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Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2024.v16.i01.pp83-90

ANTIDEPRESSANT-LIKE ACTIVITY OF BACOPA MONNIERI EXTRACT IN THE FORCED SWIM AND TAIL SUSPENSION TESTS

LOTTE BROUWER[1]*, RHYS HESKETH[2], GEORGIOS KARAGIANNIS[3]

Affiliation [1]Department of Experimental Pharmacology, Twente Institute of Pharmaceutical Technology, Hengelo, Netherlands
[2]Department of Experimental Pharmacology, Exmoor Institute of Pharmaceutical Sciences, Taunton, United Kingdom
[3]Department of Experimental Pharmacology, Macedonia Plain Institute of Pharmacy, Serres, Greece

*Corresponding author: Lotte Brouwer, l.brouwer@tipt.nl
ORCID iD: Lotte Brouwer 0000-0001-4490-4907; Rhys Hesketh 0000-0001-0237-7125; Georgios Karagiannis 0000-0001-2914-4188

Article info

Received date:11-09-2023
Accepted date:09-01-2024
Published date:26-05-2024
ISSN;0975-8216

Keywords:

Bacopa monnieri, Antidepressant-like activity, Forced swim test, Tail suspension test, Behavioural despair

Abstract

Bacopa monnieri (Brahmi) is an Ayurvedic herb best known for its effects on memory, but animal studies also report antidepressant-like behaviour. This review gathers published evidence on its extracts and constituents in the forced swim test and tail suspension test, the two rodent screens most often used to look for such activity. It describes how the tests are run, what immobility does and does not mean, which Bacopa preparations have been studied, and the mechanisms proposed, including serotonergic, stress-axis and antioxidant effects. Results are consistently positive but come from few laboratories, with limited standardisation and little control for locomotion. The review reports no new data.

1. INTRODUCTION

Major depression is common, disabling and only partly relieved by the drugs now available. A good proportion of patients do not respond fully to the first antidepressant, and side effects lead many to stop early. Understanding of the disorder points to disturbed monoamine signalling, altered stress hormone regulation and reduced synaptic plasticity in mood-related circuits [1, 2]. That mix of causes keeps interest alive in plant-derived preparations, some of which are widely used in traditional medicine for mood and cognition. Reviews of herbal products for depression, anxiety and sleep problems conclude that a few have encouraging evidence, but that most claims rest on small or poorly controlled studies [3].

Bacopa monnieri (L.) Wettst., known as Brahmi or water hyssop, is a creeping marsh herb that has been used in Ayurveda for centuries as a tonic for memory and mental clarity. Most modern work has followed that tradition, looking at learning, attention and neuroprotection [4, 5]. Mood effects have received less attention, although several animal papers and one trial in older adults point toward a benefit [6, 7, 8]. Those studies depend on behavioural screens, and anyone reading them needs to know how those screens work, what they measure and where they mislead. A review of this kind is therefore as much about the tests as about the plant.

This article reviews published evidence on the antidepressant-like activity of Bacopa monnieri extracts and isolated constituents in two rodent tests, the forced swim test and the tail suspension test. It covers the botanical and chemical background, the design and limits of the tests, the reported findings, the mechanisms that have been suggested, and the problems of interpretation. A summary of the main findings is given in table 1. No animals were used and no experiments were done for this article, so everything stated here is drawn from the cited literature, and the weaker points of that literature are described plainly.

Table 1: Behavioural tests, confounders and published evidence for Bacopa monnieri in depression-related models

Item	Reported finding or feature	Ref.
Mouse forced swim test	Six-minute session with the last four minutes scored; clinically active antidepressants shorten immobility.	[9, 10, 11]
Tail suspension test	Mice only; sensitive to strain differences and to automation settings.	[12, 13, 14]
Rat forced swim test	Pre-swim used; swimming and climbing hint at serotonergic or noradrenergic action.	[15, 16]
Locomotor confound	Stimulants can shorten immobility falsely; activity testing is needed.	[13, 17]
Meaning of immobility	May reflect adaptive coping, not despair.	[18, 19, 20]
Standardised extract in rats	Reduced immobility and improved escape performance compared with imipramine.	[7]
Triterpene saponins in mice	Decreased immobility in swim and suspension tests without raised locomotion.	[8]
Trial in older adults	Standardised extract improved depression and anxiety scores as secondary outcomes.	[6]
Serotonergic markers	Leaf extract raised TPH2 and SERT expression in rat brain.	[21]
Stress hormones	Attenuated stress-induced corticosterone and monoamine changes.	[22]
Antioxidant enzymes	Raised antioxidant enzyme activity in several brain regions.	[23]

2. BACOPA MONNIERI AND ITS CONSTITUENTS

The pharmacologically important compounds of Bacopa are triterpenoid saponins, generally called bacosides, which are dammarane-type glycosides with jujubogenin or pseudojujubogenin aglycones. Bacoside A, in fact a mixture of closely related saponins, and bacoside B have long been treated as the main active principles [4, 24]. Flavonoids, alkaloids such as brahmine, and sterols are also present. Commercial extracts are commonly standardised to a bacoside content, measured by HPLC, and the method of Deepak and colleagues for quantifying bacoside A is one of the better known [25]. The percentage differs a lot between products, which matters when comparing doses across papers.

Traditional use gives some context for the doses and forms later tested in animals. In Ayurvedic texts Brahmi belongs to the medhya rasayana group of rejuvenating agents given to support intellect and memory, and it has been taken as a fresh juice, a powder, a medicated ghee or a decoction [5, 26]. Singh and Dhawan, in an early pharmacological review, described sedative, anxiolytic and memory-supporting actions in animals and connected them with the traditional claims [26]. These accounts are not

proof, but they explain why the plant was chosen for modern screens and why anxiety and mood outcomes appear in the literature alongside the better-known cognitive ones [4, 5].

Preparation affects composition. Alcoholic extracts of the whole herb, aqueous extracts and extracts of leaves alone all appear in the literature, and each contains a different balance of saponins and polar compounds [4, 24]. Plant material also varies with season, place of growth and harvest, so two extracts called 'Bacopa' may differ widely. Good studies state the plant part, the solvent, the extraction ratio and the marker content, and deposit a voucher specimen. Many do not. In the antidepressant literature, the better-characterised work involves a standardised extract and purified triterpene saponins, whereas other reports use crude preparations whose make-up cannot be reconstructed [7, 8]. Reproducibility therefore begins at the herbarium and the extraction vessel, long before any animal is handled [4, 25].

Safety in animals appears reasonable at the doses usually tested. A study of a standardised extract in rats at repeated doses found no overt toxicity or major organ changes, which supports its use in behavioural experiments [27]. In people, trials of cognitive effects have recorded mostly mild gastrointestinal complaints [28, 29, 30]. These findings do not remove the need for dose-response designs, because a sedative or irritant effect at higher doses could change behaviour in a swim test without any antidepressant action. Reports in traditional use are not a substitute for formal toxicology, but they do suggest that the herb is tolerated over long periods [5, 26].

Cognitive results in people give a sense of the plant's pharmacology. Chronic dosing for around twelve weeks improved some memory measures in healthy adults, and meta-analyses concluded that effects were modest but consistent for speed of attention and recall [28, 29, 30]. Because the cognitive benefit builds slowly over weeks, it has been suggested that Bacopa acts through gradual changes in neurotransmission and plasticity rather than through an immediate pharmacological effect. If that is right, the design of antidepressant-like studies matters: single-dose tests might miss effects that a repeated-dose schedule could show, and some reports in rodents indeed use daily dosing for one to three weeks before testing [7, 21].

3. FORCED SWIM AND TAIL SUSPENSION TESTS

The forced swim test was introduced by Porsolt and colleagues as a quick screen for antidepressants. A rodent placed in a cylinder of water from which it cannot escape first swims and climbs and then adopts a floating posture with only the movements needed to keep its head above water [9, 10]. The time spent immobile is taken as the outcome, and drugs that are clinically effective against depression shorten it. In the standard mouse protocol the animal is in the water for six minutes and behaviour is scored in the last four [11]. Rats traditionally undergo a longer pre-swim on the day before testing, as set out in a detailed protocol paper [15].

The tail suspension test applies the same logic with a different stressor. Steru and co-workers described hanging a mouse by the tail so that it cannot reach a surface, and recording the time it spends motionless over six minutes [12]. It avoids hypothermia and the wetting of the animal, and it can be automated with strain gauges. It works in mice but is not suitable for rats, which can climb their own tails or are too heavy [13, 14]. The review by Cryan and colleagues noted marked differences between mouse strains in baseline immobility and in response to antidepressants, which means that results from one strain cannot simply be carried over to another [13].

Both tests are sensitive to many antidepressant classes, which is the main reason for their enduring use. Detke and colleagues showed in rats that serotonergic drugs tend to increase swimming whereas noradrenergic drugs increase climbing, which allows some inference about the transmitter system involved [16]. In mice the same split is less clear [17]. A major strength is that the response appears after a single or a few doses, unlike the clinical delay of weeks. A related weakness is that the tests also respond to

some drugs without clinical antidepressant action, and fail to detect some that work, so a positive result is evidence for further study, not a diagnosis of efficacy [31, 32].

Practical details of dosing shape what a swim or suspension result can tell us. Orally administered extracts are usually given by gavage in a suspension or solution, at a fixed interval before testing, commonly around thirty to sixty minutes for acute designs, with a vehicle group and a reference antidepressant such as imipramine or fluoxetine run alongside [11, 14]. Animals are habituated to the room, and the observer should not know which treatment each animal received. Repeated-dose designs add a further question: whether the last dose is given on the day of testing, because residual acute effects can mask or mimic a chronic effect [15, 33]. Papers that omit these details are hard to reproduce.

Many factors alter the outcome. Bogdanova and colleagues listed water temperature, cylinder dimensions, depth, animal strain and sex, time of day, housing, handling and the experimenter as sources of variation in the forced swim test [34]. Mouse strains differ in baseline immobility, and some are almost unresponsive to certain drugs [13, 17]. Procedures also differ in how immobility is scored, by hand, by video or by software, and in whether the observer is blind to treatment. Without such controls a difference between a treated group and vehicle may reflect the experimental set-up as much as the drug, a problem that reporting guidelines are meant to address [33].

Ethics and refinement are part of the discussion too. The forced swim test places animals in an inescapable stressful situation, and researchers are expected to justify the number of animals, use the shortest workable protocol and avoid repeat testing where possible [33]. The tail suspension test is generally seen as less severe in terms of exposure to water and cold, though it is also stressful and some strains climb their own tails, which leads to exclusion of animals [13, 14]. Sample sizes of eight to twelve per group are common, and power calculations are rarely reported. Better planning can reduce animal use without reducing the quality of evidence, which would benefit natural product research more than most fields [33, 34].

4. REPORTED ANTIDEPRESSANT-LIKE ACTIVITY

The most cited study is that of Sairam and colleagues, who tested a standardised Bacopa extract in rat models of depression, including the forced swim test and learned helplessness, and found reduced immobility and fewer escape failures, comparable to the tricyclic comparator imipramine [7]. The work is important because the extract was characterised and a reference drug was run in parallel. It is also limited. Few doses were tested, the study came from one laboratory, and the follow-up was short. It has still become the reference point for later work, and it is the basis for much of the interest in the herb as a mood agent [4, 5].

Purified constituents have been examined as well. Zhou and colleagues isolated triterpene saponins from Bacopa and tested them in two mouse models, the forced swim and tail suspension tests, reporting decreased immobility without a corresponding rise in spontaneous locomotion for the most active compounds [8]. This is helpful, since it points to the saponin fraction as the likely source of activity and speaks to the common locomotor confound. It also suggests that the antidepressant-like effect is not simply a property of the crude plant matrix. Whether the effect of whole extract equals the sum of its saponins, or involves other components, has not been settled [8, 24].

Human data are limited but relevant. In a randomised, double-blind, placebo-controlled trial in elderly volunteers, a standardised extract taken for twelve weeks improved scores on a depression scale and reduced anxiety as well as improving cognition [6]. The trial was not designed in clinically depressed people, and mood changes were secondary outcomes in a cognitive study. Even so it matches the direction of the animal work. Systematic reviews of clinical trials concentrate on cognition and say little about mood [29, 30]. The findings are brought together in table 1, which also lists the main methodological points covered above and the

mechanistic reports discussed in the next section. Counting the entries by evidence type shows them spread fairly evenly between test methods, direct Bacopa findings and proposed mechanisms, with confounders as the smallest group (fig. 1). That balance says something about the field: a large share of what can be stated with confidence concerns the tests, and a smaller share concerns the plant [13, 17, 21].

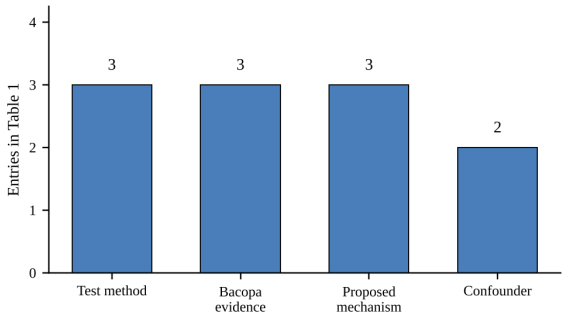


Fig. 1: Number of entries in table 1 by evidence type

How large the effect is, compared with standard drugs, is a fair question. Papers usually report a statistically significant fall in immobility relative to vehicle and then place it beside the reference drug, often with the statement that the extract is 'comparable' to the antidepressant [7, 8]. Comparability depends on the dose of the reference and on the variance in the groups. Also, a drug that reduces immobility by a fixed amount in a six-minute test does not necessarily have an equivalent clinical effect size, because the test has a ceiling and is insensitive to the dose-response shape seen in patients [31, 32]. The result is a qualitative signal of activity, which should not be converted into a claim about potency.

5. PROPOSED MECHANISMS

A serotonergic route has the most direct support. Charles and colleagues found that a Bacopa leaf extract raised expression of tryptophan hydroxylase 2 and of the serotonin transporter in rat brain, which suggests an effect on serotonin synthesis and handling [21]. Because most clinically used antidepressants act on serotonin or noradrenaline, this fits the behavioural findings. The data are from expression studies and do not show changes in extracellular serotonin or the effect of receptor antagonists on the swim result. Experiments in which a serotonin synthesis inhibitor or a receptor blocker abolishes the effect of Bacopa would be more decisive and would help to separate it from simple stimulation [16, 17].

A second explanation involves the stress axis. Chronic stress raises plasma corticosterone and disturbs brain monoamines in rodents, and Sheikh and colleagues reported that a Bacopa extract attenuated these stress-induced changes in rats [22]. Since overactivity of the hypothalamic-pituitary-adrenal axis is among the best-replicated abnormalities in depression, an adaptogen-like action could plausibly contribute to mood benefits [1, 2]. The evidence is correlative. Showing that lowered corticosterone is necessary for the behavioural effect would require adrenalectomy or receptor-blocking designs, which have not been reported for this plant [22, 26]. Dose and timing matter here too, since stress-related effects may need repeated dosing before they appear [7, 22].

Antioxidant and neuroprotective actions are the third candidate. Bhattacharya and co-workers found that Bacopa raised the activity of superoxide dismutase, catalase and glutathione peroxidase in rat frontal cortex, striatum and hippocampus [23]. Oxidative stress and inflammation are implicated in depression, so reducing them might help [1, 2]. Reviews of Bacopa's molecular pharmacology also mention effects on cholinergic function, on neurotrophic signalling and on synaptic proteins [5, 24]. These are attractive, but at present they form a list of observations, not a tested pathway, and the dose and time needed to engage each of them are not known. A cautious reader should treat the mechanisms as a list of leads, not as an explanation of what happens in a depressed person [5, 24].

Other pathways are mentioned in reviews of the plant but have little direct link to mood. Bacopa has been reported to modulate cholinergic transmission, to inhibit acetylcholinesterase weakly, to affect GABAergic signalling and to influence neurotrophic factors and synaptic proteins [4, 5, 24]. Cognitive benefit and mood benefit may share some of these steps, since

depression is accompanied by cognitive complaints and since improved plasticity could help both [1, 2]. At the same time, a broad profile makes mechanistic claims hard to pin down. A single extract that acts on many targets will always look active in a behavioural screen, so reductionist experiments with isolated saponins are needed to ask which target matters [8, 24].

6. INTERPRETATION AND LIMITATIONS

Locomotor activity is the most frequent confound. A compound that stimulates movement shortens immobility without having any antidepressant property, and a sedative may do the opposite. Good practice is to run an open field or activity test alongside the swim and suspension tests and to show that locomotion is unchanged at doses that reduce immobility [13, 17]. Some Bacopa studies include this control and others do not [7, 8]. Since the herb is described by traditional medicine as both calming and stimulating, the control matters here more than usual. Without it, a reduction of immobility cannot be accepted as an antidepressant-like effect, however significant the statistics.

Dose selection and translation are further issues. Rodent doses are usually converted to human equivalents by body surface area; for a mouse the conversion divides the dose per kilogram by roughly twelve [35]. Many rodent doses of plant extracts, when converted, would be much higher than the amounts people take as supplements, so an apparently convincing mouse result may not apply to a standard capsule. Dose-response relationships are seldom reported, and positive controls are often given by a different route or schedule. Reporting guidelines for animal research set out the information that should appear in every paper, including randomisation, blinding and sample size justification [33].

Reading the whole body of work, several gaps stand out. Chronic and sub-chronic dosing designs are underused in the swim and suspension literature, even though both the human trial and the biology of the plant suggest slow onset [6, 21]. Female animals and aged animals are seldom included. Studies in genuine models of depression, such as chronic mild stress, are few. Pharmacokinetic work on bacosides, which are large glycosides of uncertain oral absorption, would clarify which compounds reach the brain [24, 27]. Until such studies appear, claims about clinical value remain speculative and should be read as an indication for further research [3, 5].

Translation to patients brings other difficulties. Depression in people is heterogeneous, with differences in symptoms, causes and response, whereas the swim test delivers a single behavioural number in a naive animal. Animals in the tests are healthy and are not depressed before the test begins [18, 19]. A herbal product might help mild or subthreshold symptoms and do little in severe illness, or the reverse. The one trial that measured mood did so in older adults who were not selected for depression and used a self-rated scale [6]. Well-designed clinical studies would use diagnosed patients, a defined extract, adequate duration and a placebo arm, and would assess interactions with standard antidepressants [3, 29, 30].

7. CONCLUSION

Published work indicates that Bacopa monnieri extracts and their triterpene saponins reduce immobility in the forced swim and tail suspension tests, and that a trial in older adults recorded better mood scores alongside cognitive benefit. Proposed mechanisms include effects on serotonin handling, the stress axis and oxidative balance, but each rests on indirect evidence [6, 7, 8, 21, 22, 23]. Taken at face value, these findings place the herb among plants worth studying further for mood. The combined evidence is therefore encouraging but preliminary [4, 5].

Confidence should stay modest. Few laboratories are involved, extract characterisation is uneven, locomotor controls are inconsistent, and the tests themselves measure coping behaviour whose link to human depression is debated [13, 18, 19]. Future studies should use well-characterised extracts, repeated dosing, activity controls, mechanism-directed antagonist designs and

complete reporting [33, 35]. Until then Bacopa is best described as showing antidepressant-like activity in rodents, a sound basis for further research and not for clinical recommendation [3]. Replication by independent groups, using the same characterised extract, would do more for the field than a further series of single-laboratory positive reports [7, 8].

8. ABBREVIATIONS

FST-Forced swim test, TST-Tail suspension test, HPLC-High-performance liquid chromatography, TPH2-Tryptophan hydroxylase 2, SERT-Serotonin transporter, HPA-Hypothalamic-pituitary-adrenal, ARRIVE-Animal Research: Reporting of In Vivo Experiments, RCT-Randomised controlled trial

9. FUNDING

Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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