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Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Strengths and limitations of this study

- This is the first meta-analysis evaluating Ginkgo Biloba Extract (GBE) as an Acute Mountain Sickness (AMS) prophylactic.
- In pooled analyses, although GBE may tend toward AMS prophylaxis, it had no statistically significant prophylactic effect (RR =0.86; 95% CI: 0.45 to 1.04; p-value=0.07). The results of several subgroup analyses were similar.
- Only a total of 487 participants were enrolled in selected studies. Insufficient power may be an issue even in this meta-analysis.

Keywords: Ginkgo Biloba Extract (GBE), Acute Mountain Sickness (AMS)

Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

Abstract

Study objective:

Trials of ginkgo biloba extract (GBE) for the prevention of acute mountain sickness (AMS) have been published since 1996. Because of their conflicting results, the

efficacy of GBE remains unclear. We performed a systematic review and meta-analysis to assess whether GBE prevents acute mountain sickness.

Methods:

The Cochrane Library, EMBASE, Google Scholar, and PubMed databases were searched for articles published up to May 20, 2017. Only randomized controlled trials were included. The main outcome measures were the relative risks of AMS in participants receiving GBE for prophylaxis. Meta-analyses were conducted using random-effects models. Sensitivity analyses and tests for publication bias were conducted.

Results:

Six published articles with a total of 487 participants met all eligibility criteria. The pooled result found that GBE did not prevent AMS (relative risk =0.86; 95% CI: 0.45 to 1.04; p-value=0.07). The results of subgroup analyses of studies with low risk of bias, low starting altitude (<2500 m), and different starting treatments prior to ascent were similar.

Conclusions: The currently available data suggest that GBE does not prevent AMS regardless of starting altitude and pre-ascent starting treatment.

Introduction

Background

Rapid ascent from low to high altitude (> 2500 m above the sea level) is often followed by headache, fatigue, shortness of breath, sleeplessness, and anorexia, a symptom complex called acute mountain sickness (AMS).[1] AMS is more likely to happen at altitudes higher than 2500 m,[2] and worldwide studies reported incidences of AMS of 25–37% at 1900–3400 m.[1 3] Children are more prone to develop AMS, with an incidence of 59%.[4]

The pathophysiology of AMS is associated with cerebral edema, with the most compelling evidence coming from the brain MRI study of Hackett et al.,[5] which showed intense T2 signals in the white matter, particularly in the splenium and corpus callosum. Vasogenic leakage increases permeability of the endothelium, causing an elevation in intravascular pressures and inducing hypoxemia. In addition, hypoxic ventilatory response and activation of the renin-angiotensin–aldosterone system are also reported to be associated with AMS.[6] The most effective method to prevent AMS is gradual ascent. The most common pharmacologic agent used to prevent AMS is acetazolamide.[7] However, acetazolamide can cause paresthesias, dysgeusia, and sometimes nausea or drowsiness.[8] Its use is also contraindicated in patients with a history of anaphylaxis to sulfa antibiotics or acetazolamide.

Importance

Ginkgo biloba extract (GBE) is an option for those seeking a natural alternative treatment. Roncin et al. in 1996 published the first studies to suggest that GBE can prevent AMS.[9] However, not all subsequent studies have shown benefit.[10-15] To date, there is no best evidence to support the effectiveness of GBE.

Goals of This Investigation

Our study aim was to assess the effectiveness of GBE in prophylaxis of AMS by conducting a meta-analysis and systematic review of the relevant literature.

Methods

Databases and search strategy

We searched the Cochrane Library, EMBASE, Google Scholar, and PubMed databases for articles published up to May 20th, 2017. No limits were applied to our Boolean search strategy, which included keywords ('Ginkgo', 'Altitude Sickness', 'Mountain'), Medical Subject Headings (MeSH) ('Ginkgo biloba', 'Altitude Sickness'), and Emtree terms ('Ginkgo biloba', 'altitude disease'). References from retrieved articles were also examined to identify other relevant articles.

Studies were included in the systematic review if they were (1) randomized controlled trials (RCTs) of GBE for prevention of AMS; (2) compared GBE with placebo; and (3) conducted in humans. Studies were excluded if they were irrelevant to the study's aim, were animal studies, lacked a placebo group, or were published as review articles, case reports, editorials, or letters. The Institutional Review Board of Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taiwan, approved the protocol.

Data extraction and assessment of methodological quality

Two reviewers (TYT and YCS) independently screened titles and abstracts of all articles identified by the search strategy. Inter-reviewer disagreements concerning the inclusion or exclusion of a study were resolved by consensus and, if necessary, consultation with a third reviewer (SHW).

The Cochrane Collaboration's tool was used to assess the risk of selection, performance, detection, attrition, and reporting biases in the included randomized trials.[16] All co-authors discussed and made the final decisions about the overall risk of bias in the included trials. If data were not readily available or clear, we contacted first authors and corresponding authors to get further information. If studies were found to be at high risk of bias, meta-analyses stratified by study quality were performed.

Both reviewers independently extracted data from the articles selected for inclusion. The extracted data included the name of the first author, year of publication, numbers of participants, gender, starting and final altitudes, AMS scoring definitions, prescriptions of GBE, days of treatment prior to ascent, and number of individuals with AMS in the treatment and control groups.

Data collection, data processing, and primary data analysis

Pooled relative risk (RR) with corresponding 95% confidence intervals (CIs) for each outcome of interest were calculated. The main outcome measure was the RR of AMS in participants receiving GBE for prophylaxis. Random effect models were selected for these analyses.

We conducted subgroup analyses based on quality of studies, number of treatment days before ascending, and starting altitude below 2500 m.[17 18] Between-study heterogeneity was evaluated with the I^2 statistic.[19] Funnel plots, the Egger regression asymmetry test, and Begg adjusted rank correlation test were applied for assessment of potential publication bias.[20 21] We also conducted sensitivity analysis to evaluate the influence of each study on the overall pooled estimate. Analyses

were all conducted using STATA version 11.0 (StataCorp, College Station, Texas, USA). All statistical tests were two-sided and were considered significant when the P value was 0.05 or less.

Results

The literature search and study selection process are summarized in Figure 1. After the exclusion of duplicate studies, non-relevant studies, and other studies that met exclusion criteria based on a screening of article titles and abstracts, 38 potentially relevant studies were retrieved for full review.

One publication was retrieved by hand search of the references. In this study, Wang et al.[22] compared the prophylactic effect of GBE with that of other Chinese medications on AMS. However, the study had no placebo group design[23] and had to be excluded from our meta-analysis.

In the randomized double-blind study by Ke in 2013,[15] AMS was reported as a secondary outcome and the number of events in each group were not reported. We contacted the first and corresponding authors by email but (as of October 9, 2017) received no response. Since the published data could not be included for analysis, we excluded this study.

Six published articles met all eligibility criteria after a careful review process.[9-14] In the article published by Leadbetter et al.,[14] two randomized controlled trials were conducted. As a result, a total of 7 study groups with 487 participants were enrolled. The characteristics of these studies and the participants are listed in Table 1. Four study groups[9 10 13 14] demonstrated the efficacy of GBE in preventing AMS, while three[11 12 14] did not. All studies had small numbers of subjects except the one by

Gertsch and colleagues.[11] Of note, participants in the study conducted by Gertsch et al., and published in 2004, started GBE treatment at high altitude (4280–4358 m), which was different from the other studies. Further information such as study dosage, prescription frequency, number of days prior to ascending, and source of GBE are summarized in Table 2. The number of AMS events is given in Table 3. The evidence quality of these studies as assessed by Cochrane Collaboration's tool is presented in Table 4. Two of 6 articles were not double-blinded and both of them included male participants only.[9 13]

In the primary meta-analysis of all 7 study groups, GBE did not prevent AMS (RR =0.86; 95% CI: 0.45 to 1.04; p-value=0.07) (Figure 2). The I^2 statistic was 58.7% (p-value=0.02), indicating substantial heterogeneity. After excluding two high-risk-bias studies,[9 13] the I^2 statistic became 39.7% (p-value=0.16) and the result did not change (RR =0.79; 95% CI 0.58 to 1.08; p-value=0.144) (Figure 3). The funnel plot did not demonstrate asymmetry (Figure 4). The Egger's-test and Begg-test p values (0.178 and 0.462, respectively) indicate the absence of statistical evidence of publication bias after excluding our presumed high-risk-bias articles.

Sensitivity analysis was conducted by removing one trial at a time to determine what influence each low-risk bias study had on the pooled analysis. The pooled result seemed to be robust. For example, removing the study 1 conducted by Leadbetter et al. in 2009[14] only changed the pooled estimate from 0.79 to 0.88 (95% CI 0.66–1.17; P value=0.38; Figure 5).

The results of several pre-planned subgroup analyses with all 7 datasets were similar. Excluding the study by Gertsch and colleagues in 2004,[11] GBE was not prophylactic when the starting altitude was below 2500 m (RR =0.56; 95% CI 0.31 to

1.01; Figure 6). Regarding the number of days of treatment prior to ascent, GBE was not prophylactic when given "3–5 days prior to ascent" (RR =0.72; 95% CI 0.41 to 1.26; Figure 7) or "0–2 days prior to ascent" (RR =0.56; 95% CI 0.25 to 1.25; Figure 8).

Limitations

Our systematic review has several limitations. First, to limit the influence of study biases on pooled evaluation, we decided to only include RCTs. However, there were few RCTs in this field. Moreover, only 4 of 6 RCTs were double-blinded. Second, because of the difficulty in carrying out high altitude medicine studies, many studies involved only a small number of cases. In our primary pooled analysis, a total of 487 participants were enrolled. Insufficient power may be an issue even in the meta-analysis. Third, the participants were predominantly adult males and whether there is gender or age difference between treatment (GBE vs placebo) groups or response (no AMS vs AMS) groups is unknown. Fourth, GBE is a complex mixture of natural components. It is difficult to standardize all components. A lack of consistency between commercially available GBE preparations may explain these differing results. Finally, differences between studies in factors such as the strength, rate of ascent, and other characteristics of participants may also account for inconsistent results.

Discussion

To our knowledge, this is the first meta-analysis of RCTs evaluating GBE as an AMS prophylactic. In pooled analyses, we found that although GBE may tend toward AMS prophylaxis, it had no statistically significant prophylactic effect (RR =0.86; 95% CI: 0.45 to 1.04; p-value=0.07). The results of several subgroup analyses were similar. GBE also failed to show benefits in preventing AMS in low-risk bias studies, studies in which the starting altitude was low, and studies differing in the initial treatment regimen prior to ascent.

The effectiveness of GBE in AMS prophylaxis has been reported.[9 10 13 14] Zhang and colleagues in 2003 reported that GBE was the most effective of six Chinese medicines tested for AMS prophylaxis.[23] GBE has been used primarily for the treatment of dementias (e.g., Alzheimer's disease), peripheral vascular diseases (e.g., intermittent claudication), and neurosensory problems (e.g., tinnitus).[24] Hypotheses have been proposed to explain the possible role that GBE plays in preventing AMS. Hypoxia is a common feature of AMS. Several studies have suggested that nitric oxide (NO) may play a pathogenic role in AMS by mediating hypoxia-induced cerebral vasodilation in humans.[13 25 26] GBE was found to be an NO scavenger. NO scavenging can result in decreased intracellular NO level.[27] Furthermore, GBE may inhibit phosphodiesterase activity, thus enhancing relaxation of parietal smooth muscle cells and so lead to vasodilation of parietal vessels. Vasodilation in turn increases tissue perfusion and decreases local hypoxia.[27] Other potential mechanisms include increasing endogenous antioxidants,[28] reducing free-radical production,[29] and reducing lung leak during hypoxia.[30] GBE was also shown to prevent high altitude pulmonary edema in a rat model.[31]

On the other hand, several studies failed to demonstrate the benefit of GBE in AMS prophylaxis.[11 12 15] The duration of therapy before ascent and differences in the altitude at which GBE is initiated may account for the conflicts between trial results. To test these hypotheses, we conducted subgroup analyses and obtained similar results to those obtained with the original pooled data. Another explanation for the differences in efficacy may be variation in the GBE composition. For instance, Leadbetter and colleagues in 2009 compared GBE from two different sources and found they differed in composition as well as ability to reduce the incidence and severity of AMS following rapid ascent to high altitude.[14] The German Federal Institute for Drugs and Medicinal Devices Commission E recommends similar specifications for standardization of GBE. All included studies used GBE that met the German E commission standard, but most of studies use products from different companies. As an herbal supplement, more than 60% of GBE component is not mandated by law and composition may vary considerably between manufacturers. A lack of bioequivalence has been noted between brands of GBE.[32 33]

Conclusion

In the present systematic review and meta-analysis of the currently available data sources, we found that GBE may not prevent AMS. Furthermore, subgroup analysis of low-risk bias studies, studies with low starting altitude, and studies with different starting treatment regimens prior to ascent, also indicated that GBE does not prevent AMS.

Table and Figure Legends

Figure 1. Trial selection algorithm

Figure 2. Forest plot: Effect of GBE in prevention of acute mountain sickness.

Figure 3. Forest plot: Effect of GBE in prevention of acute mountain sickness in low-risk-bias studies

Figure 4. Funnel plot of low bias studies

Figure 5. Sensitivity analyses by removing one trial at a time in low-risk-bias studies

Figure 6. Forest plot of subgroup meta-analysis: studies starting altitude was below 2500m

Figure 7. Forest plot of subgroup meta-analysis: studies starting treatment 3-5 days prior to ascent

Figure 8. Forest plot of subgroup meta-analysis: studies starting treatment 0-2 days prior to ascent

Table 1. Characteristics of included studies

Table 2. Characteristics of included studies, sources, dosage and duration of GBE

Table 3. Events of acute mountain sickness between placebo and GBE

Table 4. Risk of bias in included studies.

Declarations

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests in this section.

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Authors' contributions

TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS analyzed the data and contribute in the manuscript formation. All authors read and approved the final manuscript.

Transparency declaration

We affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and

that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Table 1. Characteristics of included studies.

	Participants (number)	Male (%)	Starting altitude (m)	Altitude reached (m)	AMS scoring
GBE prevented acute mountain sickness					
Roncin, 1996	44	100	1,800	5,400	AMS-C >0.7, raw mean scores
Gertsch, 2002	26	46	0	4,205	LLS ≥3 with HA, LLS severity
Moraga, 2007	24	100	0	3,696	LLS ≥3, or 1 symptom ≥3
Leadbetter, 2009 Study 1	40	45	2,000	4,300	AMS-C ≥0. 7 plus LLS ≥3 with HA
GBE did not prevent acute mountain sickness					
Leadbetter, 2009 Study 2	37	44	2,000	4,300	AMS-C ≥0. 7 + LLS ≥3 with HA
Gertsch, 2004	279	70	4,280–4,358	4,928	LLS ≥3 with HA
Chow, 2005	37	54	1,230	3,800	LLS ≥3 with HA

GBE: ginkgo biloba extract; AMS: Acute mountain sickness; AMS-C: the Environmental Symptom Questionnaire III acute mountain sickness-cerebral (AMS-C) score; HA: headache; LLS: Lake Louise Score.

Table 2. Characteristics of included studies, sources, dosage and duration of ginkgo biloba.

	GBE source	Dose	Days of treatment prior to ascent
GBE prevented acute mountain sickness			
Roncin, 1996	Tanakan® DCI: EGb 761 Ipsen, Paris, France	60 mg BID	0
Gertsch, 2002	GK501 Memfit ® , EGb 761, Pharmaton	60 mg TID	1
Moraga, 2007	EGb 761 Rokan, Andromeco Laboratories, Chile	80 mg BID	1
Leadbetter, 2009 Study 1	Spectrum Quality, Laboratories Products, Inc.	120 mg BID	4
GBE did not prevent acute mountain sickness			
Leadbetter, 2009 Study 2	Technical Sourcing, Inc.	120 mg BID	3
Gertsch, 2004	GK501 International, Pharmaton	120 mg BID	1–2
Chow, 2005	*Gingko biloba* 120 mg Vegetarian NOW ® Foods	120 mg BID	5

BID: Bi in die=twice a day; TID: ter in die=three times a day.

Table 3. Events of acute mountain sickness compared between placebo and

ginkgo biloba.

	Placebo		GBE	
	AMS	All subjects	AMS	All subjects
GBE prevented acute mountain sickness				
Roncin, 1996	9	22	0	22
Gertsch, 2002	13	14	7	12
Moraga, 2007	7	12	0	12
Leadbetter, 2009 Study 1	13	19	7	21
GBE did not prevent acute mountain sickness				
Leadbetter, 2009 Study 2	10	22	4	15
Gertsch, 2004	40	119	43	124
Chow, 2005	12	20	11	17

Table 4. Risk of bias in included studies.

Risk of bias domain	Roncin, 1996	Gertsch, 2002	Gertsch, 2004	Chow, 2005	Moraga, 2007	Leadbetter, 2009
Random-sequence generation (selection bias)	Unclear	Low	Low	Low	Low	Low
Allocation concealment (selection bias)	Unclear	Low	Low	Low	Unclear	Low
Blinding of participants (performance bias)	High	Low	Low	Low	High	Low
Blinding of outcome assessment (detection bias)	High	Low	Low	Low	High	Low
Incomplete outcome data (attrition bias)	High	Low	Low	Low	Low	Low
Selective outcome reporting (reporting bias)	Low	Low	Low	Low	Low	Low
Other source of bias	High	Low	High	Low	High	Low
Overall risk of bias	**High**	**Low**	**Low**	**Low**	**High**	**Low**

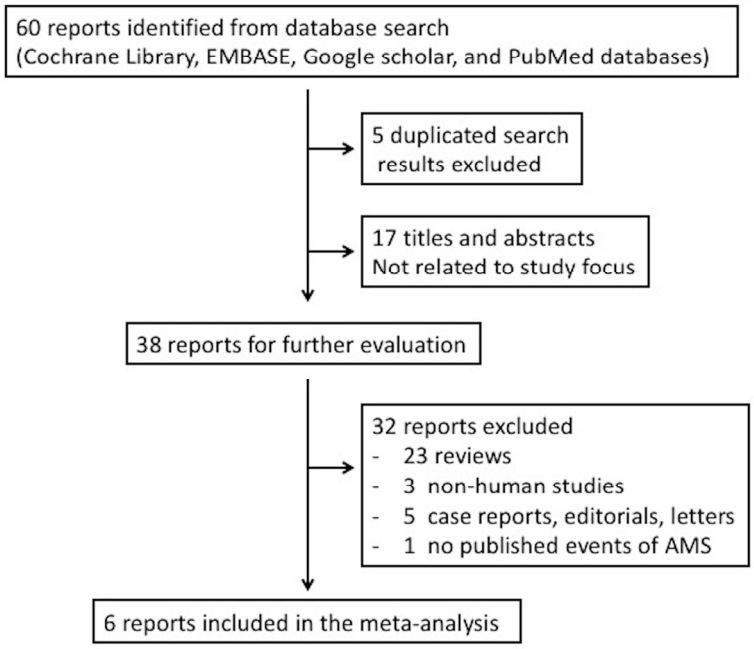

Figure 1

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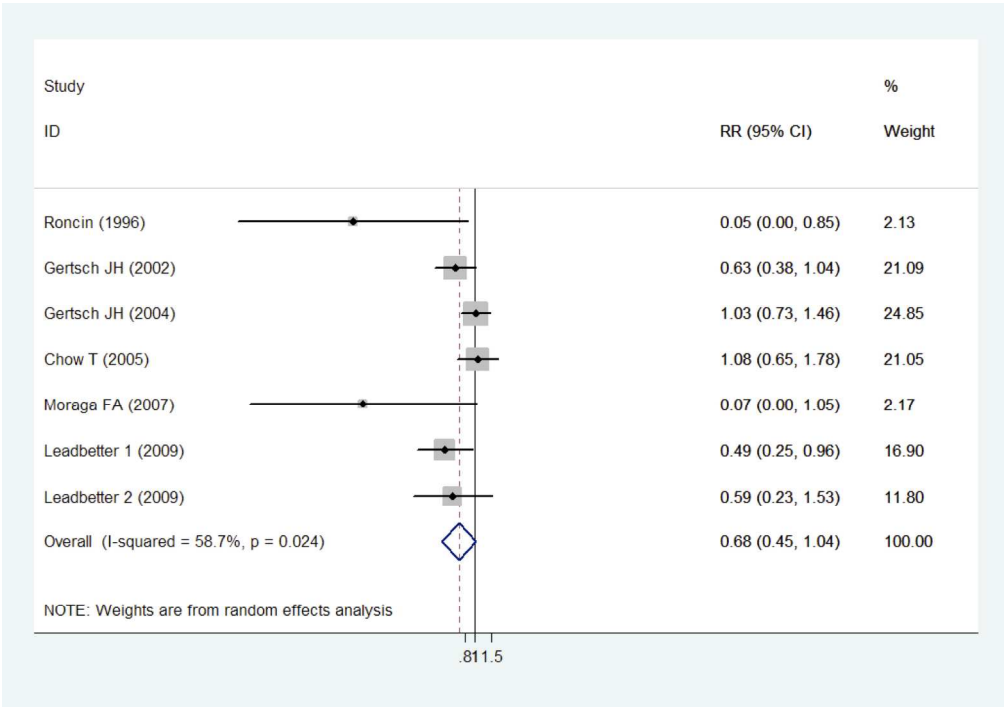

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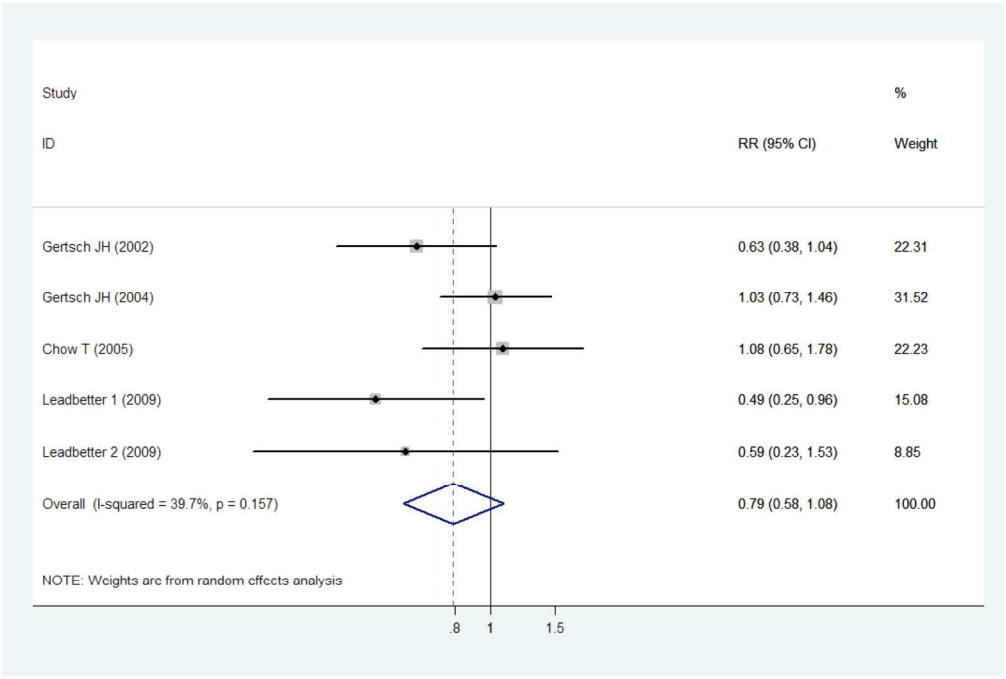

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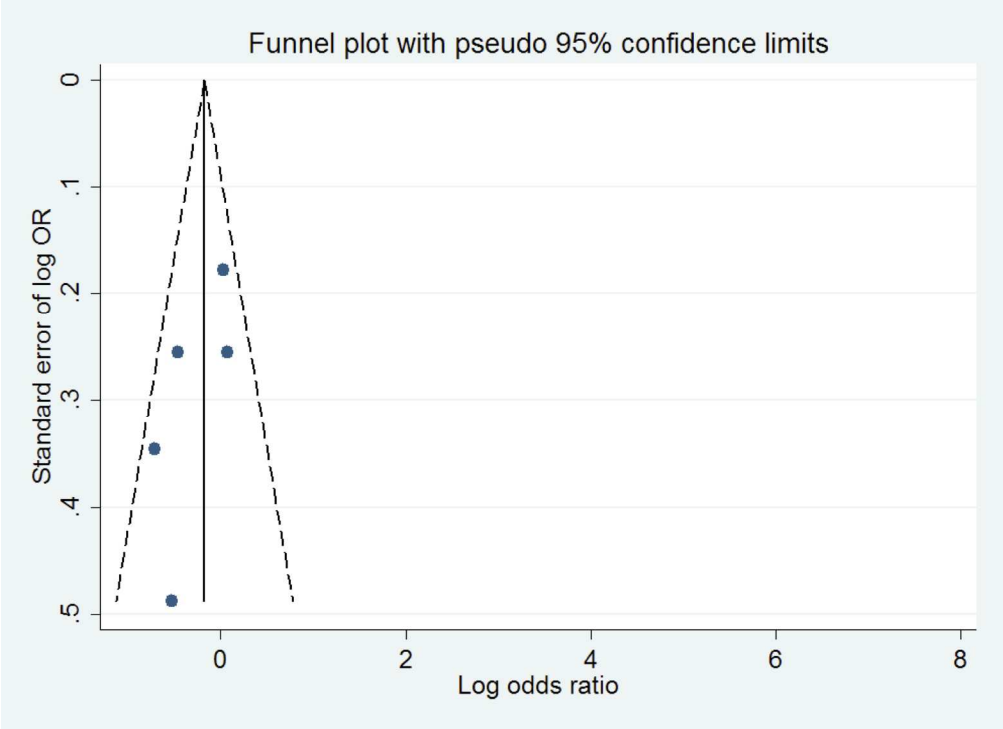

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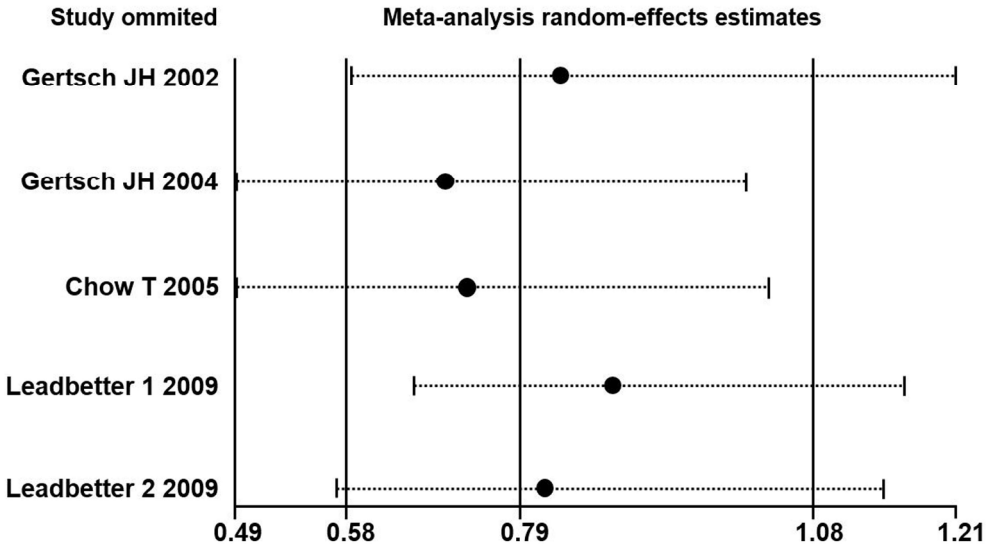

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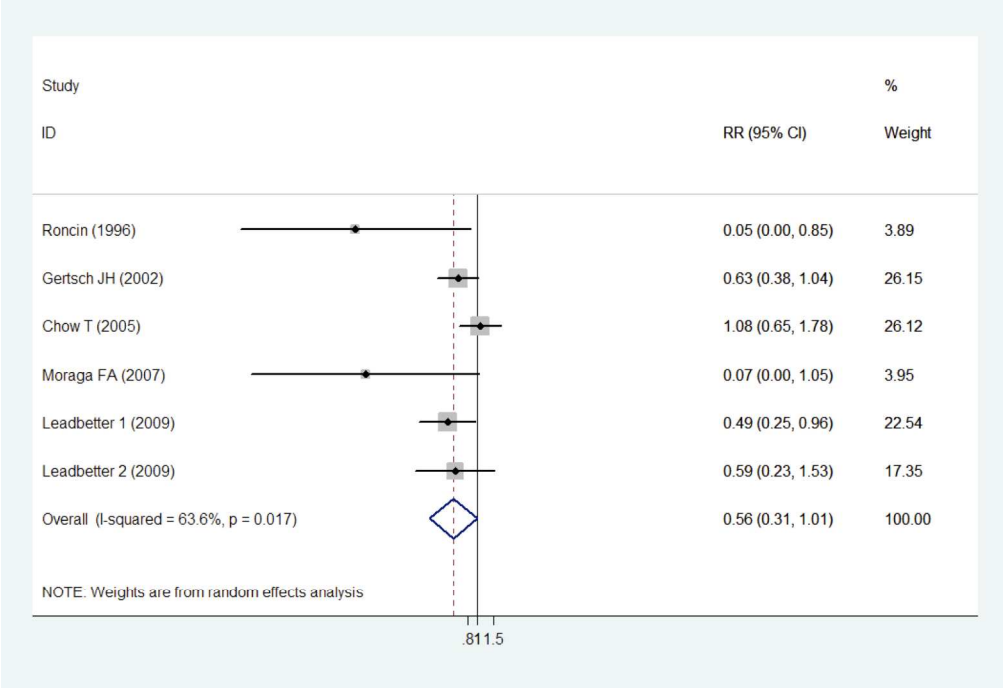

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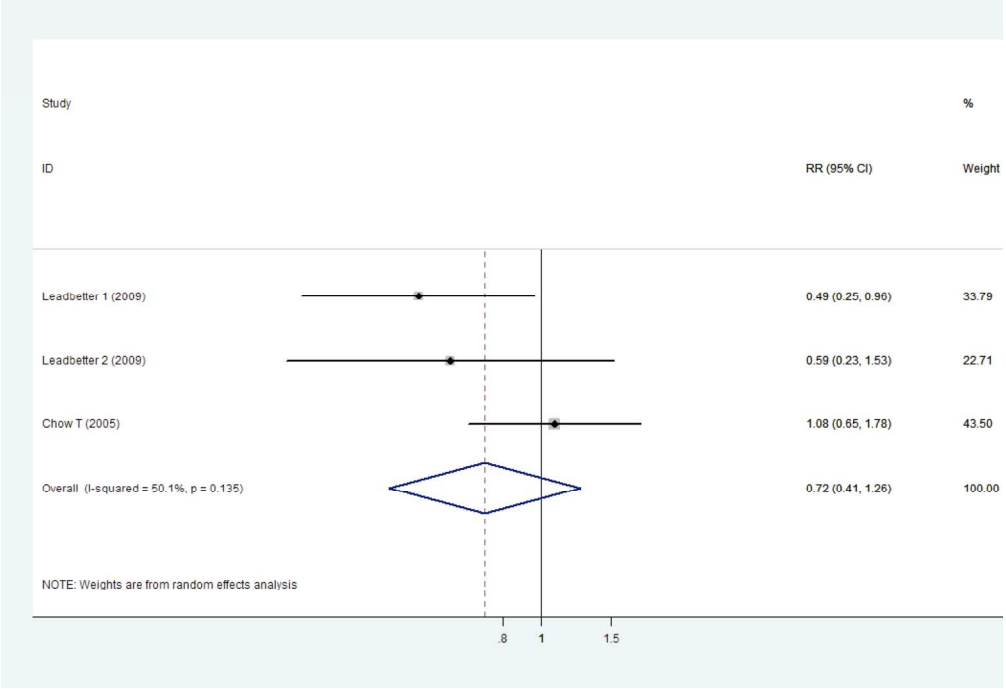

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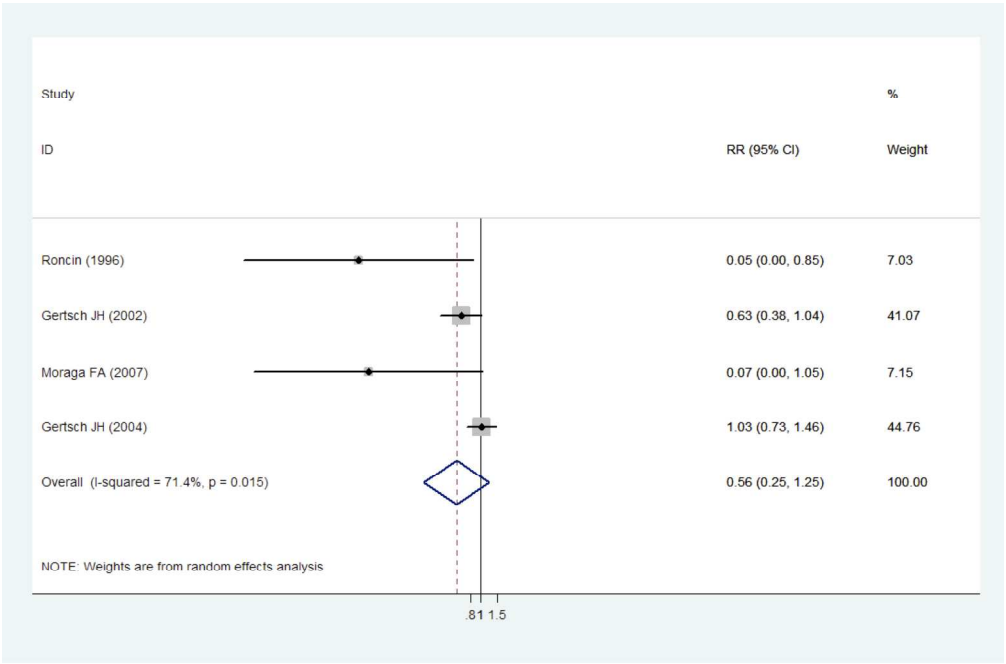

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N.A.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N.A.

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Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Primary Subject Heading:	Sports and exercise medicine
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Keywords:	Ginkgo Biloba Extract, Acute Mountain Sickness, meta-analysis

Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Author contributions:

TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Keywords: Ginkgo Biloba Extract (GBE), Acute Mountain Sickness (AMS)

Abstract

Study objective:

Trials of ginkgo biloba extract (GBE) for the prevention of acute mountain sickness (AMS) have been published since 1996. Because of their conflicting results, the efficacy of GBE remains unclear. We performed a systematic review and meta-analysis to assess whether GBE prevents acute mountain sickness.

Methods:

The Cochrane Library, EMBASE, Google Scholar, and PubMed databases were searched for articles published up to May 20, 2017. Only randomized controlled trials were included. AMS defined as acute mountain sickness–cerebral(AMS-C) score≧0.7 or Lake Louise Score (LLS)≧3 with headache. The main outcome measures were the relative risks of AMS in participants receiving GBE for prophylaxis. Meta-analyses were conducted using random-effects models. Sensitivity analyses, subgroup analyses and tests for publication bias were conducted.

Results:

Six published articles with a total of 451 participants met all eligibility criteria. In the primary meta-analysis of all 7 study groups, GBE showed trend of AMS prophylaxis, but it is not statistically significant (RR =0.68; 95% CI: 0.45 to 1.04; p-value=0.08). The I^2 statistic was 58.7% (p-value=0.02), indicating substantial heterogeneity. The results of subgroup analyses of studies with low risk of bias, low starting altitude (<2500 m), number of treatment days before ascending and dosage of GBE were similar.

Conclusions:

The currently available data suggest that although GBE may tend toward AMS prophylaxis, there are not enough data to show the statistically significant effect of GBE for preventing

AMS. Further large randomized control studies are warranted.

Strengths and limitations of this study

- This study is, to date, the first systematic review and meta-analysis evaluating Ginkgo Biloba Extract (GBE) as an Acute Mountain Sickness (AMS) prophylactic, strengthened by a thorough quality assessment of each enrolled study and comprehensive subgroup analyses.
- In the pooled analyses, although GBE may tend toward AMS prophylaxis, it is not statistically significant. Subgroup analyses of low-risk bias studies, studies with low starting altitude, number of treatment days before ascending and dosage of GBE also revealed the similar results.
- Insufficient power may be an issue in this meta-analysis. Further large randomized control studies are warranted.

Introduction

Background

Rapid ascent from low to high altitude (> 2500 m above the sea level) is often followed by headache, fatigue, shortness of breath, sleeplessness, and anorexia, a symptom complex called acute mountain sickness (AMS).[1] Lake Louise Score (LLS) Questionnaires[2] and Environmental Symptom Questionnaire III[3] are two tools to diagnose and evaluate severity of AMS. AMS is more likely to happen at altitudes higher than 2500 m,[4] and worldwide studies reported incidences of AMS of 25–37% at 1900–3400 m.[1 5] Children are more prone to develop AMS, with an incidence of 59%.[6]

The pathophysiology of AMS is associated with cerebral edema, with the most

compelling evidence coming from the brain MRI study of Hackett et al.,[7] which showed intense T2 signals in the white matter, particularly in the splenium and corpus callosum. Vasogenic leakage increases permeability of the endothelium, causing an elevation in intravascular pressures and inducing hypoxemia. In addition, hypoxic ventilatory response and activation of the renin-angiotensin–aldosterone system are also reported to be associated with AMS.[8] The most effective method to prevent AMS is gradual ascent. The most common pharmacologic agent used to prevent AMS is acetazolamide.[9] However, acetazolamide can cause paresthesia, dysgeusia, and sometimes nausea or drowsiness.[10] Its use is also contraindicated in patients with a history of anaphylaxis to sulfa antibiotics or acetazolamide.

Importance

Ginkgo biloba extract (GBE) is an option for those seeking a natural alternative treatment. GBE is found to decreases the tissue hypoxia, induces vasodilation, reduces free-radical production and lung leak, which may in turn prevent AMS. [11-14] Roncin et al. in 1996 published the first studies to suggest that GBE can prevent AMS.[15] However, not all subsequent studies have shown benefit.[13 16-20] To date, there is no best evidence to support the effectiveness of GBE.

Goals of This Investigation

Our study aim was to assess the effectiveness of GBE in prophylaxis of AMS by conducting a meta-analysis and systematic review of the relevant literature.

Methods

Databases and search strategy

We searched the Cochrane Library, EMBASE, Google Scholar, and PubMed databases for

articles published up to May 20th, 2017. No limits were applied to our Boolean search strategy, which included keywords ('Ginkgo', 'Altitude Sickness', 'Mountain'), Medical Subject Headings (MeSH) ('Ginkgo biloba', 'Altitude Sickness'), and Emtree terms ('Ginkgo biloba', 'altitude disease'). References from retrieved articles were also examined to identify other relevant articles.

Studies were included in the systematic review if they were (1) randomized controlled trials (RCTs) of healthy non-acclimatized adult between age 18 and 60 years; (2) compared GBE with placebo; (3) conducted in humans; and (4) studies diagnosing AMS with the Lake Louise Score or AMS-C. We excluded studies which subjects were pregnant, had symptoms consistent with AMS at baseline. Studies were also excluded if they were irrelevant to the study's aim, were animal studies, lacked a placebo group, or were published as review articles, case reports, editorials, or letters. The systematic review and the meta-analysis was conducted under the PRISMA guidelines (see online supplementary Checklist). The Institutional Review Board of Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taiwan, approved the protocol.

Outcome measures

AMS defined as AMS-C score≧0.7 or an LLS≧3 with headache. Primary outcome were the relative risks of AMS in participants receiving GBE for prophylaxis. Secondary outcomes in the enrolled studies are summarized as the supplementary table. We only extracted data when they were available in dichotomous form.

Data extraction and assessment of methodological quality

Two reviewers (TYT and YCS) independently screened titles and abstracts of all articles identified by the search strategy. Inter-reviewer disagreements concerning the inclusion or exclusion of a study were resolved by consensus and, if necessary, consultation with a third

reviewer (SHW).

The Cochrane Collaboration's tool was used to assess the risk of selection, performance, detection, attrition, and reporting biases in the included randomized trials.[21] We defined studies as "high risk of bias" if one or more key domains is taken as high risk in the checklist. All co-authors discussed and made the final decisions about the overall risk of bias in the included trials. If data were not readily available or clear, we contacted first authors and corresponding authors to get further information. If studies were found to be at high risk of bias, meta-analyses stratified by study quality were performed.

Both reviewers independently extracted data from the articles selected for inclusion. The extracted data included the name of the first author, year of publication, numbers of participants, gender, starting and final altitudes, AMS scoring definitions, prescriptions of GBE, days of treatment prior to ascent, and number of individuals with AMS in the treatment and control groups.

Patient and Public Involvement

patients and or public were not involved directly in the systemic reviews.

Data collection, data processing, and primary data analysis

Pooled relative risk (RR) with corresponding 95% confidence intervals (CIs) for each outcome of interest were calculated. The main outcome measure was the RR of AMS in participants receiving GBE for prophylaxis. Random effect models with DerSimonian and Laird method were selected for these analyses.

We conducted subgroup analyses based on quality of studies, starting altitude, number of treatment days before ascending, and dosage of GBE.[22-24] Between-study heterogeneity was evaluated with the I^2 statistic.[25] The Egger regression asymmetry test and Begg adjusted rank correlation test were applied for assessment of potential publication bias.[26 27] We also

conducted sensitivity analysis to evaluate the influence of each study on the overall pooled estimate. Analyses were all conducted using STATA version 11.0 (StataCorp, College Station, Texas, USA). All statistical tests were two-sided and were considered significant when the P value was 0.05 or less.

Results

The literature search and study selection process are summarized in Figure 1. After the exclusion of duplicate studies, non-relevant studies, and other studies that met exclusion criteria based on a screening of article titles and abstracts, 38 potentially relevant studies were retrieved for full review.

One publication was retrieved by hand search of the references. In this study, Wang et al.[28] compared the prophylactic effect of GBE with that of other Chinese medications on AMS. However, the study had no placebo group design[29] and had to be excluded from our meta-analysis.

In the randomized double-blind study by Ke in 2013,[20] AMS was reported as a secondary outcome and the number of events in each group were not reported. We contacted the first and corresponding authors by email but (as of October 9, 2017) received no response. Since the published data could not be included for analysis, we excluded this study.

Six published articles met all eligibility criteria after a careful review process.[13 15-19] In the article published by Leadbetter et al.,[19] two randomized controlled trials were conducted. As a result, a total of 7 study groups with 451 participants were enrolled. The characteristics of these studies and the participants are listed in Table 1. Four study groups[13 15 16 19] demonstrated the efficacy of GBE in preventing AMS, while three[17-19] did not. All

studies had small numbers of subjects except the one by Gertsch and colleagues.[17] Of note, participants in the study conducted by Gertsch et al. published in 2004, started GBE treatment at high altitude (4280–4358 m), which was different from the other studies. Further information such as study dosage, prescription frequency, number of days prior to ascending, and source of GBE are summarized in Table 2. The number of AMS events and its incidence are summarized in Figure 2. The evidence quality of these studies as assessed by Cochrane Collaboration's tool is presented in Table 3. Two of 6 articles were not double-blinded and both of them included male participants only.[13 15] The study conducted by Gertsch et al.in 2002, used "first-come first-served basis" after receiving signed consent. Therefore, we judge it as "unclear random-sequence generation".[16] In addition, we appraisal it as incomplete outcome data (attrition bias) because the study presented data on only 26 subjects when the intention was to enroll 100 subjects.

In the primary meta-analysis of all 7 study groups, GBE showed trend of AMS prophylaxis, but it is not statistically significant (RR =0.68; 95% CI: 0.45 to 1.04; p-value=0.08) (Figure 2). The I^2 statistic was 58.7% (p-value=0.02), indicating substantial heterogeneity. Pooled risk difference is summarized in the additional supplementary figure 1. After excluding three high-risk-bias studies,[13 15 16] the I^2 statistic became 40.2% (p-value=0.17) and the result did not change (RR =0.84; 95% CI 0.59 to 1.21; p-value=0.36). The Egger's-test and Begg-test (p-values, 0.22 and 0.31, respectively) indicate the absence of statistical evidence of publication bias after excluding our presumed high-risk-bias articles.

Sensitivity analysis was conducted by removing one trial at a time to determine what influence each study had on the pooled analysis. The pooled result seemed to be robust. For example, removing the study conducted by Leadbetter et al. in 2009[19] only changed the pooled estimate from 0.79 to 0.74 (95% CI 0.48–1.16; p-value=0.19; see supplementary

figure 2).

The results of several pre-planned subgroup analyses were similar. Excluding the study by Gertsch and colleagues in 2004,[17] GBE was not prophylactic when the starting altitude was below 2500 m (RR =0.56; 95% CI 0.31 to 1.01)[13 15 16 18 19]. Regarding the number of treatment days before ascending, GBE was not prophylactic when given "3–5 days prior to ascent"[18 19] (RR =0.72; 95% CI 0.41 to 1.26) or "0–2 days prior to ascent" [13 15-17](RR =0.56; 95% CI 0.25 to 1.25). Dosage of GBE was also not prophylactic for AMS when given "less than 200mg per day"[13 15 16] (RR =0.16; 95% CI 0.01 to 2.57) or "more than 200mg per day"[17-19] (RR =0.84; 95% CI 0.59 to 1.21).

Discussion

To our knowledge, this is the first meta-analysis of RCTs evaluating GBE as an AMS prophylactic. In pooled analyses, we found that although GBE may tend toward AMS prophylaxis, it had no statistically significant prophylactic effect (RR =0.68; 95% CI: 0.45 to 1.04; p-value=0.08). The results of several subgroup analyses were similar. GBE also failed to show benefits in preventing AMS in low-risk bias studies, studies in which the starting altitude was low, studies differing in the initial treatment regimen prior to ascent, and different dosage of GBE.

The effectiveness of GBE in AMS prophylaxis has been reported.[13 15 16 19] Zhang and colleagues in 2003 reported that GBE was the most effective of six Chinese medicines tested for AMS prophylaxis.[29] GBE has been used primarily for the treatment of dementias (e.g., Alzheimer's disease), peripheral vascular diseases (e.g., intermittent claudication), and neurosensory problems (e.g., tinnitus).[30] Hypotheses have been proposed to explain the possible role that GBE plays in preventing AMS. Hypoxia is a common feature of AMS. Several studies have suggested that nitric oxide (NO) may play a pathogenic role in AMS by

mediating hypoxia-induced cerebral vasodilation in humans.[11-13] GBE was found to be an NO scavenger. NO scavenging can result in decreased intracellular NO level.[14] Furthermore, GBE may inhibit phosphodiesterase activity, thus enhancing relaxation of parietal smooth muscle cells and so lead to vasodilation of parietal vessels. Vasodilation in turn increases tissue perfusion and decreases local hypoxia.[14] Other potential mechanisms include increasing endogenous antioxidants,[31] reducing free-radical production,[32] and reducing lung leak during hypoxia.[33] GBE was also shown to prevent high altitude pulmonary edema in a rat model.[34]

On the other hand, several studies failed to demonstrate the benefit of GBE in AMS prophylaxis.[17 18 20] The duration of therapy before ascent, dosage of GBE, and differences in the altitude at which GBE is initiated may account for the conflicts between trial results. To test these hypotheses, we conducted subgroup analyses and obtained similar results to those obtained with the original pooled data. Another explanation for the differences in efficacy may be variation in the GBE composition. For instance, Leadbetter and colleagues in 2009 compared GBE from two different sources and found they differed in composition as well as ability to reduce the incidence and severity of AMS following rapid ascent to high altitude.[19] The German Federal Institute for Drugs and Medicinal Devices Commission E recommends similar specifications for standardization of GBE. All included studies used GBE that met the German E commission standard, but most of studies use products from different companies. As an herbal supplement, more than 60% of GBE component is not mandated by law and composition may vary considerably between manufacturers. A lack of bioequivalence has been noted between brands of GBE.[35 36]

Limitations

Our systematic review has several limitations. First, to limit the influence of study biases

on pooled evaluation, we decided to only include RCTs. However, there were few RCTs in this field. Moreover, only 4 of 6 RCTs were double-blinded. Second, because of the difficulty in carrying out high altitude medicine studies, many studies involved only a small number of cases. In our primary pooled analysis, a total of 451 participants were enrolled. Insufficient power may be an issue in this meta-analysis. There are not enough data to show the statistically significant effect of GBE for preventing AMS, and further studies are warranted. Third, the participants were predominantly adult males and whether there is gender or age difference between treatment (GBE vs placebo) groups or response (no AMS vs AMS) groups is unknown. Fourth, GBE is a complex mixture of natural components. It is difficult to standardize all components. A lack of consistency between commercially available GBE preparations may explain these differing results. Finally, differences between studies in factors such as the strength, rate of ascent, and other characteristics of participants may also account for inconsistent results.

Conclusion

The currently available data suggest that although GBE may tend toward AMS prophylaxis, there are not enough data to show the statistically significant effect of GBE for preventing AMS. Further large randomized control studies are warranted.

Table and Figure Legends

Figure 1. Trial selection algorithm

Figure 2. Events of acute mountain sickness between placebo and GBE, and forest plot of meta-analysis.

Table 1. Characteristics of included studies

Table 2. Characteristics of included studies, sources, dosage and duration of GBE

Table 3. Risk of bias in included studies.

Declarations

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests in this section.

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Author contributions

TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS analyzed the data and contribute in the manuscript formation. All authors read and approved the final manuscript.

Transparency declaration

We affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Table 1. Characteristics of included studies.

	Participants (number)	Male (%)	Starting altitude (m)	Altitude reached (m)	Ascent rate(m/h)	AMS definition
Roncin, 1996	44	100	1,800	5,400	15	AMS-C >0.7
Gertsch, 2002	26	46	0	4,205	1402	LLS ≥3 with HA
Gertsch, 2004	243	70	4,280–4,358	4,928	10-20	LLS ≥3 with HA
Chow, 2005	37	54	1,230	3,800	1285	LLS ≥3 with HA
Moraga, 2007	24	100	0	3,696	435	LLS ≥3, or 1 symptom score≥3
Leadbetter, 2009 Study 1	40	45	2,000	4,300	1150	AMS-C ≥0. 7 + LLS ≥3 with HA
Leadbetter, 2009 Study 2	37	44	2,000	4,300	1150	AMS-C ≥0. 7 + LLS ≥3 with HA

GBE: ginkgo biloba extract; AMS: Acute mountain sickness; AMS-C: the Environmental Symptom Questionnaire III acute mountain sickness-cerebral (AMS-C) score; HA: headache; LLS: Lake Louise Score.

Table 2. Characteristics of included studies, sources, dosage and duration of ginkgo biloba.

	GBE source	Dose	Days of treatment prior to ascent
Roncin, 1996	Tanakan® DCI: EGb 761 Ipsen, Paris, France	60 mg BID	0
Gertsch, 2002	GK501 Memfit ® , EGb 761, Pharmaton	60 mg TID	1
Gertsch, 2004	GK501 International, Pharmaton	120 mg BID	1–2
Chow, 2005	*Gingko biloba* 120 mg Vegetarian NOW ® Foods	120 mg BID	5
Moraga, 2007	EGb 761 Rokan, Andromeco Laboratories, Chile	80 mg BID	1
Leadbetter, 2009 Study 1	Spectrum Quality, Laboratories Products, Inc.	120 mg BID	4
Leadbetter, 2009 Study 2	Technical Sourcing, Inc.	120 mg BID	3

BID: Bi in die=twice a day; TID: ter in die=three times a day.

Table 3. Risk of bias in included studies.

Risk of bias domain	Roncin, 1996	Gertsch, 2002	Gertsch, 2004	Chow, 2005	Moraga, 2007	Leadbetter, 2009
Random-sequence generation (selection bias)	Unclear	Unclear	Low	Low	Low	Low
Allocation concealment (selection bias)	Unclear	Low	Low	Low	Unclear	Low
Blinding of participants (performance bias)	High	Low	Low	Low	High	Low
Blinding of outcome assessment (detection bias)	High	Low	Low	Low	High	Low
Incomplete outcome data (attrition bias)	High	High	Low	Low	Low	Low
Selective outcome reporting (reporting bias)	Low	Low	Low	Low	Low	Low
Other source of bias	High	Low	High	Low	High	Low
Overall risk of bias	**High**	**High**	**Low**	**Low**	**High**	**Low**

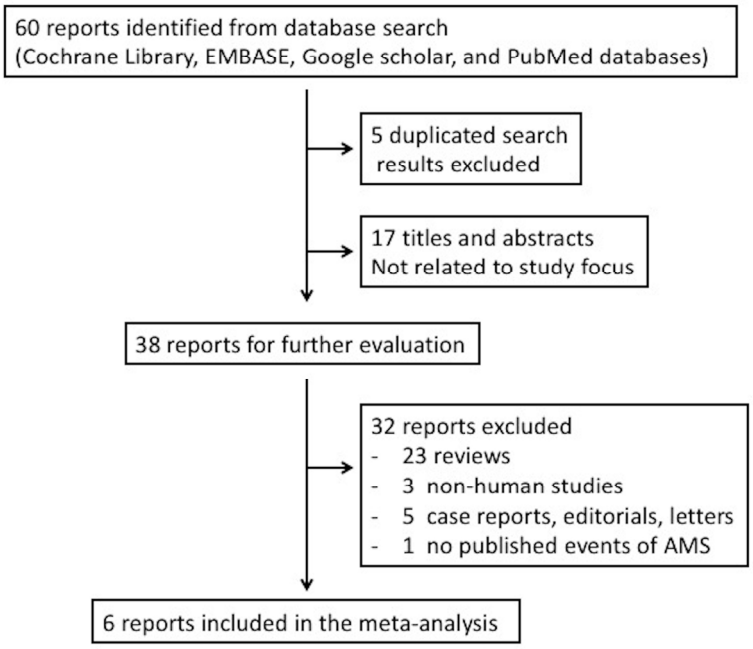

Figure 1

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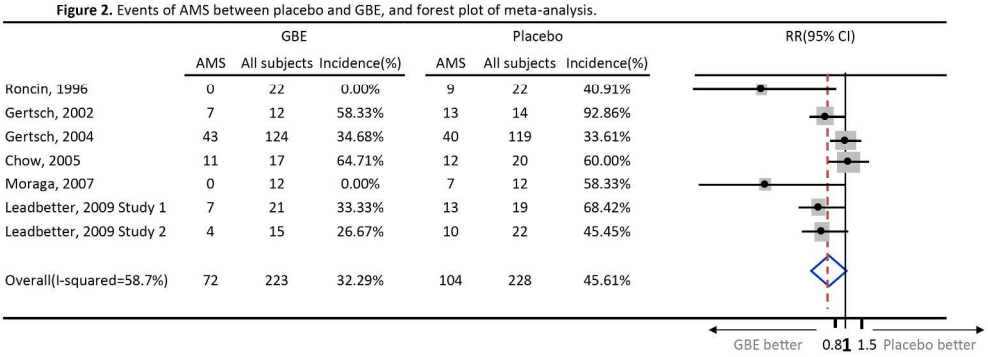

Figure 2. Events of acute mountain sickness between placebo and GBE, and forest plot of meta-analysis.

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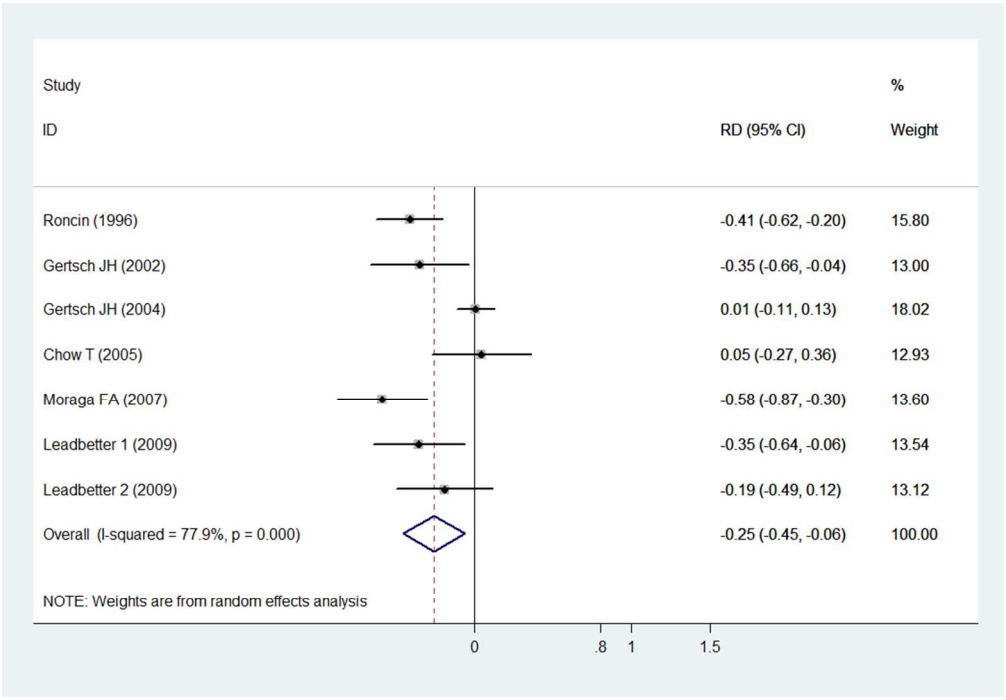
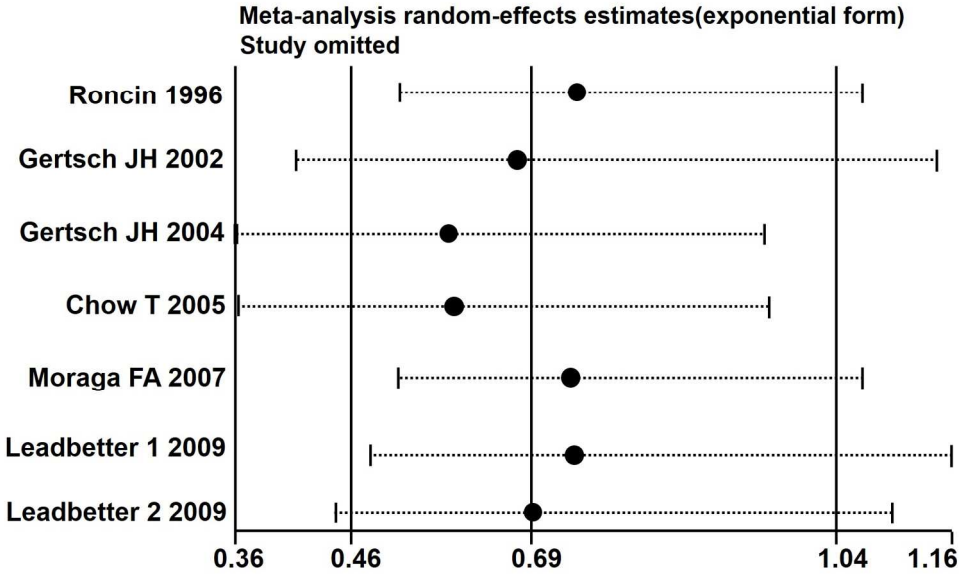


88x61mm (300 x 300 DPI)



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Appendix Table. Secondary outcomes of included studies

		Incidence of severe AMS	Headache	Severe headache	Oxygen saturation	Pulmonary edema	Adverse events
Roncin, 1996	GBE	X	22/22 (100%)	0/22 (0%)	X	AMS-R >0.6 3/21 (13.6%)	GBE(18.2%) is less urine than Placebo(77.3%)
	Placebo	X	22/22 (100%)	0/22 (0%)	X	AMS-R >0.6 18/22 (81.8%)	
Gertsch, 2002	GBE	2/12 (16.7%)	X	1/12 (8%)	81%	X	No side effect in GBE
	Placebo	9/14 (64.3%)	X	1/14 (7%)	80%	X	
Gertsch, 2004	GBE	23/124 (18%)	72/124 (58%)	24/124 (19%)	79.5%	Non occurred	No side effect in GBE
	Placebo	22/119 (18%)	63/119 (53%)	16/119 (13%)	82.1% P<0.01		
Chow, 2005	GBE	X	GBE is 5% less than placebo		X	Non occurred	No side effect in GBE
	Placebo	X			X		
Moraga, 2007	GBE	X	LLS score, headache item 0.19±0.41		92±2%	X	X
	Placebo	X	1.28±0.14 P<0.05		84±3% P<0.01	X	X
Leadbetter, 2009(Study 1)	GBE	0/21 (0%)	X	X	X	X	X
	Placebo	3/19 (16%)	X	X	X	X	X
Leadbetter, 2009(Study 2)	GBE	3/15 (20%)	X	X	X	X	X
	Placebo	4/22 (18%)	X	X	X	X	X

AMS: Acute mountain sickness ; AMS-R: the Environmental Symptom Questionnaire III acute mountain sickness-Respiratory (AMS-R) score; GBE: ginkgo biloba extract; LLS: Lake Louise Score; X: not mentioned in the study.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N.A.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N.A.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Keywords:	Ginkgo Biloba Extract, Acute Mountain Sickness, meta-analysis

Ginkgo Biloba Extract for Prevention of Acute Mountain Sickness: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Author contributions:

TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: 10.5061/dryad.35h13bg.

Keywords: Ginkgo Biloba Extract (GBE), Acute Mountain Sickness (AMS)

Abstract

Study objective:

Trials of ginkgo biloba extract (GBE) for the prevention of acute mountain sickness (AMS) have been published since 1996. Because of their conflicting results, the efficacy of GBE remains unclear. We performed a systematic review and meta-analysis to assess whether GBE prevents acute mountain sickness.

Methods:

The Cochrane Library, EMBASE, Google Scholar, and PubMed databases were searched for articles published up to May 20, 2017. Only randomized controlled trials were included. AMS defined as acute mountain sickness–cerebral(AMS-C) score≧0.7 or Lake Louise Score (LLS)≧3 with headache. The main outcome measures were the relative risks of AMS in participants receiving GBE for prophylaxis. Meta-analyses were conducted using random-effects models. Sensitivity analyses, subgroup analyses and tests for publication bias were conducted.

Results:

Seven study groups in 6 published articles met all eligibility criteria, including the article published by Leadbetter et al. which two randomized controlled trials were conducted. Overall, 451 participants were enrolled. In the primary meta-analysis of all 7 study groups, GBE showed trend of AMS prophylaxis, but it is not statistically significant (RR =0.68; 95% CI: 0.45 to 1.04; p-value=0.08). The I^2 statistic was 58.7% (p-value=0.02), indicating substantial heterogeneity. The pooled risk difference (RD) revealed a significant risk reduction in participants with GBE use. (RD= -25%; 95% CI, from a reduction of 45% to 6%; p-value=0.011) The results of subgroup analyses of studies with low risk of bias, low starting altitude (<2500 m), number of treatment days before ascending and dosage of GBE are not

statistically significant.

Conclusions:

The currently available data suggest that although GBE may tend toward AMS prophylaxis, there are not enough data to show the statistically significant effect of GBE for preventing AMS. Further large randomized control studies are warranted.

Strengths and limitations of this study

- This meta-analysis is the first systematic review and meta-analysis evaluating Ginkgo Biloba Extract (GBE) as an Acute Mountain Sickness (AMS) prophylactic.
- This meta-analysis strengthened by a thorough quality assessment of each enrolled study and comprehensive subgroup analyses.
- There is notable heterogeneity and the small number of studies limits the analyses, but heterogeneity decreased after excluding studies with high risk of bias.
- Insufficient power may be an issue in this meta-analysis. Further large randomized control studies are warranted.

Introduction

Background

Rapid ascent from low to high altitude (> 2500 m above the sea level) is often followed by headache, fatigue, shortness of breath, sleeplessness, and anorexia, a symptom complex called acute mountain sickness (AMS).[1] Lake Louise Score (LLS) Questionnaires[2] and Environmental Symptom Questionnaire III[3] are two tools to diagnose and evaluate severity of AMS. AMS is more likely to happen at altitudes higher than 2500 m,[4] and worldwide studies reported incidences of AMS of 25–37% at 1900–3400 m.[1 5] Children are more prone

to develop AMS, with an incidence of 59%.[6]

The pathophysiology of AMS is associated with cerebral edema, with the most compelling evidence coming from the brain MRI study of Hackett et al.,[7] which showed intense T2 signals in the white matter, particularly in the splenium and corpus callosum. Vasogenic leakage increases permeability of the endothelium, causing an elevation in intravascular pressures and inducing hypoxemia. In addition, hypoxic ventilatory response and activation of the renin-angiotensin–aldosterone system are also reported to be associated with AMS.[8] The most effective method to prevent AMS is gradual ascent. The most common pharmacologic agent used to prevent AMS is acetazolamide.[9] However, acetazolamide can cause paresthesia, dysgeusia, and sometimes nausea or drowsiness.[10] Its use is also contraindicated in patients with a history of anaphylaxis to sulfa antibiotics or acetazolamide.

Importance

Ginkgo biloba extract (GBE) is an option for those seeking a natural alternative treatment. GBE is found to decreases the tissue hypoxia, induces vasodilation, reduces free-radical production and lung leak, which may in turn prevent AMS. [11-14] Roncin et al. in 1996 published the first studies to suggest that GBE can prevent AMS.[15] However, not all subsequent studies have shown benefit.[13 16-20] To date, there is no best evidence to support the effectiveness of GBE.

Goals of This Investigation

Our study aim was to assess the effectiveness of GBE in prophylaxis of AMS by conducting a meta-analysis and systematic review of the relevant literature.

Methods

Databases and search strategy

We searched the Cochrane Library, EMBASE, Google Scholar, and PubMed databases for articles published up to May 20th, 2017. No limits were applied to our Boolean search strategy, which included keywords ('Ginkgo', 'Altitude Sickness', 'Mountain'), Medical Subject Headings (MeSH) ('Ginkgo biloba', 'Altitude Sickness'), and Emtree terms ('Ginkgo biloba', 'altitude disease'). The full search strategy for database is provided in the supplementary file. References from retrieved articles were also examined to identify other relevant articles.

Studies were included in the systematic review if they were (1) randomized controlled trials (RCTs) of healthy non-acclimatized adult between age 18 and 60 years; (2) compared GBE with placebo; (3) conducted in humans; and (4) studies diagnosing AMS with the Lake Louise Score or AMS-C. We excluded studies which subjects were pregnant, had symptoms consistent with AMS at baseline. Studies were also excluded if they were irrelevant to the study's aim, were animal studies, lacked a placebo group, or were published as review articles, case reports, editorials, or letters. The systematic review and the meta-analysis was conducted under the PRISMA guidelines (see online supplementary Checklist). The Institutional Review Board of Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taiwan, approved the protocol.

Outcome measures

AMS defined as AMS-C score≧0.7 or an LLS≧3 with headache. Primary outcome were the relative risks of AMS in participants receiving GBE for prophylaxis. We only extracted data when they were available in dichotomous form. Secondary outcomes of included studies were summarized in supplementary Table 1.

Data extraction and assessment of methodological quality

Two reviewers (TYT and YCS) independently screened titles and abstracts of all articles identified by the search strategy. Inter-reviewer disagreements concerning the inclusion or exclusion of a study were resolved by consensus and, if necessary, consultation with a third reviewer (SHW).

The Cochrane Collaboration's tool was used to assess the risk of selection, performance, detection, attrition, and reporting biases in the included randomized trials.[21] We defined studies as "high risk of bias" if one or more key domains is taken as high risk in the checklist. All co-authors discussed and made the final decisions about the overall risk of bias in the included trials. If data were not readily available or clear, we contacted first authors and corresponding authors to get further information. If studies were found to be at high risk of bias, meta-analyses stratified by study quality were performed.

Both reviewers independently extracted data from the articles selected for inclusion. The extracted data included the name of the first author, year of publication, numbers of participants, gender, starting and final altitudes, AMS scoring definitions, prescriptions of GBE, days of treatment prior to ascent, and number of individuals with AMS in the treatment and control groups.

Data collection, data processing, and primary data analysis

Pooled relative risks (RR) with corresponding 95% confidence intervals (CIs) are derived for all studies and different subgroups of interest. The main outcome measure was the RR of AMS in participants receiving GBE for prophylaxis. Random effect models with DerSimonian and Laird method were selected for these analyses. The pooled risk difference (RD) was also measured as the alternative outcome. The pooled RD is the difference between the observed risks (proportions of participants with AMS) in the two groups.

We conducted subgroup analyses based on quality of studies, starting altitude, number

of treatment days before ascending, and dosage of GBE.[22-24] Between-study heterogeneity was evaluated with the I^2 statistic.[25] The Egger regression asymmetry test and Begg adjusted rank correlation test were applied for assessment of potential publication bias.[26 27] We also conducted sensitivity analysis to evaluate the influence of each study on the overall pooled estimate. For the zero cells dealing we add 0.5 to all cells of the 2 × 2 table for the study. Analyses were all conducted using STATA version 11.0 (StataCorp, College Station, Texas, USA). All statistical tests were two-sided and were considered significant when the P value was 0.05 or less.

Patient and Public Involvement statement

Participants and the public sector were not directly involved in the design and conduct of this study.

Results

The literature search and study selection process are summarized in Figure 1. After the exclusion of duplicate studies, non-relevant studies, and other studies that met exclusion criteria based on a screening of article titles and abstracts, 38 potentially relevant studies were retrieved for full review.

One publication was retrieved by hand search of the references. In this study, Wang et al.[28] compared the prophylactic effect of GBE with that of other Chinese medications on AMS. However, the study had no placebo group design[29] and had to be excluded from our meta-analysis.

In the randomized double-blind study by Ke in 2013,[20] AMS was reported as a secondary outcome and the number of events in each group were not reported. We contacted the first and corresponding authors by email but (as of June 12, 2018) received no

response. Since the published data could not be included for analysis, we excluded this study.

Six published articles met all eligibility criteria after a careful review process.[13 15-19] In the article published by Leadbetter et al.,[19] two randomized controlled trials were conducted. As a result, a total of 7 study groups with 451 participants were enrolled. The characteristics of these studies and the participants are listed in Table 1. Four study groups[13 15 16 19] demonstrated the efficacy of GBE in preventing AMS, while three[17-19] did not. All studies had small numbers of subjects except the one by Gertsch and colleagues.[17] Of note, participants in the study conducted by Gertsch et al. published in 2004, started GBE treatment at high altitude (4280–4358 m), which was different from the other studies. Further information such as study dosage, prescription frequency, number of days prior to ascending, and source of GBE are summarized in Table 2. The number of AMS events and its incidence are summarized in Figure 2. The evidence quality of these studies as assessed by Cochrane Collaboration's tool is presented in Table 3. Two of 6 articles were not double-blinded and both of them included male participants only.[13 15] The study conducted by Gertsch et al.in 2002, used "first-come first-served basis" after receiving signed consent. Therefore, we judge it as "unclear random-sequence generation".[16] In addition, we appraisal it as incomplete outcome data (attrition bias) because the study presented data on only 26 subjects when the intention was to enroll 100 subjects.

In the primary meta-analysis of all 7 study groups, GBE showed trend of AMS prophylaxis, but it is not statistically significant (RR =0.68; 95% CI: 0.45 to 1.04; p-value=0.08) (Figure 2). The I^2 statistic was 58.7% (p-value=0.02), indicating substantial heterogeneity. The pooled RD revealed a significant risk reduction in participants with GBE use. (RD= -25%; 95% CI, from a reduction of -45% to -6%; p-value<0.001) (Figure 3). After excluding three high-risk-bias

studies,[13 15 16] the I^2 statistic became 40.2% (p-value=0.17) and the result did not change (RR =0.84; 95% CI 0.59 to 1.21; p-value=0.36). In the same subgroup the pooled RD are also not statistically significant. (RD= -9.7%; 95% CI, from a reduction of -27.4% to 7.9%; p-value=0.28). The Egger's-test and Begg-test (p-values, 0.22 and 0.31, respectively) indicate the absence of statistical evidence of publication bias after excluding our presumed high-risk-bias articles.

Sensitivity analysis was conducted by removing one trial at a time to determine what influence each study had on the pooled analysis. The pooled result seemed to be robust. For example, removing the study conducted by Leadbetter et al. in 2009[19] only changed the pooled estimate from 0.68 to 0.74 (95% CI 0.48–1.16; p-value=0.19; see supplementary figure 1).

The results of several pre-planned subgroup analyses were similar. Excluding the study by Gertsch and colleagues in 2004,[17] GBE was not prophylactic when the starting altitude was below 2500 m (RR =0.56; 95% CI 0.31 to 1.01)[13 15 16 18 19]. Regarding the number of treatment days before ascending, GBE was not prophylactic when given "3–5 days prior to ascent"[18 19] (RR =0.72; 95% CI 0.41 to 1.26) or "0–2 days prior to ascent" [13 15-17](RR =0.56; 95% CI 0.25 to 1.25). Dosage of GBE was also not prophylactic for AMS when given "less than 200mg per day"[13 15 16] (RR =0.16; 95% CI 0.01 to 2.57) or "more than 200mg per day"[17-19] (RR =0.84; 95% CI 0.59 to 1.21). Information regarding number of participants and enrolled studies in each subgroup are summarized in supplementary table 2.

Discussion

To our knowledge, this is the first meta-analysis of RCTs evaluating GBE as an AMS prophylactic. In pooled analyses, we found that although GBE may tend toward AMS prophylaxis, it had no statistically significant prophylactic effect (RR =0.68; 95% CI: 0.45 to

1.04; p-value=0.08). The results of several subgroup analyses were similar. GBE also failed to show benefits in preventing AMS in low-risk bias studies, studies in which the starting altitude was low, studies differing in the initial treatment regimen prior to ascent, and different dosage of GBE.

The effectiveness of GBE in AMS prophylaxis has been reported.[13 15 16 19] Zhang and colleagues in 2003 reported that GBE was the most effective of six Chinese medicines tested for AMS prophylaxis.[29] GBE has been used primarily for the treatment of dementias (e.g., Alzheimer's disease), peripheral vascular diseases (e.g., intermittent claudication), and neurosensory problems (e.g., tinnitus).[30] Hypotheses have been proposed to explain the possible role that GBE plays in preventing AMS. Hypoxia is a common feature of AMS. Several studies have suggested that nitric oxide (NO) may play a pathogenic role in AMS by mediating hypoxia-induced cerebral vasodilation in humans.[11-13] GBE was found to be an NO scavenger. NO scavenging can result in decreased intracellular NO level.[14] Furthermore, GBE may inhibit phosphodiesterase activity, thus enhancing relaxation of parietal smooth muscle cells and so lead to vasodilation of parietal vessels. Vasodilation in turn increases tissue perfusion and decreases local hypoxia.[14] Other potential mechanisms include increasing endogenous antioxidants,[31] reducing free-radical production,[32] and reducing lung leak during hypoxia.[33] GBE was also shown to prevent high altitude pulmonary edema in a rat model.[34]

On the other hand, several studies failed to demonstrate the benefit of GBE in AMS prophylaxis.[17 18 20] The duration of therapy before ascent, dosage of GBE, and differences in the altitude at which GBE is initiated may account for the conflicts between trial results. To test these hypotheses, we conducted subgroup analyses and obtained similar results to those obtained with the original pooled data. Another explanation for the differences in

efficacy may be variation in the GBE composition. For instance, Leadbetter and colleagues in 2009 compared GBE from two different sources and found they differed in composition as well as ability to reduce the incidence and severity of AMS following rapid ascent to high altitude.[19] The German Federal Institute for Drugs and Medicinal Devices Commission E recommends similar specifications for standardization of GBE. All included studies used GBE that met the German E commission standard, but most of studies use products from different companies. As an herbal supplement, more than 60% of GBE component is not mandated by law and composition may vary considerably between manufacturers. A lack of bioequivalence has been noted between brands of GBE.[35 36]

Limitations

Our systematic review has several limitations. First, to limit the influence of study biases on pooled evaluation, we decided to only include RCTs. However, there were few RCTs in this field. Moreover, only 4 of 6 RCTs were double-blinded. Second, because of the difficulty in carrying out high altitude medicine studies, many studies involved only a small number of cases. In our primary pooled analysis, a total of 451 participants were enrolled. Insufficient power may be an issue in this meta-analysis. There are not enough data to show the statistically significant effect of GBE for preventing AMS, and further studies are warranted. Third, the participants were predominantly adult males and whether there is gender or age difference between treatment (GBE vs placebo) groups or response (no AMS vs AMS) groups is unknown. Fourth, GBE is a complex mixture of natural components. It is difficult to standardize all components. A lack of consistency between commercially available GBE preparations may explain these differing results. Finally, differences between studies in factors such as the strength, rate of ascent, and other characteristics of participants may also account for inconsistent results.

Conclusion

The currently available data suggest that although GBE may tend toward AMS prophylaxis, there are not enough data to show the statistically significant effect of GBE for preventing AMS. Further large randomized control studies are warranted.

Table and Figure Legends

Figure 1. Trial selection algorithm

Figure 2. Events of acute mountain sickness between placebo and GBE, and forest plot of meta-analysis.

Figure 3. Pooled risk difference of enrolled studies

Table 1. Characteristics of included studies

Table 2. Characteristics of included studies, sources, dosage and duration of GBE

Table 3. Risk of bias in included studies

Declarations

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests in this section.

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Author contributions

TYT analyzed and interpreted the data and was a major contributor in writing the manuscript. SHW interpreted the data. YKL supervised the study and interpreted the data. YCS analyzed the data and contribute in the manuscript formation. All authors read and approved the final manuscript.

Transparency declaration

We affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Table 1. Characteristics of included studies.

	Participants (number)	Male (%)	Starting altitude (m)	Altitude reached (m)	Ascent rate(m/h)	AMS definition
Roncin, 1996	44	100	1,800	5,400	15	AMS-C >0.7
Gertsch, 2002	26	46	0	4,205	1402	LLS ≥3 with HA
Gertsch, 2004	243	70	4,280–4,358	4,928	10-20	LLS ≥3 with HA
Chow, 2005	37	54	1,230	3,800	1285	LLS ≥3 with HA
Moraga, 2007	24	100	0	3,696	435	LLS ≥3, or 1 symptom score≥3
Leadbetter, 2009 Study 1	40	45	2,000	4,300	1150	AMS-C ≥0. 7 + LLS ≥3 with HA
Leadbetter, 2009 Study 2	37	44	2,000	4,300	1150	AMS-C ≥0. 7 + LLS ≥3 with HA

GBE: ginkgo biloba extract; AMS: Acute mountain sickness; AMS-C: the Environmental Symptom Questionnaire III acute mountain sickness-cerebral (AMS-C) score; HA: headache; LLS: Lake Louise Score.

Table 2. Characteristics of included studies, sources, dosage and duration of ginkgo biloba.

	GBE source	Dose	Days of treatment prior to ascent
Roncin, 1996	Tanakan® DCI: EGb 761 Ipsen, Paris, France	60 mg BID	0
Gertsch, 2002	GK501 Memfit ® , EGb 761, Pharmaton	60 mg TID	1
Gertsch, 2004	GK501 International, Pharmaton	120 mg BID	1–2
Chow, 2005	*Gingko biloba* 120 mg Vegetarian NOW ® Foods	120 mg BID	5
Moraga, 2007	EGb 761 Rokan, Andromeco Laboratories, Chile	80 mg BID	1
Leadbetter, 2009 Study 1	Spectrum Quality, Laboratories Products, Inc.	120 mg BID	4
Leadbetter, 2009 Study 2	Technical Sourcing, Inc.	120 mg BID	3

BID: Bi in die=twice a day; TID: ter in die=three times a day.

Table 3. Risk of bias in included studies.

Risk of bias domain	Roncin, 1996	Gertsch, 2002	Gertsch, 2004	Chow, 2005	Moraga, 2007	Leadbetter, 2009
Random-sequence generation (selection bias)	Unclear	Unclear	Low	Low	Low	Low
Allocation concealment (selection bias)	Unclear	Low	Low	Low	Unclear	Low
Blinding of participants (performance bias)	High	Low	Low	Low	High	Low
Blinding of outcome assessment (detection bias)	High	Low	Low	Low	High	Low
Incomplete outcome data (attrition bias)	High	High	Low	Low	Low	Low
Selective outcome reporting (reporting bias)	Low	Low	Low	Low	Low	Low
Other source of bias	High	Low	High	Low	High	Low
Overall risk of bias	**High**	**High**	**Low**	**Low**	**High**	**Low**

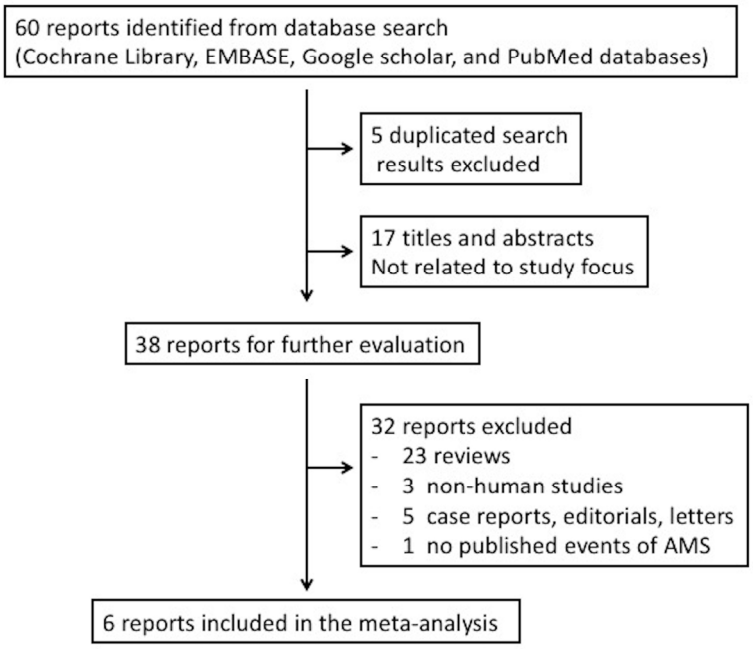

Figure 1

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Table 3. Events of acute mountain sickness compared between placebo and ginkgo biloba.

	Placebo			GBE			RR(95% CI)	% Weight
	AMS	All subjects	Incidence(%)	AMS	All subjects	Incidence(%)		
Roncin, 1996	9	22	40.91%	0	22	0.00%	0.05 (0.00 , 0.85)	2.13
Gertsch, 2002	13	14	92.86%	7	12	58.33%	0.63 (0.38 , 1.04)	21.09
Gertsch, 2004	40	119	33.61%	43	124	34.68%	1.03 (0.73 , 1.46)	24.85
Chow, 2005	12	20	60.00%	11	17	64.71%	1.08 (0.65 , 1.78)	21.05
Moraga, 2007	7	12	58.33%	0	12	0.00%	0.07 (0.00 , 1.05)	2.17
Leadbetter, 2009 Study 1	13	19	68.42%	7	21	33.33%	0.49 (0.25 , 0.96)	16.90
Leadbetter, 2009 Study 2	10	22	45.45%	4	15	26.67%	0.59 (0.23 , 1.53)	11.80
Overall(I-squared=58.7%, P=0.024)							0.68 (0.45 , 1.04)	100.00

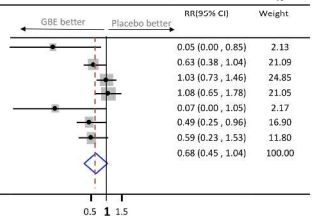

Figure 2. Events of acute mountain sickness between placebo and GBE, and forest plot of meta-analysis.

419x297mm (300 x 300 DPI)

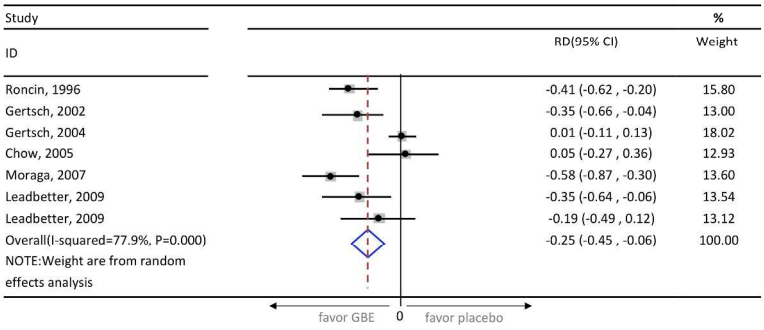

Figure 3. Pooled risk difference of enrolled studies

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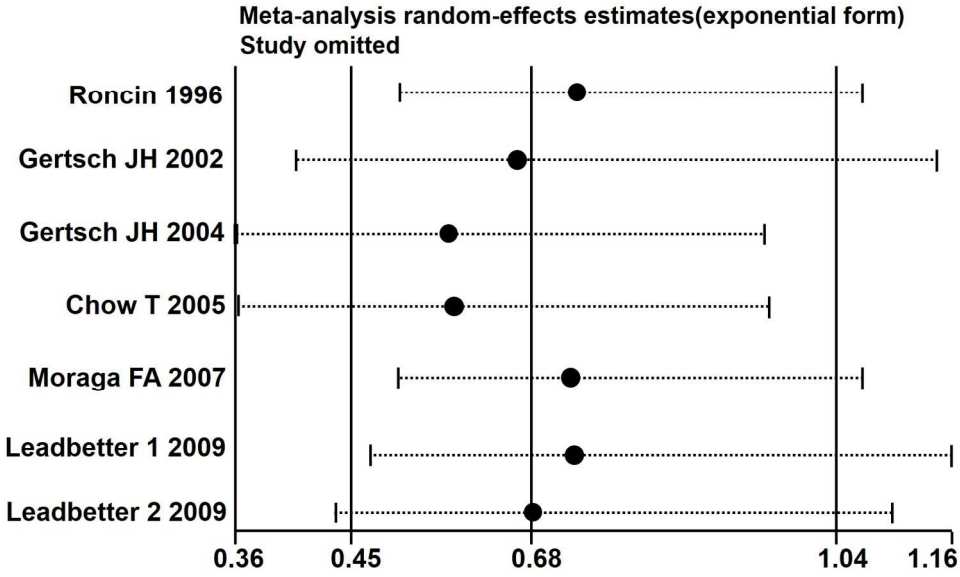

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The full search strategy for Pubmed is as followings:

We use the following search string: ("Ginkgo biloba"[Mesh] or "ginkgo"[tiab]) AND("Altitude Sickness"[Mesh] or "Altitude Sickness"[tiab] or "mountain"[tiab])

Supplementary Table 1. Secondary outcomes of included studies

		Incidence of severe AMS	Headache	Severe headache	Oxygen saturation	Pulmonary edema	Adverse events
Roncin, 1996	GBE	X	22/22 (100%)	0/22 (0%)	X	AMS-R >0.6 3/21 (13.6%)	GBE(18.2%) is less urine than Placebo(77.3%)
	Placebo	X	22/22 (100%)	0/22 (0%)	X	AMS-R >0.6 18/22 (81.8%)	
Gertsch, 2002	GBE	2/12 (16.7%)	X	1/12 (8%)	81%	X	No side effect in GBE
	Placebo	9/14 (64.3%)	X	1/14 (7%)	80%	X	
Gertsch, 2004	GBE	23/124 (18%)	72/124 (58%)	24/124 (19%)	79.5%	Non occurred	No side effect in GBE
	Placebo	22/119 (18%)	63/119 (53%)	16/119 (13%)	82.1% P<0.01		
Chow, 2005	GBE	X	GBE is 5% less than placebo		X	Non occurred	No side effect in GBE
	Placebo	X			X		
Moraga, 2007	GBE	X	LLS score, headache item 0.19±0.41		92±2%	X	X
	Placebo	X	1.28±0.14 P<0.05		84±3% P<0.01	X	X
Leadbetter, 2009(Study 1)	GBE	0/21 (0%)	X	X	X	X	X
	Placebo	3/19 (16%)	X	X	X	X	X
Leadbetter, 2009(Study 2)	GBE	3/15 (20%)	X	X	X	X	X
	Placebo	4/22 (18%)	X	X	X	X	X

AMS: Acute mountain sickness ; AMS-R: the Environmental Symptom Questionnaire III acute mountain sickness-Respiratory (AMS-R) score; GBE: ginkgo biloba extract; LLS: Lake Louise Score; X: not mentioned in the study.

Supplementary Table 2. Number of participants and enrolled studies in each subgroup

Subgroup	Number of participants
Low risk of bias studies	357
Gertsch, 2004	
Chow, 2005	
Leadbetter, 2009	
Starting altitude below 2500m	208
Roncin, 1996	
Gertsch, 2002	
Chow, 2005	
Moraga, 2007	
Leadbetter, 2009	
3–5 days prior to ascent	114
Chow, 2005	
Leadbetter, 2009	
0–2 days prior to ascent	337
Roncin, 1996	
Gertsch, 2002	
Gertsch, 2004	
Moraga, 2007	
Dosage less than 200mg per day	94
Roncin, 1996	
Gertsch, 2002	
Moraga, 2007	
Dosage more than 200mg per day	357
Gertsch, 2004	
Chow, 2005	
Leadbetter, 2009	

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N.A.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N.A.

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