronic databases with English language restriction.

Message for the Clinic: The decision to include berberine(BBR)–silymarin on the list of complementary medicines recommended for Fatty liver disease, is not evidence based. Patients who are using (or considering using) berberine (BBR)–silymarin for management of these symptoms should be provided with current evidence of effectiveness. Whilst a lack of evidence does not mean that berberine(BBR)–silymarin isn't ineffective, further clinical trials with high quality seem to be helpful to use BBR–silymarin as a supplement for improving liver function.

1 | INTRODUCTION

The liver plays central roles in the maintenance of body homeostasis and is important to multiple metabolic functions and physiological processes such as bile production, energy generation, vitamin storage, and the metabolism of carbohydrates, proteins, and lipids [1]. Fatty liver disease is becoming progressively prevalent in numerous parts of the world, affecting about 25% of people globally [2]. High concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in hepatocytes are used as biochemical indicators of hepatocellular disease [3]. Results from several studies suggest that certain vitamins, herbs and dietary supplements may help lessen liver fat and reduce the risk of liver disease progression [4]. Furthermore, among supplements that may ameliorate fatty liver, silymarin; berberine has been great of interest [5-9]. BBR is an isoquinoline derivative alkaloid isolated from Rhizoma Coptidis (RC). The composition of BBR in RC is about 5.2% to 7.7%. BBR has been widely used as a drug in traditional Indian and Chinese medicinal plants [10, 11]. This plant is useful as cholesterol-lowering action and anti-hyperglycemic effect

[12-16]. But BBR due to the presence of P-glycoprotein (P-gp) have low oral bioavailability. It has been recently formulated along with silymarin, and this component rich in flavolignanes (60–80%) and flavolignanes inhibits the transport of P-gp substrates, thus improving oral bioavailability of BBR [17]. Then, silymarin considered a fine candidate for combine with berberis aristate to improve the efficacy of liver function [18].

BBR by anti-dyslipidemic [19, 20], anti-hyperglycemic [20] and anti-inflammatory effect [21-25] may play a role in improving the nonalcoholic fatty liver disease (NAFLD). Silymarin has anti-inflammatory effect such as reduction of Tumor Necrosis Factor Alpha (TNF-α) [26, 27], and alleviate oxidative stress by (Superoxide Dismutase (SOD), Glutathione (GSH), Glutathione Peroxidase (GPx) activity) [28], antifibrotic activity [26]. Also BBR inhibited the 5-lipoxygenase pathway [29]. In the past study we observed improve in lipid and glucose profile with silymarin; berberine [30]. Also, BBR [31, 32] and silymarin [33-36] lonely effect on the liver function and improve in liver enzymes.

The aim of our meta-analysis was to systematically summarize the effects of the BBR–silymarin supplementation on liver enzymes using randomized controlled trials (RCTs).

2 | METHODS

This meta-analysis was conducted consistent with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Guidelines [37].

2.1. | Search strategy

Systematic search was conducted within the PubMed/Medline, Scopus, ISI Web of Science, EMBASE and Google Scholar up to June 2020 with English language restriction for identifying eligible studies.

The following combination of search terms was used (Silymarin[tiab] OR silimarin[tiab] OR SIL[tiab] OR "milk thistle"[tiab] OR Silybum[tiab] OR Silymarina[tiab] OR Silimarina[tiab] OR Silymarin[Mesh] OR "Milk Thistle"[Mesh]) AND (berberine[tiab] OR BBR[tiab] OR Berberis[tiab] OR Berberina[tiab] OR Berberol[tiab] OR Berberine[Mesh] OR Berberis[Mesh]) AND ("liver enzyme"[tiab] OR "liver enzymes"[tiab] OR "Liver function"[tiab] OR aminotransferase[tiab] OR ALT[tiab] OR "alanine aminotransferase"[tiab] OR "Alanine Transaminase"[tiab] OR "serum glutamic-pyruvic transaminase"[tiab] OR SGPT[tiab] OR AST[tiab] OR "Aspartate Aminotransferases"[tiab] OR "Aspartate transaminase"[tiab] OR SGOT[tiab] OR "serum glutamic-oxaloacetic transaminase"[tiab] OR ALP[tiab] OR "Alkaline phosphatase"[tiab] OR GGT[tiab] OR "gamma-Glutamyltransferase"[tiab] OR "Liver Function Tests"[Mesh] OR "Alanine Transaminase"[Mesh] OR "Aspartate Aminotransferases"[Mesh] OR "Alkaline Phosphatase"[Mesh] OR "gamma-Glutamyltransferase"[Mesh]). Literature searches were downloaded into EndNote (version X8, for Windows, Thomson Reuters, Philadelphia, PA, USA) to begin the review process.

2.2. | Study selection criteria

All titles and abstracts were screened to evaluate eligibility for inclusion. For a study to be included in the systematic review, it had to be a) RCTs with either parallel or cross-over design, b) participants aged 18 years or older, c) intervention with berberies aristate/silybum marianum, d) assessment of liver enzymes. Exclusion criteria were; a) they were conducted on animals, pregnant women or children; also b) unpublished data, books, letters, comments, congress abstracts, reviews, meta-analyses and observational studies were excluded.

2.3. | Data extraction

Study selection and data extraction was performed by two independent researchers (FM and MRA). Any disagreements about eligibility were discussed with a third reviewer (SS-b). Data abstracted from the eligible studies were (a) first author's name; (b) year of publication; (c) study design; (d) intervention (type, dose and duration of supplementation); (e) number of participants; (g) subjects' information, age, sex, and health status; and (h) outcomes (ALT, AST) assessed, before and after the intervention of serum ALT, AST.

2.4. | Quality assessment of studies

The risk of bias for RCTs in the included studies was performed using the Cochrane criteria [38]. The objects applied for the evaluation of any study were as follows: adequacy of sequence generation, allocation concealment, blinding addressing of dropouts (incomplete outcome data), selective outcome reporting, and other expected sources of bias. According to the Cochrane Handbook recommendation, studies were determining to low risk of bias, high risk of bias or unclear regarding any parameters **(Table 1).**

2.5. | Data analysis

Effect sizes were indicated as MD and 95% confidence interval (CI) and P[?]0.05 were considered as statistically significant for each parameter in this meta-analysis. Net changes in the explored parameters (change scores) were computed by subtracting the value at baseline from the one after intervention, in the treatment group, and in the control one. Standard Deviation (SDs) of the Weighted Mean Differences (WMD) were obtained as reported by Follmann, Elliott, Suh, and Cutler (1992): SD $_{\text{difference}}$ = square root [(SD $_{\text{pre-treatment}}$)^2+(SD$_{\text{post-treatment}}$)^2 - (2× R × SD$_{\text{pre-treatment}}$× SD $_{\text{post-treatment}}$)], assuming a correlation coefficient (R) = 0.8 as it is a conservative estimate for an expected range of 0-1. If the trials did not reported means and (SDs) of outcome measures, we converted the available statistical data into means and (SDs) by suitable formula: SD = SEM × [?]n, being "n" the number of subjects in any group. If medians and inter-quartile range were reported, mean and SD values were computed by the method described by Hozo et al[39].

I2 testing performed find the potential sources of between-study heterogeneity. Fixed effect model chosen for meta-analysis due to (I^2 was below 50% with p-value <0.1)[40], and selected the random-effects model if (I^2 was above 50%) [41]. All analyses were performed by STATA software (version 14.0). Potential publication bias was explored using Egger's regression test (Egger's test) (21).

3 | RESULTS

3.1. | Study selection

Out of 79 identified publications in the initial search, 15 duplicated studies were excluded. After screening by title and abstract, 51 unrelated studies discarded due to the primary evaluation of inclusion criteria: unrelated title (n=33), animal study (n=16) and review (n=2) and 14 papers were retained for full-text review. One additional trial was extracted through hand-searching of reference lists of related reviews. Of these articles, 9 were excluded because of the following reasons: irrelevant (n=5), has no placebo-controlled group (n=1), without sufficient data for outcomes (n=3). Finally, 5 studies met all our inclusion criteria. **Figure 1** demonstrates the process by which articles were selected.

3.2. | Characteristics of included studies

The characteristics of included studies were abstracted in **Table 2** . Selected eligible trials enrolled 549 participants with age ranging from 30.7 to 59.4 years old and were conducted on both genders. All trials were conducted in Italy, which involved 549 patients with dyslipidemic patients and overweight [5] euglycemic with statins at high doses and overweight [7] dyslipidemic and euglycemic with statins at high doses and overweight [6], type 2 diabetes mellitus [9], type 1 diabetic [42]. Publication dates ranged from 2013 and 2016. All of the included studies had parallel designs. The participants of all studies were of both genders. All studies used berberis aristata/silybum marianum for intervention. The cases received a daily dose of berberis aristata from 1176 mg/day plus silybum marianum from 210 mg/day except Guarino study with dose of BBR-aristata from 1000 mg/day plus silybum marianum from 300 mg/day [9] and the control group received a placebo. The intervention duration was from 12 to 24 weeks.

3.3. | Effect of BBR and silymarin on liver enzymes The effect of BBR-silymarin on serum ALT and AST was reported in all studies. The combined supplementation was found to not significantly reduce in ALT (WMD: -0.39 mg/dl; 95% CI: -1.67 to 0.89, P=0.55) (I^2=39.3%, P<0.001) (**Figure 2)** and AST (WMD: -0.44 mg/dl; 95% CI: -2.02 to 1.14, P=0.58) (I^2=35.9%, P<0.001) (**Figure 3).**

3.4. | Publication biases

Egger's weighted regression tests were performed to explore the publication bias. The results of Egger's test showed no evidence of publication bias for ALT (P =0.421) and AST (P=0.357).

4 | DISCUSSION

In this systematic review and meta-analysis, we summarized published evidence from five RCTs that investigated effect of BBR–silymarin on liver enzymes (ALT and AST) levels. To the best of our knowledge, this systematic review and meta-analysis is the first one that examined the effect of BBR–silymarin supplementation on liver enzyme levels. The results of the review showed that supplementation with BBR–silymarin had no significant effect of on ALT and AST, in supplementation group compared with placebo group in both sexes including men and women with liver complications.

In the previous studies the effect of BBR and silymarin investigated separately and observed beneficial effects to decrease liver enzymes with BBR [31, 32]. Also studies represented that BBR was effective to treat NAFLD [43]. The half-life of BBR in liver is longer than that in other tissues, and these results may explain that liver is the main target tissue of BBR [44]. BBR due to the presence of P-gp have low oral bioavailability and low oral bioavailability can be improve by P-gp inhibitors such as silymarin. recently trials are experimenting on the nutraceutical combination of berberis aristata plus-silybum marianum to treat NAFLD [45].

BBR has been previously shown that decreases transaminase levels, reducing liver necrosis in hepatitis C infection [31] and has an effectiveness in decreasing serum triglyceride and serum level of ALT and AST within 48 hours in patients with NAFLD [32]. In case of total cholesterol [9, 46, 47] it seems that the hypocholesterolemic potential of BBR supplementation intensifies when is combined with statin therapy [6, 48, 49].

There were also few studies that did not report the effectiveness of silymarin on liver enzymes. A clinical trial of silymarin treatment in patients with cirrhosis of the liver showed liver function tests, such as ALT, bilirubin and alkaline phosphatase had no difference between the two study groups [50]. But other studies have also reported different result; a double blind study in 60 female patients were treated with antipsychotics (phenothiazines or butyrophenones) drugs plus 800 mg/day silymarin represent serum levels of AST or ALT decreased at least twice the normal values after 90 days, in receiving silymarin versus the placebo group, and no significant change within the γGT levels [33]. Moreover, in another trial 140 mg, Livergol capsule per day for 30 days reported a statistically significant difference between the two groups in terms of AST and ALT enzymes as the level of liver enzymes in the intervention group was lower than that in the control one, at the end of the study [34]; and a review article which published in 2001 showed in person with chronic alcoholic liver, silymarin treatment, normalized serum bilirubin, AST , ALT values, and γ-GT activity levels decreased in the silymarin supplementation groups [35, 36].

Dosage and duration are two important factors which may affect final results of clinical trials. Included studies in this met-analysis used different doses for their intervention. Silymarin; berberine supplementation was used in a study [9] with a dose of 300 mg silymarin compare to other study which its participants used 210 mg/day. Studies have found that milk thistle, alone or in combination with vitamin E, may help reduce insulin resistance, inflammation and liver damage in people with NAFLD [4, 51-53] and the dosages of silymarin utilized in these studies was 140–800 mg/day, so increase in silymarin dosage may be helpful but further studies are needed to test to prove it.

BBR by regulation of hepatic lipids [19, 20], glucose metabolism [20], and anti-inflammatory effect [21-25] play a role in improving the NAFLD. BBR significantly decreased Hepatic Fat Content (HFC) in the rats with high fat diet induced NAFLD by decreasing methylation of the microsomal triglyceride transfer protein (MTTP) promoter (13). Regulation of AMP-activated protein kinase (AMPK) independent mechanism for BBR to suppress obesity-associated inflammation and alleviate hepatic steatosis [21-23] decrease Cyclooxygenase-2 (COX-2), and mRNA levels of proinflammatory cytokines, resulting in an anti-inflammation effect [24, 25]. BBR also reduced rates of glucose appearance (Raglu), gluconeogenesis (GNG) and hepatic lipogenesis [20] and alleviated insulin sensitivity via activation of AMPK. Silymarin is another component used along with

BBR may increase SOD, GSH, and GPx activity. Silymarin is another component of berberol, increases Superoxide dismutase (SOD) glutathione and glutathione peroxidase (GPx) activity. Additionally Silymarin improved hepatic fibrosis [26], decreased the hepatic levels of hydroxyproline and connective tissue growth factor (CTGF) [28], and inhibition of the 5-lipoxygenase pathway particularly LTB4 are the major targets of Silymarin in the treatment of NAFLD [29].

Despite the present study was the first to investigate the effects of berberis aristate and Silybum marianum on liver enzymes, our meta-analysis has some limitations. First, there were only a few eligible RCTs in this meta-analysis, and most of them had a relatively small population, thus performing further studies with a bigger population is needed to determine whether BBR–silymarin is not effective on controlling/lowering ALT and AST levels. Second, studies that were included had heterogeneous patient characteristics. Ultimately, the included studies enrolled only on adult subjects, so we cannot directly infer our studies' results to children and the elderly.

CONCLUSION

In conclusion, we did not find any significant effect of BBR–silymarin on the ALT and AST. Further large and well-designed RCTs are needed to confirm these findings.

AUTHOR CONTRIBUTIONS

FM, SS-b and KD designed the study. MRA and FM did the literature search and screening data. MRA performed data extraction and quality assessment, independently. FM interpreted data and wrote the manuscript. SS-b supervised the study. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest

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