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Safety evaluation of BacoMind™ in healthy volunteers: A phase I study

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Abstract

BacoMind™ is an enriched phytochemical composition of *Bacopa monniera* (*B. monniera*), a common medicinal plant used in the traditional systems of medicine as a memory-enhancing agent. BacoMind™ was standardized with reference to bioactive compounds and was evaluated for short-term safety and tolerability in healthy adult volunteers. The study plan employed randomized, open label, dose escalation design. Each of 23 participants were orally given one single capsule of BacoMind™ daily for 30 days, i.e., 300 mg for first 15 days and 450 mg for next 15 days. Detailed examination of clinical, hematological, biochemical and electrocardiographic parameters done in pre and post-treatment periods did not indicate any untoward effects in any of the treated volunteers. Mild adverse events related to gastrointestinal system were observed in the trial, which subsided spontaneously. BacoMind™ was found to meet the safety criteria at the dose administered for the given duration of trial period in healthy adult volunteers.

Keywords: *Bacopa monniera*; BacoMind™; Safety; Tolerability; Healthy volunteers

Introduction

Dementia, including Alzheimer's disease (AD) is a common and devastating neurodegenerative disorder of the elderly and is one of the leading health problems worldwide. However, symptoms of cognitive decline can begin much earlier in some individuals and often go unrecognized until later in life. Delphi Consensus study (Ferri et al., 2005) estimated that 24.3 million people suffer from dementia worldwide, with 4.6 million new cases of dementia every year (one new case in every 7 s). The number of people affected would double every 20 years to 81.1 million by 2040. The same study estimated that the incidence of dementia is rapidly increasing in developing countries (60% in 2001, rising to 71% by 2040).

Biological evaluation of plants based on their use in the traditional systems of medicine is a sound and cost-effective strategy to develop new agents from plants. The survey conducted in Australian community revealed that *Ginkgo biloba* and *Bacopa monniera* were the most widely used medicinal plants for memory-enhancing effect (Jorm et al., 2004). *B. monniera*, also referred to as *Herpestis monniera*, water hyssop, and "Brahmi" (Fig. 1), has been used in the traditional and Ayurvedic

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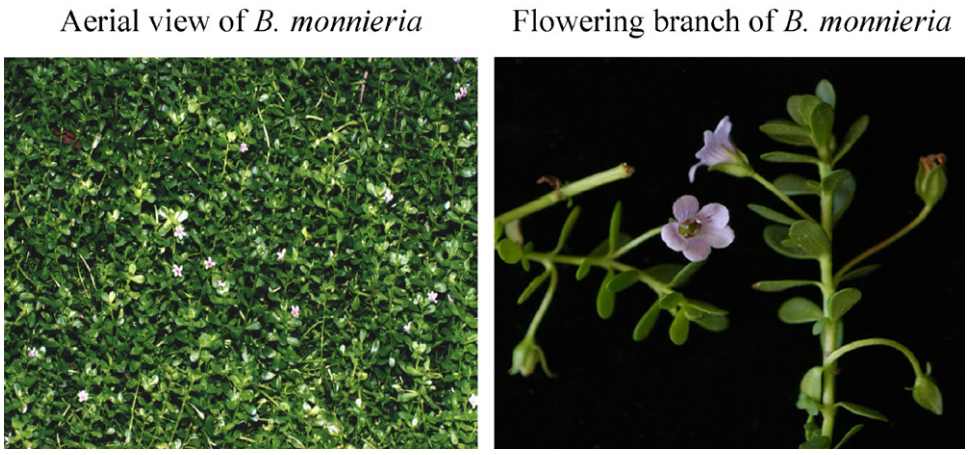

Fig. 1. Aerial parts of *B. monniera* (Brahmi).

systems of medicine for centuries. Traditionally it is used as a brain tonic to enhance learning and memory, to improve concentration, and to provide relief to patients with anxiety or epileptic disorders (Udupa and Singh, 1993). Many experimental studies were conducted to evaluate the neuropharmacological effects of *B. monniera*; the study results on memory, learning and concentration supported the traditional and Ayurvedic claims (Malhotra and Das, 1959; Singh and Dhawan, 1982; Das et al., 2002). Clinical studies have been conducted to evaluate short and long-term effects of *B. monniera* on cognitive function of different population (Negi et al., 2000; Nathan et al., 2001; Roodenrys et al., 2002).

The chemistry of *B. monniera* was investigated in detail and it was observed that the cognitive effects were possibly related to the modulatory effects on cholinergic (Bhattacharya et al., 1999, 2000) and serotonergic (Meneses, 1999; Parker and Medora, 2005) systems in the brain along with the antioxidant effect (Bhattacharya et al., 2000; Russo et al., 2003). Compounds responsible for the neuropharmacological effects include alkaloids, saponins, and sterols (Singh et al., 1988; Bhattacharya et al., 1999; Deepak and Amit, 2004). Bacosides, the important constituents identified, have been responsible for facilitatory effect of *B. monniera* on learning and memory (Singh and Dhawan, 1997).

B. monniera has been used safely in Ayurvedic medicine for several 100 years. Clinical studies have demonstrated that therapeutic doses of different extracts of *B. monniera* were not associated with any major adverse effects (Sharma et al., 1987; Stough et al., 2001). A double-blind, placebo-controlled clinical trial on healthy male volunteers investigated the safety of pharmacological doses of bacosides, the active constituents of *B. monniera*. Concentrated bacosides given in single dose (20–30 mg) and multiple (100–200 mg) daily doses over a 4-week period were well tolerated and were found to be devoid of any untoward reaction or side effects (Singh and Dhawan, 1997).

An enriched phytochemical composition, BacoMind^{TM} was developed from *B. monniera* for use as a memory-enhancing agent and it differs from the previously reported standardized extracts, in that it has been identified and/or standardized to different bioactive constituents. Hence in order to establish the safety and tolerability of the unique phytochemical composition, the present study was undertaken in healthy adult volunteers with the objective of evaluating the clinical safety and also to examine the short-term tolerance at the given dose levels by employing subjective and objective evaluation of relevant clinical and laboratory parameters, to detect the occurrence of any adverse drug reactions (ADRs) associated with BacoMind^{TM}.

Materials and methods

Study design

A dose tolerance study was planned as randomized, open label, dose escalation study in healthy adults with pre and post-test comparison of clinical and laboratory parameters for safety and tolerance of BacoMind^{TM}.

Study centre

The Phase-I clinical trial of BacoMind^{TM} was carried out at Anasuya Ayurveda Clinic, in association with Department of Pharmacology and Toxicology, Natural Remedies after obtaining ethical committee approval.

Test substance

BacoMind^{TM}, an enriched phytochemical composition of *B. monniera*, developed by Natural Remedies Pvt. Ltd.,

(patent pending) was standardized to the content of the following bioactive constituents viz., bacoside A_3 (>5.0% w/w), bacopaside I (>7.0% w/w), bacopaside II (>5.5% w/w), jujubogenin isomer of bacopasaponin C (>7.0% w/w), bacopasaponin C (>4.5% w/w), bacosine (>1.5% w/w), luteolin (>0.2% w/w), apigenin (>0.1% w/w) and β-sitosterol-D-glucoside (>0.3% w/w). It was further standardized using the following *in vitro* bioassays viz., lipoxygenase inhibition assay (IC_{50} < 600 μg/ml), ABTS radical scavenging assay (IC_{50} < 100 μg/ml), DPPH assay (IC_{50} < 200 μg/ml) and butyrylcholinesterase inhibition assay (IC_{50} < 3000 μg/ml).

HPLC fingerprint of BacoMind™ (Batch no. BM/03/LOT15) is shown in Figs. 2 and 3; Fig. 4 describes the HPTLC profile of BacoMind™. The individual compounds present in BacoMind™ were identified and quantified using respective standards. Quantification of the compounds was carried out on dry weight basis. BacoMind™ contained bacoside A_3 (57 mg/g), bacopaside I (71 mg/g), bacopaside II (56 mg/g), jujubogenin isomer of bacopasaponin C (82 mg/g), bacopasaponin C (54 mg/g), bacosine (19 mg/g), luteolin (5 mg/g), apigenin (3 mg/g) and β-sitosterol-D-glucoside (9 mg/g).

Volunteers

Healthy adult volunteers of either sex, willing to participate in the study were enrolled and written informed consent was obtained from each volunteer. Screening procedure involved detailed medical history, clinical examination and laboratory (hematology and blood biochemistry) investigations including urine analysis and Electrocardiogram (ECG). Unless proven healthy by clinical examination, nobody was subjected to undergo laboratory evaluation. A fasting blood sample of 5 ml was collected for conducting hematology and blood biochemistry and urine sample was collected for urine analysis. It was followed by test substance administration for a period of 30 days for the selected volunteers, based on inclusion and exclusion criteria.

Inclusion criteria

Healthy adult volunteers aged 18–45 years and willing to give voluntary written informed consent were selected to participate in this study. The volunteers were proven to be healthy through clinical examination by the

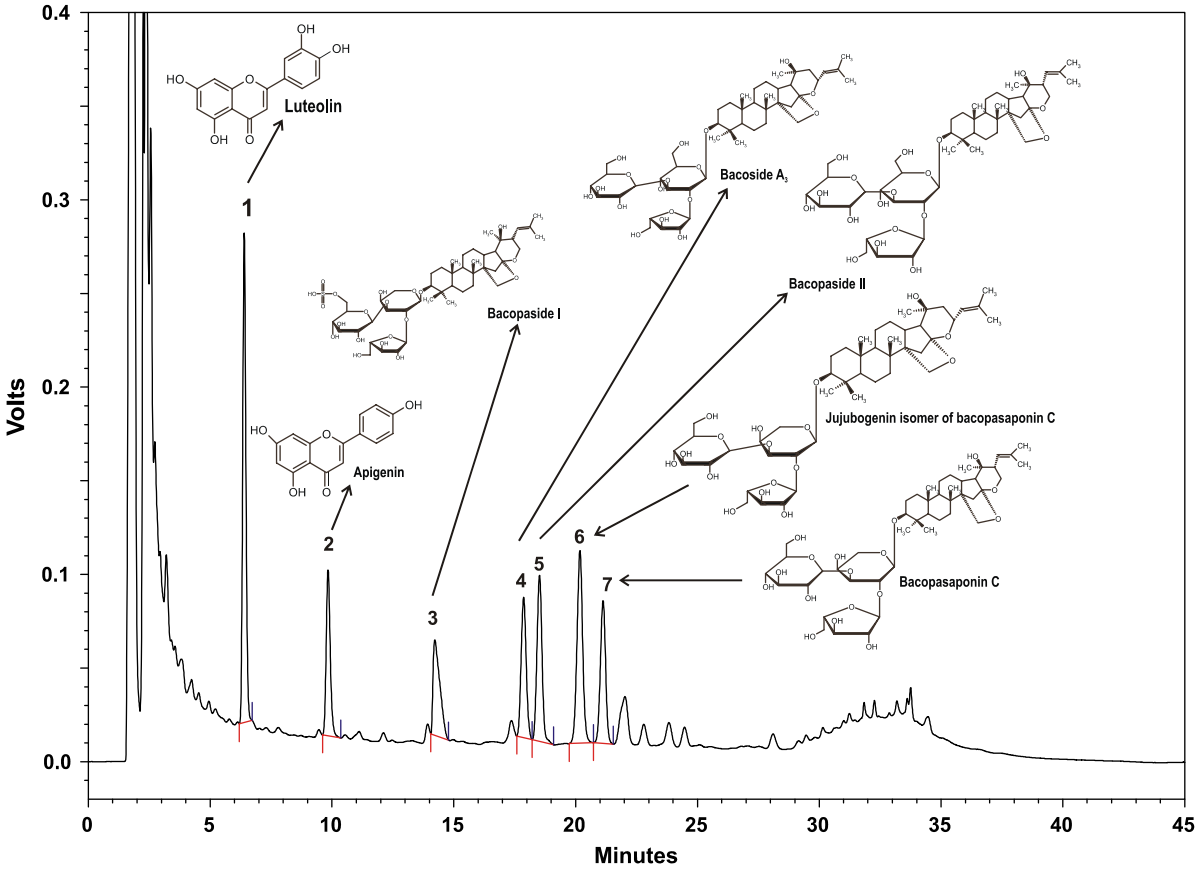

Fig. 2. HPLC fingerprint of BacoMind™. HPLC conditions: Column: Pinnacle DB C18 5 μm 250 mm × 4.6 mm, Restek; mobile phase: mixture of phosphate buffer (A) + Acetonitrile (B); solvent A linear gradient from 70% to 60% in 25 min; phosphate buffer: 0.01 N potassium dihydrogen phosphate in HPLC grade water + 0.5 ml orthophosphoric acid; temperature: 25 °C; flow rate: 1.5 ml/min throughout; UV: 205 nm.

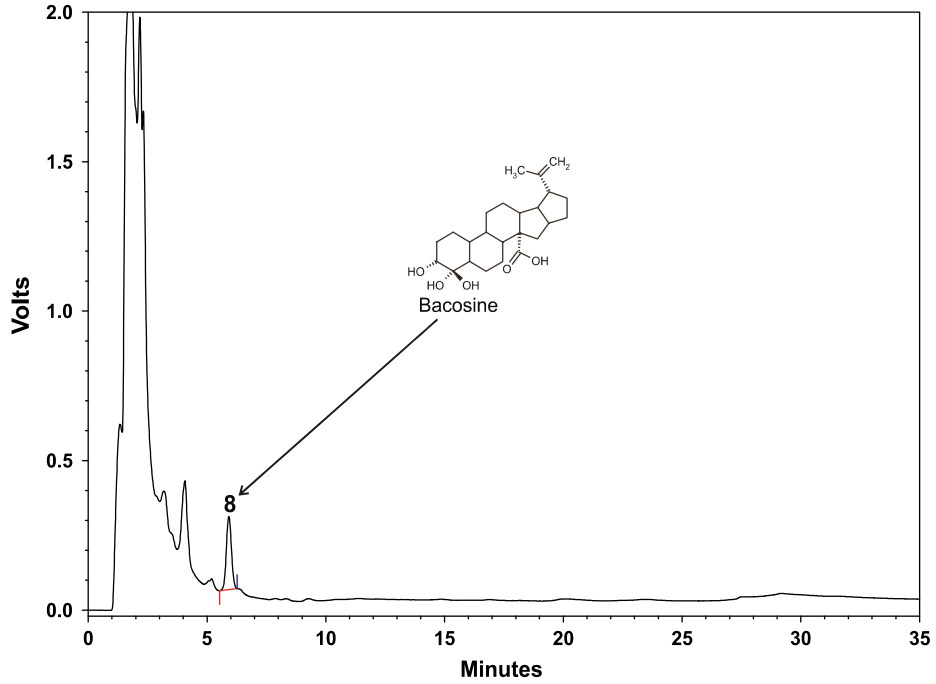

Fig. 3. HPLC fingerprint of BacoMind^{TM}. HPLC conditions: Column: Pinnacle DB C18 5 μm 250 mm × 4.6 mm, Restek; mobile phase: 5% acetonitrile in 0.2% aqueous orthophosphoric acid and methanol (1:9); temperature: 25 °C; flow rate: 1.5 ml/min throughout; UV: 205 nm.

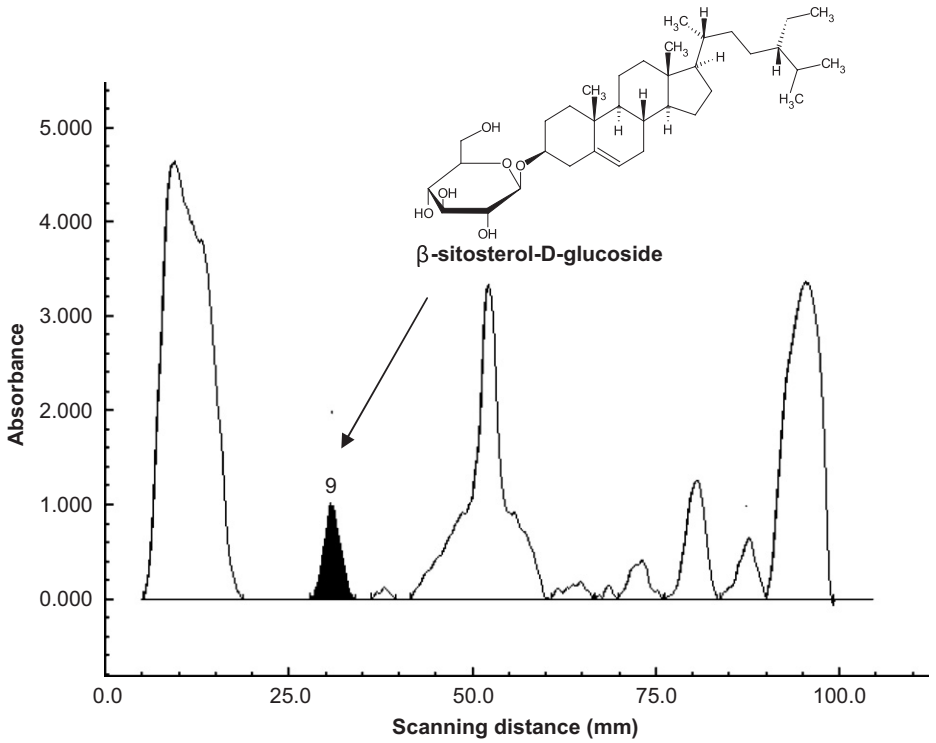

Fig. 4. HPTLC profile of BacoMind^{TM}. HPTLC condition: TLC plate: Silica gel 60F254, 20 cm × 20 cm, Merck, Germany; spotting device: Linomat IV, Camag; mobile phase: Chloroform : Methanol (9:1); spraying agent: Anisaldehyde sulfuric acid reagent; Densitometer: CS 9301 PC, Shimadzu; UV: 530 nm.

Table 1. Demographic data of volunteers who completed the study

Group	Number of volunteers
Male	19
Female	4
Total	23

physician along with hematological and biochemical investigations as well as ECG.

Exclusion criteria

Subjects were excluded from the study if any of the following criteria applied at the time of screening: evidence of significant clinical abnormalities detected during the screening procedure, history of any acute/chronic disorders, history of chronic medication within the past 6 weeks, history of hypersensitivity to any medication, history of use of drugs known to affect CNS performance, regular smokers who smoke more than 20 cigarettes daily or have difficulty in abstaining from smoking during the study duration, history of drug dependence or chronic alcohol use associated with altered hepatic functions, use of any microsomal enzyme modifying drugs within 30 days and/or systemic medication including OTC preparation 14 days prior to day 1 of the study, participation in any clinical trial within 6 weeks preceding day 1 of the study, women with anticipated pregnancy/pregnant/lactating/using oral contraceptives and abnormal clinical and biochemical examination and ECG findings.

In addition to the health examination, only those who fulfilled the inclusion and exclusion criteria were included in the study.

BacoMind^{TM} administration schedule

All the volunteers were instructed to take one capsule of BacoMind^{TM} with a glass (100–200 ml) of water after the breakfast between 8.00 a.m. and 9.00 a.m. as a single dose daily for the duration of 30 days. In the first instance, each one was provided with the first container of 15 capsules (each capsule with 300 mg actives). First administration was done under medical supervision. At the end of 15 days, they were followed up to enquire about the side effects, compliance of medication and willingness to continue the participation. If affirmed, they were issued the second container of 15 capsules (each capsule with 450 mg actives). After 30 days, the medication was stopped without taper.

Compliance assessment

The compliance of study medication was assured by medication diaries along with personal supervision in some cases. The volunteers were followed up on daily basis in the morning to find out any adverse events during trial period. The old container along with diary was collected during the respective follow-up visits. There was no restriction placed on normal and routine activity or diet during the study period. No medication other than BacoMind^{TM} was allowed except under exceptional conditions with the permission of investigator.

End point measurements

The study outcome measures were evaluated on day 31. Each volunteer was subjected to detailed clinical examination as per the protocol. Individual systemic examination, especially on nervous system along with general physical examination including vital signs (body temperature, supine resting pulse rate, supine blood pressure and respiratory rate) was carried out by qualified medical professional.

They also underwent laboratory investigations as before viz., hematological tests such as hemoglobin (Hb), total leucocyte count (TLC), differential leucocyte count (DLC), red blood cell (RBC) count; biochemical tests such as fasting blood sugar (FBS), serum creatinine, liver function tests (serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum bilirubin – total and direct) and serum proteins (total protein and albumin). Qualitative (protein, sugar, urobilinogen, epithelial cells, pus cells, RBCs, hemoglobin) and quantitative (volume, pH and specific gravity) evaluations were conducted on urine samples. ECG too was recorded in 12 leads.

Safety reporting

Adverse events were recorded based on either self-reporting by subject or by identification of clinical signs and symptoms including changes in vital and laboratory parameters (blood chemistry, blood cell count and urinalysis). During the follow up, enquiries were made without any leading questions to find out the occurrence of any expected ADRs of *B. monniera* as reported in published literature (Stough et al., 2001).

Statistics

The data obtained from clinical and laboratory evaluation before and after the test substance administration were analyzed and calculated as mean $\pm$ SEM. Statistical analysis was done by using Student's paired *t*-test with SPSS software. The statistical significance was set at $p \leqslant 0.05$.

Results

A total of 31 volunteers aged between 22 and 42 years, who were willing to participate in the study were screened. A total of 23 volunteers (19 males and 4 females) with mean age of 31.09 ± 1.27 yr, certified as healthy without any major clinical abnormalities were recruited (Table 1). Each subject was given appropriate instructions on test substance administration. All the participants completed 30 days of BacoMind™ administration with good compliance.

Clinical examination

Final assessment was done after completion of the study medication. Thorough clinical examination by physician showed no gross changes in clinical signs and symptoms in all the volunteers after the treatment. No significant differences were noted in the mean values of body weight, supine systolic and diastolic blood pressure in the volunteers before and after the treatment except for resting pulse rate (Table 2). Though the pulse rate increased from 75.61 ± 1.42 to 78.17 ± 1.15 per minute ($p \leqslant 0.05$), it was within normal limits. No signs of any systemic including neurological abnormalities were detected. There were no signs of liver enlargement or palpable liver in any of the volunteers.

Table 2. Details of vital parameters recorded in healthy adult volunteers treated with BacoMind™

Parameters	Pre-treatment (Day 0)	After treatment (Day 31)
Body weight (kg)	63.43 ± 1.97	63.75 ± 1.96
Pulse rate (per minute)	75.61 ± 1.42	78.17 ± 1.15*
Systolic blood pressure (mmHg)	122.39 ± 1.82	124.87 ± 1.54
Diastolic blood pressure (mmHg)	78.96 ± 1.31	79.48 ± 0.85

Values are expressed in mean ± SEM; $n = 23$, except body weight $n = 22$.
*$p \leqslant 0.05$ significant vs. pre-treatment values.

Laboratory investigations

Hematological and biochemical parameters including hemoglobin, total and differential leukocyte count, liver and kidney function tests revealed no significant difference compared to that of baseline values (Table 3) except for FBS, serum creatinine, SGOT which showed significant decline ($p \leqslant 0.05$) in the post-treatment values; mean SGPT-value elevated to 32.0 ± 2.50 IU/l from the baseline value of 23.39 ± 1.49 IU/l ($p \leqslant 0.05$). But all these values were within the normal reference ranges. ECG recordings detected no abnormalities in all treated volunteers and were within normal limits. Urinalysis results were found to be in the normal physiological range and revealed no abnormalities.

Safety reporting

Reporting of adverse events was either volunteered statements or probed by the investigators. No major

Table 3. Hematological and biochemical investigations done in healthy adult volunteers

Parameters	Pre-treatment (Day 0)	After treatment (Day 31)	Normal range
Hematology			
Hemoglobin (g %)	14.52 ± 0.18	14.50 ± 0.19	12.5–16.0
Total WBC count (cells/cu mm)	8147.83 ± 173.76	8308.70 ± 170.20	4000–11000
Polymorphs (%)	58.30 ± 0.26	58.96 ± 0.32	40–70
Lymphocytes (%)	37.17 ± 0.20	36.74 ± 0.30	20–40
Eosinophils (%)	2.70 ± 0.13	2.61 ± 0.15	00–06
Monocytes (%)	1.78 ± 0.17	1.74 ± 0.16	00–08
RBC count (millions/cu mm)	4.80 ± 0.06	4.82 ± 0.06	3.8–5.8
Biochemistry			
Fasting blood glucose (mg/dl)	86.10 ± 1.72	78.55 ± 1.39*	60–110
Creatinine (mg/dl)	1.00 ± 0.02	0.95 ± 0.01*	0.8–1.5
Bilirubin total (mg/dl)	0.57 ± 0.02	0.57 ± 0.01	Up to 1.0
Bilirubin direct (mg/dl)	0.16 ± 0.01	0.17 ± 0.01	Up to 0.25
SGOT (IU/l)	27.39 ± 1.35	24.35 ± 1.10*	0–37
SGPT (IU/l)	23.39 ± 1.49	32.0 ± 2.50*	0–40
Alkaline phosphatase (U/l)	75.13 ± 2.35	77.17 ± 1.92	39–130
Total proteins (g/dl)	7.48 ± 0.04	7.38 ± 0.05	6.0–8.0
Albumin (g/dl)	4.31 ± 0.06	4.22 ± 0.04	3.5–5.0

Values are expressed in mean ± SEM; $n = 23$; except fasting blood glucose $n = 20$.
*$p \leqslant 0.05$ significant vs. pre-treatment values.

adverse events were reported during the trial. However, mild gastrointestinal disturbances, i.e., epigastric burning sensation in one volunteer (4.35% of 23 volunteers) and nausea in one volunteer (4.35% of 23 volunteers) with 300 mg dosage; fullness and bloating sense of abdomen in one volunteer (4.35% of 23 volunteers) with 450 mg dosage were observed in total of three volunteers. These symptoms subsided spontaneously without any need for discontinuation of treatment.

Drop outs

There were no drop outs during study period.

Discussion

The present study was designed to evaluate the safety and tolerability of BacoMind™, an enriched phytochemical composition of *B. monniera,* in the healthy adult volunteers. The dose levels of 300 and 450 mg once a day orally was based on pre-clinical safety and efficacy studies conducted. The published literature on clinical studies of *B. monniera* also contributed towards the selection of dose and duration for the current study (Stough et al., 2001; Sharma et al., 1987; Singh and Singh, 1980).

BacoMind™, an enriched phytochemical composition from *B. monniera*, when given orally to Sprague Dawley rats showed an LD_{50} of 2400 mg/kg b.w. BacoMind™ given orally for 90 days to Sprague Dawley rats, did not cause any mortality and treatment-related clinical abnormalities along with no observed adverse effect level (NOAEL) of 500 mg/kg b.w. (unpublished data).

Nootropic activity of BacoMind™ was evaluated in rodents. A significant increase in the inflexion ratio was noticed in scopolamine treated Swiss albino mice at 60 mg/kg b.w. in elevated plus maze model. Similarly, in the passive shock avoidance test, normal as well as scopolamine-treated mice demonstrated significant improvement when treated with 60 mg/kg b.w. of BacoMind™. In object recognition test, normal as well as scopolamine treated Wistar rats when treated with 40 mg/kg b.w. of BacoMind™ exhibited significant increase in discrimination index as compared to that of vehicle control (Kasture et al., 2007).

The safety studies of BacoMind™ strengthen the available pre-clinical safety data of *B. monniera.* Aqueous extract given upto 5 g/kg b.w. did not reveal any toxicity while the oral LD_{50} of alcoholic extract was found to be 17 g/kg b.w. in rats (Martis et al., 1992). Bacosides A and B injected intraperitoneally at the doses of 20, 40 and 80 mg/kg b.w. did not induce any chromosomal aberration in bone marrow cells of mice; there were no significant differences observed in sister chromatid exchange and micronuclei formation tests at the same dose levels. Bacosides A and B did not reveal any genotoxic effect when administered up to 80 mg/kg b.w. (i.p.) and had an LD_{50} value of 300 mg/kg (i.p.) in mice (Giri and Khan, 1996). Ethanolic extract of *B. monniera* (10–200 mg/kg b.w.) did not cause any behavioral change in mice and LD_{50} of the extract was found to be 520 mg/kg of mouse body weight (Dar and Channa, 1997).

The findings of present study indicated that BacoMind™ did not induce any significant physiological, biochemical or electrocardiographic untoward effects or abnormalities in any of the healthy adult volunteers at the given oral dosage of 300 and 450 mg. Though there were statistically significant ($p \leqslant 0.05$) reduction in FBS, serum creatinine and SGOT values and increase in SGTP value following BacoMind™ supplementation, these values were found to be within the normal reference ranges. In conjunction with other liver function tests and clinical findings, the borderline changes in SGOT and SGPT values following 30 days of administration were considered to be of no clinical relevance. Also, it was evident from the study that there were no signs of neurotoxicity.

An interesting observation in the pilot safety study of BacoMind™ was that the adverse events reported by all three volunteers were related to gastrointestinal system. The findings match with the ADRs observed with *B. monniera* given at 300 mg once a day oral dose over a period of 12 weeks in healthy adults (Stough et al., 2001).

The present dose escalation study provides evidence to the safety and tolerability of BacoMind™ in healthy adult individuals at the oral dosage of 300 and 450 mg given for the duration of 30 days.

The subjective and objective evaluation of BacoMind™ in healthy adult volunteers revealed that, at the given oral dose of 300 mg once a day for first 15 days and 450 mg once a day for next 15 days, the herbal supplement was found to be safe and tolerable. Though minor gastrointestinal side effects were reported in 3 out of 23 volunteers, the general physical, systemic, hematological, biochemical and electrocardiographic parameters were within the normal limits. The clinical information projected a relatively safe and normal profile of herbal supplement in exposed individuals at the given doses within the trial period and further supported the safety profile of BacoMind™.

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